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(54) Title: NF-kappaB INHIBITORS

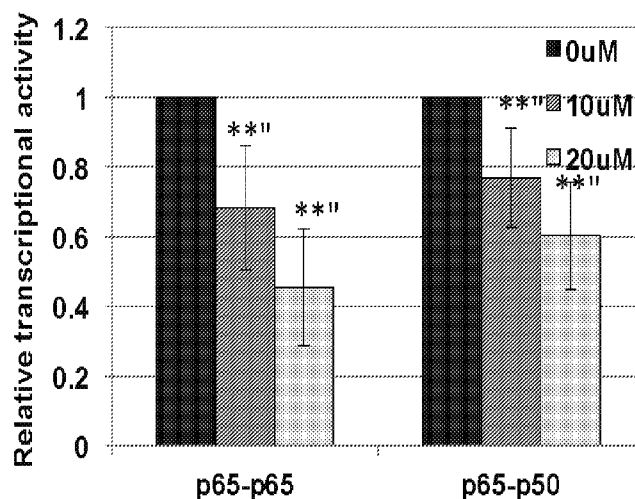


FIG. 8B

(57) Abstract: Disclosed herein are compounds capable of inhibiting dimerization of NF- κ B, for example, p65-p65 homodimerization and/or p50-p65 heterodimerization, the compounds being represented by formula I: X-L₁-L₂-Y Formula I wherein X, Y, L₁, L₂ and L₂ are as defined herein, the compound being for use in inhibiting dimerization of NF- κ B. Further disclosed are compounds capable of disrupting a hydrophobic core domain of p65, for use in inhibiting dimerization of NF- κ B comprising p65. Further disclosed herein is a method of identifying an inhibitor of NF- κ B dimerization, utilizing a first NF- κ B monomer attached to a first fragment of a luciferase protein and a second NF- κ B monomer attached to a second fragment of a luciferase protein, wherein dimerization of the first NF- κ B monomer and the second NF- κ B monomer increases luciferase activity.



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NF-kappaB INHIBITORS

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to a therapy and, more particularly, but not exclusively, to compounds usable in inhibiting NF-κB dimerization and/or activity and to methods of identifying such compounds.

The NF-κB family is family of dimeric transcription factors that plays key roles in innate and adaptive immune responses, cell proliferation, cell death, and inflammation. The proteins of the NF-κB family, including p65/RelA, cRel, RelB, p50 and p52, are responsible for transcription activation of a large number of inflammatory genes, immune response genes, and genes promoting cell survival of normal and cancer cells [Gilmore, *Cancer Treat Res* 2003, 115:241-265; DiDonato et al., *Immunol Rev* 2012, 246:379-400]. These proteins share a highly conserved DNA-binding and dimerization domain called Rel homology region (RHR). NF-κB proteins can form homodimers and heterodimers which bind to specific DNA sequences called κB sites [Ghosh et al., *Ann Rev Immunol* 1998, 16:225-260]. This combinatorial diversity contributes to the regulation of distinct but overlapping sets of genes, as individual dimers display affinity towards a collection of related κB sites [Smale, *Immunol Rev* 2012, 246:193-204; Wang et al., *Cell Rep* 2012, 2:824-839; Siggers et al., *Nat Immunol* 2012, 13:95-102; Tsui et al., *Nat Commun* 2015, 6:7068]. The relative abundance of different NF-κB proteins varies between different cell types with the exception of p50-p65/RelA heterodimer, which is ubiquitous and the most readily detected complex in most cells [Hoffman et al., *Oncogene* 2006, 25:6706-6716]. In this dimer, the p65/RelA is responsible for the transcriptional activity through a composite transactivation domain located within the last 120 C-terminal amino acids.

The activity of NF-κB is modulated by many extra-cellular signals including cytokines, tumor promoters, carcinogens and chemotherapeutic agents. In unstimulated cells, NF-κB is retained in the cytoplasm in an inactive form by IκB proteins. Signals that activate NF-κB trigger phosphorylation of IκB by IκB kinase complexes (IKKs) and subsequent ubiquitination and degradation of IκB by the proteasome, resulting in transport of NF-κB into the nucleus and activation of responsive genes [Karin & Lin, *Nat Immunol* 2002, 3:221-227; Hayden & Ghosh, *Genes Dev* 2004, 18:2195-2224].

Under normal conditions, NF- κ B activity is transient, as several of its early target genes are negative regulators of NF- κ B signaling.

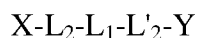
Aberrant regulation of NF- κ B is associated with chronic inflammation, cancer development and progression, and resistance to chemotherapy and radiotherapy [Ben-Neriah & Karin, *Nat Immunol* 2011, 12:715-723]. In many lymphoid malignancies, certain solid tumors and chronic inflammatory conditions, NF- κ B activity becomes persistent and contributes to or causes disease [Colotta et al., *Carcinogenesis* 2009, 30:1073-1081; Hanahan & Weinberg, *Cell* 2011, 144:646-674; Mantovani et al., *Nature* 2008, 454:436-444; Nagel et al., *Oncogene* 2014, 33:5655-5665].

A common strategy for inhibiting NF- κ B activation is to target the signaling cascade that leads to NF- κ B activation. Inhibitors of the 26S proteasome or IKK β have been shown to inhibit I κ B degradation and NF- κ B nuclear translocation, and some are already in use in the clinic.

Additional background art includes Ashkenazi et al. [*Mol Cell Biol* 2016, 36:1237-1247]; Jiang et al. [*J Biol Chem* 2010, 285:21023-21036]; Rai et al. [*Pharm Biol* 2016, 54:189-197]; Xia et al. [*Cancer Immunol Res* 2014, 2:823-830]; and U.S. Patent Nos. 6,410,516, and 8,552,206.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the invention, there is provided a compound capable of inhibiting p65-p65 homodimerization and/or p50-p65 heterodimerization, the compound being represented by formula I:



Formula I

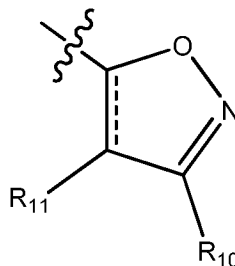
wherein:

X is a heteroaryl or aryl;

L₁ is selected from the group consisting of -CR₁R₂-A-C(=O)-B-, -A-C(=O)-B-, -CR₅R₆-NR₇-CR₈R₉- and phenylene, wherein A is NR₃ or absent, and B is NR₄ or absent;

L₂ and L'₂ are each independently absent, a linker of up to two atoms in length, or a linker of 3-7 atoms in length which comprises a heteroalicyclic ring;

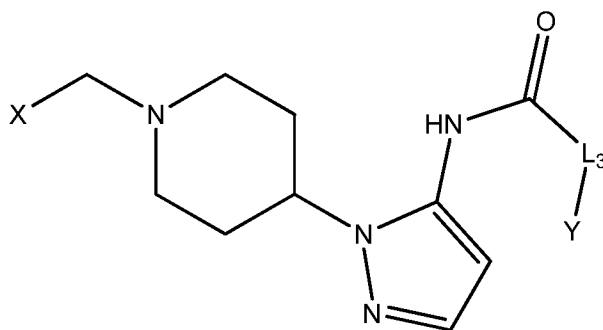
Y is selected from the group consisting of optionally substituted phenyl, optionally substituted pyridinyl, optionally substituted cycloalkyl, and a heterocyclic moiety having the general formula II:



Formula II

wherein the dashed line represents a saturated bond or an unsaturated bond; and R_1 - R_{11} are each independently selected from the group consisting of hydrogen and alkyl.

According to an aspect of some embodiments of the invention, there is provided a compound represented by Formula Ia:



Formula Ia

wherein:

X is a heteroaryl or aryl;

L_3 is absent or is selected from the group consisting of CH_2 and NH ; and

Y is selected from the group consisting of optionally substituted phenyl and cycloalkyl,

for use in inhibiting dimerization of NF- κ B.

According to an aspect of some embodiments of the invention, there is provided a compound capable of disrupting a hydrophobic core domain of a p65 unit of NF- κ B,

for use in inhibiting dimerization of an NF- κ B comprising the p65 unit, with the proviso that the compound is not withaferin A.

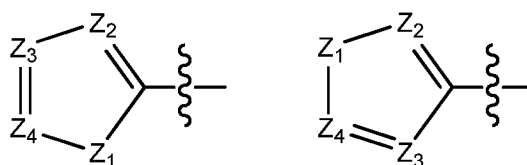
According to an aspect of some embodiments of the invention, there is provided a method of identifying an inhibitor of NF- κ B dimerization, the method comprising:

5 contacting at least one compound with a first NF- κ B monomer and a second NF- κ B monomer, wherein the first NF- κ B monomer is attached to a first fragment of a luciferase protein and the second NF- κ B monomer is attached to a second fragment of a luciferase protein, and wherein dimerization of the first NF- κ B monomer and the second NF- κ B monomer increases luciferase activity, and

10 identifying a compound which inhibits the increase in luciferase activity.

According to some embodiments of the invention according to any of the embodiments described herein, the compound according to any of the aspects described herein is for use in treating a medical condition in which an onset and/or progression of the condition is associated with NF- κ B dimerization and/or activity.

15 According to some embodiments of the invention according to any of the embodiments described herein pertaining to formula I, X in formula I has the general formula IIIa or general formula IIIb:



Formula IIIa

Formula IIIb

wherein:

Z₁ is selected from the group consisting of O, S, NR₂₁, and -CR₂₂=CR₂₃-;

Z₂ is selected from the group consisting of N and CR₂₄;

25 Z₃ is selected from the group consisting of N and CR₂₅;

Z₄ is selected from the group consisting of N and C₂₆; and

R₂₁-R₂₆ are each independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, and hydroxy, or alternatively, R₂₅ and R₂₆ together form a six-membered aromatic, heteroaromatic, alicyclic or

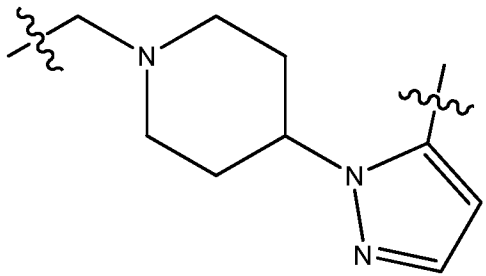
heteroalicyclic ring and/or any one of R₂₁-R₂₆ together with L₁ or L₂ forms a five-membered or six-membered alicyclic or heteroalicyclic ring.

According to some embodiments of the invention according to any of the embodiments described herein pertaining to formula I, Y in formula I is phenyl or pyridinyl, each being unsubstituted or substituted by one or more substituent selected from the group consisting of methyl, hydroxy, halo, and heteroaryl.

According to some embodiments of the invention according to any of the embodiments described herein pertaining to formula I, R₁₀ is C₁₋₅-alkyl.

According to some embodiments of the invention according to any of the embodiments described herein pertaining to formula I, R₁₁ is hydrogen.

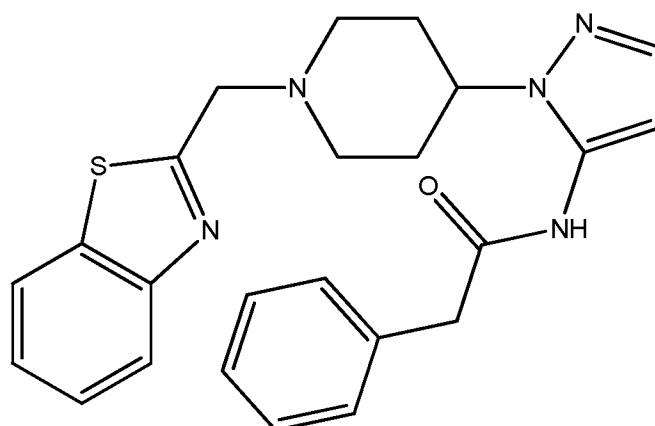
According to some embodiments of the invention according to any of the embodiments described herein pertaining to formula I, L₂ has the formula:



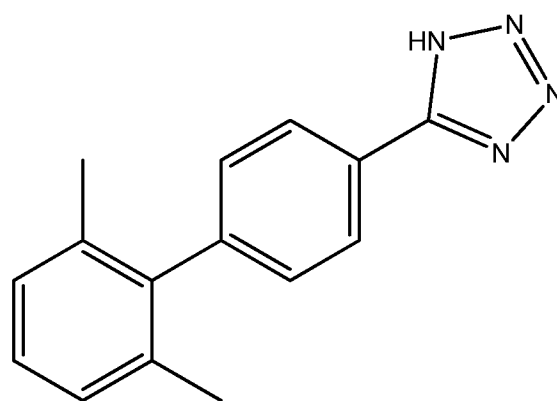
According to some embodiments of the invention according to any of the embodiments described herein pertaining to formula I, L₁ is selected from the group consisting of -NH-C(=O)-, -NH-C(=O)-CH₂-, and -NH-C(=O)-NH-.

According to some embodiments of the invention according to any of the embodiments described herein pertaining to formula I, Y is selected from the group consisting of optionally substituted phenyl and optionally substituted cycloalkyl.

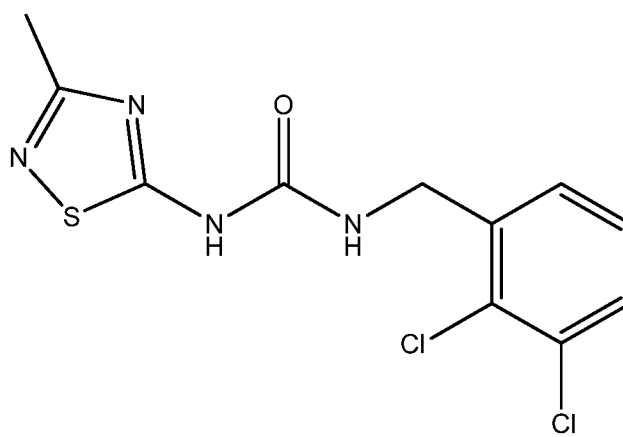
According to some embodiments of the invention, the compound is selected from the group consisting of:



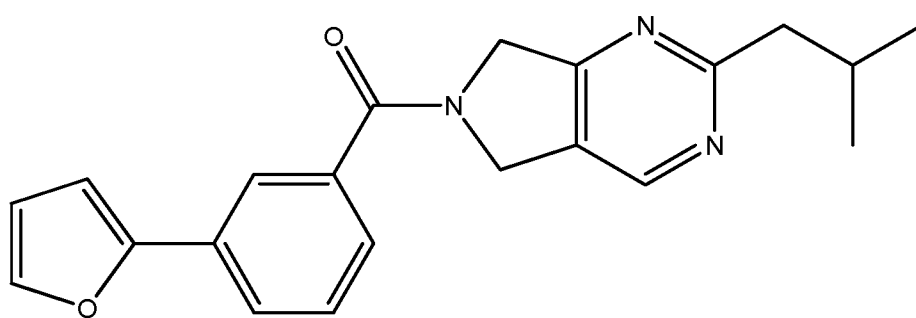
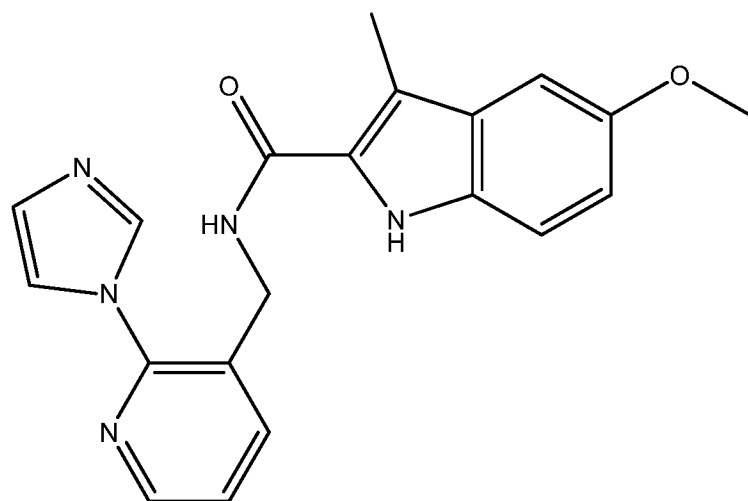
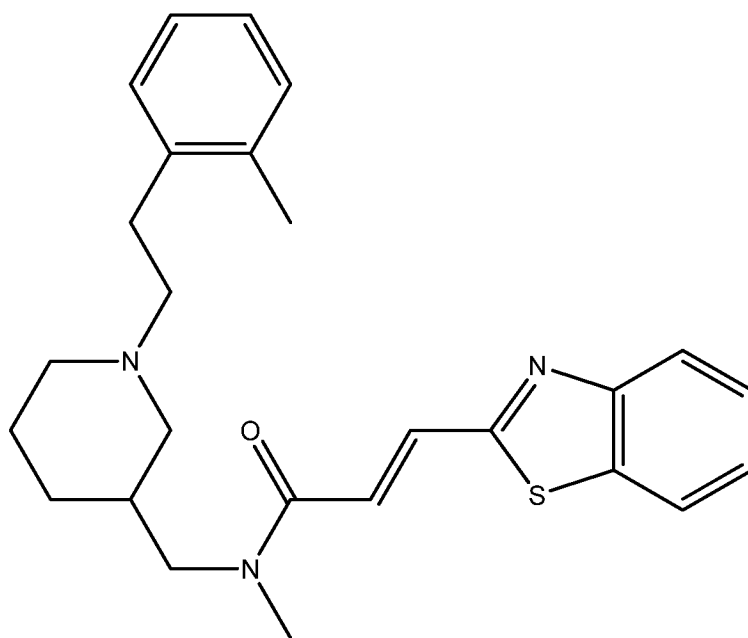
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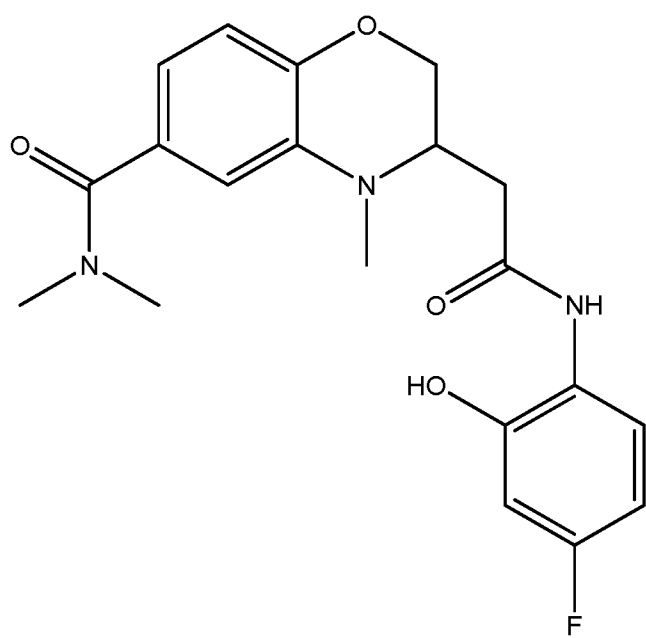
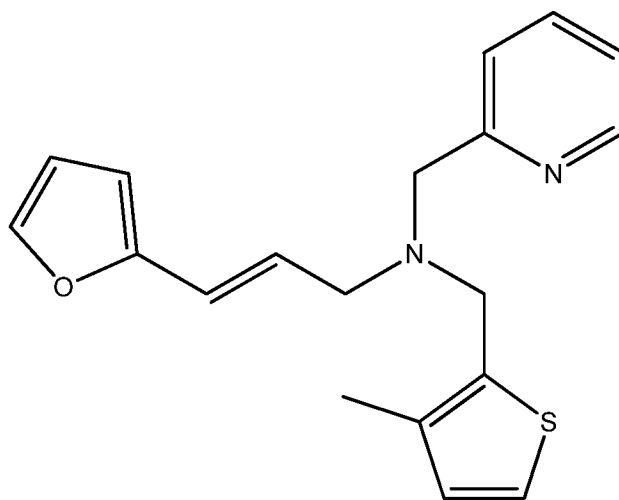
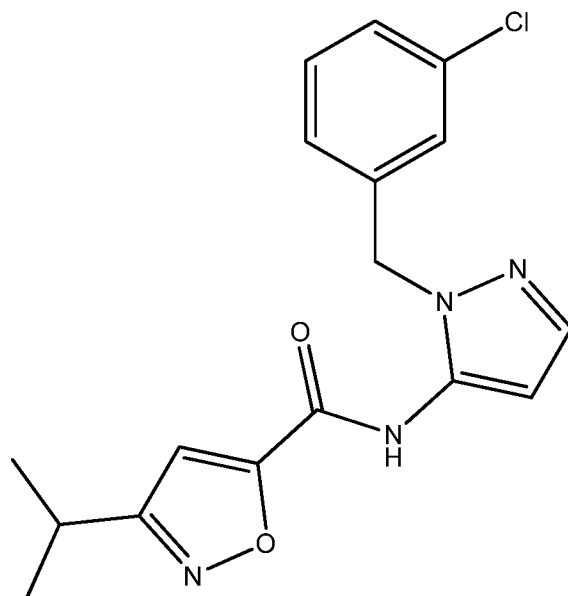


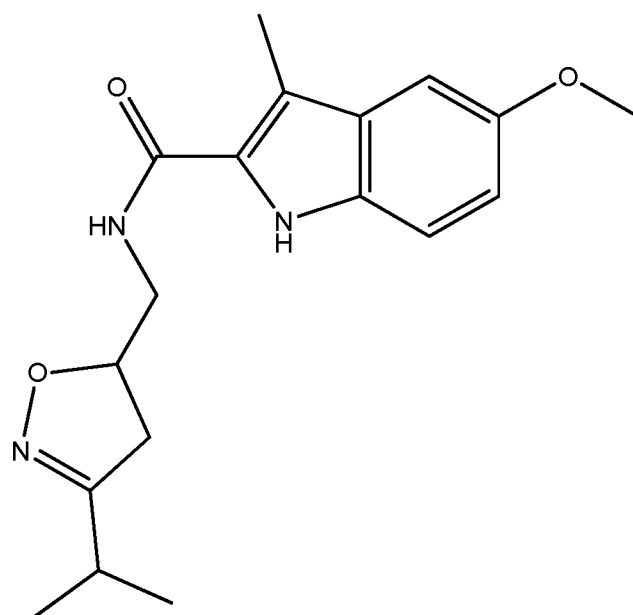
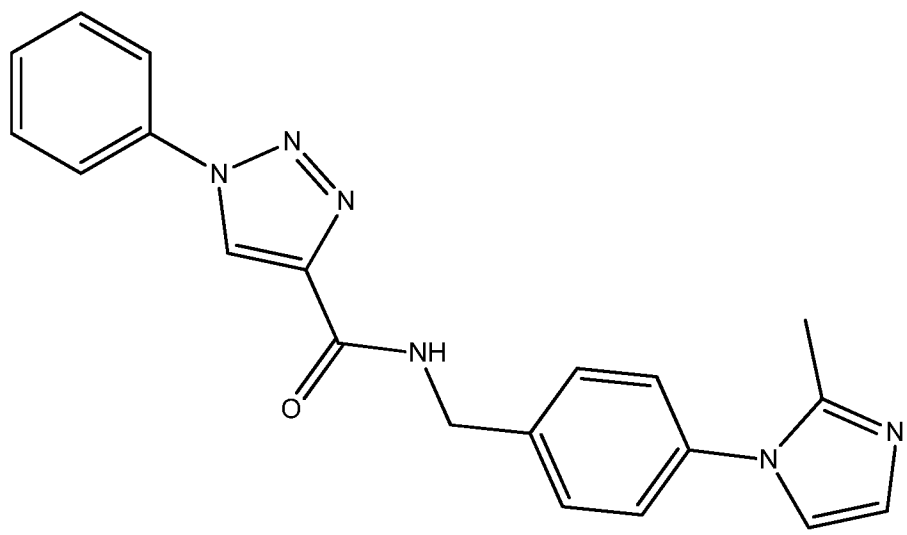
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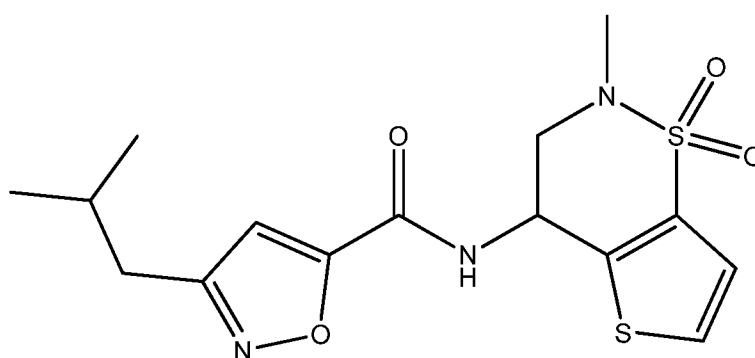
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5 According to some embodiments of the invention according to any of the embodiments described herein, the compound is capable of binding to an amino acid

residue of a p65 unit of NF- κ B, the amino acid residue being selected from the group consisting of F228, W233, T254, Y257, V268, F286, and Y288.

According to some embodiments of the invention according to any of the embodiments described herein, the compound is capable of binding to an E285 amino
5 acid residue and/or Q287 amino acid residue of a p65 unit of NF- κ B.

According to some embodiments of the invention according to any of the embodiments described herein pertaining to a medical condition, the medical condition is selected from the group consisting of an inflammatory diseases or disorder, an autoimmune disease or disorder, cancer, adult respiratory distress syndrome,
10 Alzheimer's disease, ataxia telangiectasia, atherosclerosis, cachexia, diabetes, glomerulonephritis, restenosis, and substance abuse.

According to some embodiments of the invention, the medical condition is a lymphoid cancer.

According to some embodiments of the invention, the cancer is multiple
15 myeloma.

According to some embodiments of the invention which relate to any of the embodiments described herein pertaining to a method of identifying an inhibitor, the plurality of compounds comprises a library comprising at least 100 compounds, the method comprising screening the library.

According to some embodiments of the invention which relate to any of the
20 embodiments described herein pertaining to a method of identifying an inhibitor, the method further comprises measuring luminescence at a wavelength suitable for detecting an activity of luciferase, wherein luminescence is indicative of the luciferase activity.

According to some embodiments of the invention, the method further comprises
25 contacting the compound which inhibits the increase in luciferase activity with an active luciferase protein having a luciferase activity, and

determining an effect of the compound on the luciferase activity of the active luciferase protein,

wherein inhibition of a luciferase activity of the active luciferase protein by the
30 compound is indicative of the compound not being an inhibitor of NF- κ B dimerization.

According to some embodiments of the invention, the first NF- κ B monomer and/or the second first NF- κ B monomer described herein is a fragment of p65.

According to some embodiments of the invention, the first fragment of a luciferase protein is attached to an N-terminus of the first NF- κ B monomer and/or the second fragment of a luciferase protein is attached to an N-terminus of the second NF- κ B monomer.

5 Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent
10 specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example
15 only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

20 In the drawings:

FIGs. 1A-E present the design and application of a high-throughput screen for direct inhibitors of p65 dimerization. FIG. 1A presents an illustration of p65-p65 homodimer structure (aa 191-304) (PDB ID: 1MY5) with labeled dimerization interface residues (left) and data obtained after WT and dimerization interface mutants of p65
25 were transfected into cells together with the NF- κ B target reporter A20-luciferase and the RSV-Renilla, which served as a control for transfection efficiency, twenty-four hours after transfection cells. The cells were harvested and luciferase activities are shown. Bars represent the mean $\pm$ SE of 3 independent experiments. FIG. 1B presents a schematic illustration of the p65-split-*Renilla* luciferase principle. FIG. 1C is a bar
30 graph showing RL and FL activities measured 24 hours after N- and C- termini of the RL fused to p65 were transfected into cells together with miR-22- *firefly* luciferase (FL), which served as an internal control. Bars represent the mean of 3 $\pm$ SEM

independent experiments. The activity of the WT p65-C/N-RL pair was set to 1. FIG. 1D is a bar graph showing RL enzymatic activity measured after *E. Coli* was transformed with either an empty plasmid or with a plasmid directing expression of p65-N/C-RL fusion proteins. Following induction cell lysates were analyzed. FIG. 1E presents Western Blot analyses of the p65-N/C RL proteins, with the antibodies indicated at the bottom.

FIGs. 2A-F present a flow chart summarizing the high-throughput screen for direct inhibitors of p65 dimerization (FIG. 2A); the chemical structure of Withaferin A (WFA) (FIG. 2B); a dose-response to WFA of the empty and p65-N/C-RL (FIG. 2C); Western blot analysis using α -p65 antibody of immune complexes formed after cells were co-transfected with p65 and GFP-p65 and harvested 48 hours later and the cell lysates were immunoprecipitated either with α -GFP and treated with 10 μ M or 30 μ M WFA for one hour (FIG. 2D); a bar graph showing data obtained when N- and C-termini of the RL fused to p65 or to p50 were transfected into cells and RL activity was measured after 24 hours (Bars represent the mean of $3 \pm$ SEM independent experiments, and the activity of the WT p65-C/N-RL pair was set to 1) (FIG. 2E); and comparative plots showing a dose-response to WFA of the empty, a homodimer of p50-N/C-RL and a heterodimer of p65-C/p50-N-RL (FIG. 2F).

FIGs. 3A-C present data showing inhibition of NF- κ B activity in cells by WFA. In FIG. 3A, untreated or WFA treated (10 μ M, 1 hour) cells were induced by TNF α for 1 hour and levels of A20, I κ B α and GAPDH mRNAs were determined by RT-qPCR using gene specific primers. Bars represent the mean $\pm$ SD of A20 and I κ B α levels normalized to GAPDH of 2 independent experiments. In FIG. 3B, Untreated or WFA treated cells (10 μ M, 1 hour) were induced by TNF α for 2 hours and level of A20 protein was determined by western blot. The WFA lanes were spliced to bring them close to the control lanes. In FIG. 3C, cells, pretreated with WFA for 60 minutes and then treated with TNF α for 30 minutes or left untreated, were subjected to chromatin immunoprecipitation (ChIP) using anti-p65 or control (for background levels) antibodies. Analysis was performed by qPCR. Graphs show occupancy levels normalized to the input levels. The un-induced sample was set to 1. The results represent the average $\pm$ SEM of 3 independent experiments.

FIGs. 4A-E present data for WFA-bound p65 indicating that E285 and Q287 are allosteric modulators of p65 dimerization. In FIG. 4A, Computational models of p65:p65 homodimer (PDB ID: 1MY5) in a complex with WFA using Schrodinger program are shown. Surface and ribbon models are shown on the left and right, respectively. Contacting residues are labeled on the right. In FIG. 4B, data obtained in split Renilla dimerization assay of WT p65 and the indicated mutants are presented. In FIG. 4C, the effect of mutations in E285 and Q287 on p65 on transcriptional activity was determined as described in FIG. 1A. Bars represent the mean $\pm$ SE of 3 independent experiments. Expression of the WT and mutant proteins is shown at the bottom. In FIG. 4D, a dose-response to WFA of the WT p65 and E285A/Q287A mutant N/C-RL pairs is presented. The results represent the mean $\pm$ SE of 3 independent protein preparations. In FIG. 4E, purified p65 WT or E285A/Q287A (3.4 μ M) were incubated with increasing concentrations of WFA and the intrinsic fluorescence levels were measured using Monolith NT.LabelFree instrument (NanoTemper). The graph shows the changes in fluorescence intensities in response to the indicated concentration of WFA.

FIGs. 5A-G show that the effect of the non-conserved E285 and Q287 on dimerization is linked to the highly conserved F286 and Y288. In FIG. 5A, amino acid sequence alignment of five NF- κ B (SEQ ID NO: 1 and 6-9) and two NFAT (SEQ ID NO: 10 and 11) proteins is shown. Surface residues E285 and Q287 are marked by asterisks and adjacent HCD residues with arrowheads. In FIG. 5B, a bar graph showing that F286 and Y288, which are adjacent to E285 and Q287, are essential for dimerization is presented. A split Renilla dimerization assay was performed using WT p65 and F286A and Y288A mutants. In FIG. 5C, it is shown that p65 Y288A mutation impairs dimerization. Cells were co-transfected with p65 and GFP-p65 (both WT or Y288A mutant) and harvested 48 hours later. Lysates were immunoprecipitated either with α -GFP antibody or with a control antibody. The input (10 %) and the immune complexes were analyzed by Western blot using α -p65 antibody. The lanes of the Y288A IP were spliced to bring them close to the WT lanes. In FIG. 5D, activation of the A20 promoter-luciferase by WT, Y288A and Y288F p65 variants is demonstrated. Bars represent the mean $\pm$ SE of 3 independent experiments. In FIG. 5E, HEK293T cells were transfected with p65 WT and Y288A and 24 hours later total RNA was

extracted. Levels of A20, I κ B α and GAPDH mRNAs were determined by RT-qPCR. Bars represent the mean $\pm$ SD of A20 and I κ B α levels normalized to GAPDH of 2 independent experiments. In FIG. 5F, it is shown that Y288 mutations impair DNA binding. WT, Y288A and Y288F p65 variants were synthesized *in vitro* in rabbit
5 reticulocytes lysate and then subjected to electrophoresis mobility shift assay (EMSA) using a fluorescently labeled DNA probe. The positions of p65-DNA complex and the free DNA are indicated. In FIG. 5G, HEK293T cells were transfected with p65 WT and Y288A and 24 hours later subjected to chromatin immunoprecipitation (ChIP) using anti-p65 or control (for background levels) antibodies. Analysis was performed by
10 qPCR. Graphs show occupancy levels normalized to the input levels. The un-induced sample was set to 1. The results represent the average $\pm$ SEM of at least 3 independent experiments.

FIGs. 6A-G present characterization of a highly conserved hydrophobic core domain (HCD) as a dimerization scaffold in NF- κ B and NFAT. In FIG. 6A, structural
15 illustration of a single central domain of p65 (aa 191-304) (PDB ID: 1MY5), with HCD residues marked, is presented. In FIG. 6B, analysis of transcriptional activity of HCD mutants F228A, W233A, T254, Y257A and Y288A, is presented. In FIG. 6C, the effect of HCD mutants on the expression of I κ B α , an endogenous target gene, is shown. HEK293T cells were transfected with the indicated HCD mutants and harvested 24
20 hours post transfection. Levels of endogenous I κ B α in mock and p65 variants transfected cells were monitored by Western blot. In FIG. 6D, The effect of HCD and T254 mutants on DNA binding was analyzed. Arrowheads point to the p65-DNA complex. In FIG. 6E, the effect of T254 mutants on transcriptional activity is shown. The bars represent the mean $\pm$ SE of 4 independent experiments. In FIG. 6F, analysis of
25 dimerization activity of HCD mutants F228A, W233A, T254, Y257A and Y288A using the split-Renilla assay is presented. In FIG. 6G, structural illustration of NFAT1 dimer (PDB ID: 2O93) is shown on the left. Residues of the HCD that correspond to those mutated in NFAT2 are marked in cyan. NFAT2 WT and HCD mutants were co-transfected into cells with a luciferase reporter gene driven by the IL-2 promoter and
30 analyzed. The results represent the mean $\pm$ SE of 4 independent experiments.

FIG. 7 is a flow chart describing a high-throughput screen of compounds from the Chembridge library for direct inhibitors of p65 dimerization, according to some embodiments of the invention.

FIGs. 8A and 8B show dose response of 293T cells to the exemplary compound SA788. FIG. 8A presents a graph showing relative dimerization of empty, p65-p65 and p65-p50-N/C-RL as a function of SA788 concentration in 293T cell lysate. FIG. 8B presents a bar graph showing relative transcriptional activity in 293T cells transfected with p65-p65 or p-65-p50 along with NF- κ B target reporter A20-luciferase and RSV-Renilla, which served as a control for transfection efficiency; after 5 hours, cells were treated with 10 or 20 μ M SA788 or with DMSO (0 μ M SA788) as control, and 24 hours after transfection, cells were harvested and luciferase activities measured (bars represent mean $\pm$ SE of 3 independent experiments; ** indicates $p < 0.01$).

FIG. 9 presents a graph showing fluorescence intensities as a function of SA788 concentration upon incubating of purified p65 (1-298) labeled with the fluorescent dye maleimide with increasing concentrations of SA788 in a representative experiment (fluorescence levels measured using Monolith NT.115 (NanoTemper); the average calculated K_d was $50.5 \pm$ SD of 19).

FIG. 10 presents bar graphs showing relative mRNA levels of TNF α , ICAM-1, CXCL-10, CXCL-11 and CXCL-1 (normalized to GAPDH mRNA levels) in MEF cells pre-treated with 25 μ M SA788 for 1 hour and then incubated with TNF α for 24 hours, and in control cells without pre-Sa788 treatment and/or TNF α incubation (bars represent mean $\pm$ SE of 3 independent experiments; RNA was extracted from cells and mRNA levels were determined by RT-qPCR using gene-specific primers; * indicates $p < 0.05$, ** indicates $p < 0.01$).

FIGs. 11A and 11B show dose response of 293T cells to the exemplary compound SA321. FIG. 11A presents a graph showing luminescence levels of p65-p65 (diamonds), indicating relative dimerization, as well as full length *Renilla* luciferase (RL) (squares) as a control, as a function of SA321 concentration in 293T cell lysate. FIG. 11B presents a bar graph showing relative transcriptional activity in 293T cells transfected with p65-p65 along with NF- κ B target reporter A20-luciferase and RSV-Renilla, which served as a control for transfection efficiency; after 5 hours, cells were

treated with 10, 25 or 50 μ M SA321 or with DMSO (0 μ M SA321) as control, and 24 hours after transfection, cells were harvested and luciferase activities measured (bars represent mean $\pm$ SE of 4 independent experiments).

5 DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to a therapy and, more particularly, but not exclusively, to compounds usable in inhibiting NF- κ B dimerization and/or activity and to methods of identifying such compounds.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

While investigating the activity and properties of NF- κ B, the inventors have uncovered a novel methodology for identifying inhibitors of NF- κ B dimerization, which may be effected in a rapid and efficient manner, thereby allowing screening of large numbers of compounds.

While reducing the present invention to practice, the inventors have uncovered exemplary compounds capable of inhibiting NF- κ B dimerization, and shown that inhibition of NF- κ B dimerization is a promising mechanism for therapy.

According to an aspect of some embodiments of the invention, there is provided a compound capable of inhibiting dimerization of NF- κ B.

Herein and in the art, the term "NF- κ B" refers to a protein which is commonly in a form of a dimer of protein units, and encompasses the proteins p65/RelA, cRel, RelB, p50 and p52 (each of which may be a unit of NF- κ B), as well as homodimers and heterodimers of any of the units of NF- κ B.

According to some embodiments of any of the embodiments described herein, the compound capable of inhibiting dimerization of an NF- κ B which comprises at least one p65 unit (i.e., dimerization of a p65 unit with any other NF- κ B unit). In exemplary embodiments, the compound is capable of inhibiting dimerization of a p65-p65 homodimer or a p65-p50 heterodimer.

Herein, the terms "p65", "RelA" and "p65/RelA" are used interchangeably. The canonical amino acid sequence of the p65 protein, as recognized in the art, is presented in SEQ ID NO: 1.

An exemplary amino acid of cRel is presented in SEQ ID NO: 6.

5 An exemplary amino acid of RelB is presented in SEQ ID NO: 7.

An exemplary amino acid of p50 is presented in SEQ ID NO: 8.

An exemplary amino acid of p52 is presented in SEQ ID NO: 9.

Herein, "dimerization" of an NF- κ B refers to the formation of a dimer form of an NF- κ B, from two NF- κ B units. The dimerization may optionally be homodimerization
10 (i.e., dimerization of two of the same unit) or heterodimerization (i.e., dimerization of two units which are different from one another).

According to some embodiments of any of the embodiments described herein, the compound is for use in inhibiting dimerization of NF- κ B (e.g., according to any of the respective embodiments described herein).

15 A compound may optionally be identified as being capable of inhibiting dimerization of an NF- κ B according to any suitable technique known in the art, optionally a method of identifying an inhibitor of NF- κ B dimerization according to some embodiments of the invention, as described herein (e.g., according to procedures exemplified in the Examples section herein).

20 ***Compounds:***

According to some embodiments of any of the embodiments described herein, the compound capable of inhibiting dimerization (according to any of the respective embodiments described herein) is represented by formula I:



Formula I

wherein X, L₁, L₂, L'₂ and Y are as defined herein.

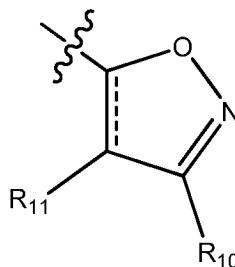
The moiety represented by the variable Y in formula I represents an aryl,
30 heteroaryl, cycloalkyl or heteroalicyclic, each being substituted or unsubstituted.

In some embodiments of any of the embodiments described herein, Y is a substituted or unsubstituted phenyl and/or substituted or unsubstituted pyridinyl (according to any of the respective embodiments described herein).

In some embodiments of any of the embodiments described herein, Y is a substituted or unsubstituted phenyl or a substituted or unsubstituted cycloalkyl (according to any of the respective embodiments described herein).

In some embodiments of any of the embodiments described herein, Y is an aryl, heteroaryl, cycloalkyl or heteroalicyclic (according to any of the respective embodiments described herein) which is unsubstituted or is substituted by an alkyl, hydroxy, halo or heteroaryl substituent. In some embodiments, the alkyl substituent is C₁₋₄-alkyl, optionally methyl. In some embodiments, the halo substituent is chloro or fluoro. In some embodiments, the heteroaryl substituent of Y is imidazol-1-yl, 2-methyl-imidazol-1-yl or furan-2-yl.

In some embodiments of any of the embodiments described herein, the heteroaryl or heteroalicyclic (represented by Y) is a substituted or unsubstituted pyridinyl (a heteroaryl) or a heterocyclic (i.e., heteroaryl or heteroalicyclic) having formula II:



Formula II

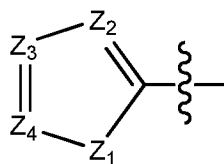
wherein the dashed line represents a saturated bond or an unsaturated bond, and R₁₀ and R₁₁ are each independently hydrogen, alkyl, alkenyl or alkynyl.

In some embodiments of any of the embodiments described herein, R₁₀ is hydrogen or alkyl. In some embodiments, R₁₀ is a C₁₋₅-alkyl, optionally unsubstituted. Exemplary C₁₋₅-alkyls include isopropyl and isobutyl.

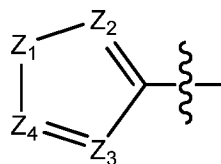
In some embodiments of any of the embodiments described herein, R₁₁ is hydrogen.

The moiety represented by the variable X in formula I represents a heteroaryl or aryl.

In some embodiments of any of the embodiments described herein, the X moiety is represented by formula IIIa or formula IIIb:



Formula IIIa



Formula IIIb

wherein:

Z₁ is O, S, NR₂₁ or -CR₂₂=CR₂₃-;

Z₂ is N or CR₂₄;

Z₃ is N or CR₂₅;

Z₄ is N or CR₂₆; and

R₂₁-R₂₆ are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, or hydroxy; or alternatively, R₂₅ and R₂₆ together form a six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring and/or any one of R₂₁-R₂₆ together with L₁ or L₂ forms a five-membered or six-membered alicyclic or heteroalicyclic ring.

In some embodiments of any of the respective embodiments described herein, at least one of Z₁ and Z₂ in formula IIIa is a heteroatom.

In some embodiments of any of the respective embodiments described herein, Z₁ in formula IIIb is O, S, or NR₂₁. In some embodiments, Z₁ in both formula IIIb and formula IIIa is O, S, or NR₂₁.

In some embodiments of any of the respective embodiments described herein, R₂₁ is hydrogen, alkyl, aryl or heteroaryl. In some embodiments, the aryl is a (substituted or unsubstituted) phenyl, optionally unsubstituted phenyl. In some embodiments, the heteroaryl is a (substituted or unsubstituted) pyridinyl, optionally pyridin-2-yl. In some embodiments, the alkyl is a C₁₋₄-alkyl, optionally methyl or ethyl. In some embodiments, the alkyl is unsubstituted. In alternative embodiments, the alkyl (optionally a C₁₋₄-alkyl) is an arylalkyl (i.e., alkyl substituted by an aryl or heteroaryl).

In some embodiments, the arylalkyl is a (substituted or unsubstituted) phenylalkyl. In some embodiments, the arylalkyl is an aryl-substituted methyl. In some embodiments, the arylalkyl is a (substituted or unsubstituted) phenylmethyl, for example, o-chlorophenyl-methyl.

5 In some embodiments of any of the respective embodiments described herein, R₂₁ is hydrogen, aryl (e.g., phenyl) or arylalkyl (e.g., o-chlorophenyl-methyl), according to any of the respective embodiments described herein.

In some embodiments of any of the respective embodiments described herein, R₂₁ is hydrogen, alkyl (e.g., methyl or ethyl), aryl (e.g., phenyl) or heteroaryl (e.g.,
10 pyridine-2-yl), according to any of the respective embodiments described herein.

In some embodiments of any of the respective embodiments described herein, R₂₂ and R₂₃ are each independently hydrogen or hydroxy or R₂₂ together with L₁ or L₂ forms a five- or six-membered alicyclic or heteroalicyclic ring (optionally a heteroalicyclic ring). In some such embodiments, X is represented by formula IIIa, and
15 C₂₃ is attached to the carbon atom adjacent to Z₄; and R₂₂ is hydrogen or together with L₁ or L₂ forms a five- or six-membered alicyclic or heteroalicyclic ring.

In some embodiments of any of the respective embodiments described herein, R₂₄ is hydrogen, alkyl (e.g., methyl) or alkoxy, or R₂₄ together with L₁ or L₂ forms a five- or six-membered alicyclic or heteroalicyclic ring (optionally a heteroalicyclic ring).

20 In some embodiments, the alkyl or alkoxy is a C₁₋₄-alkyl or C₁₋₄-alkoxy, for example, a substituted or unsubstituted methyl or methoxy (e.g., difluoromethoxy). In some embodiments, R₂₄ is hydrogen or methyl, or R₂₄ together with L₂ forms a six-membered alicyclic or heteroalicyclic ring (optionally a heteroalicyclic ring), according to any of the respective embodiments described herein. In some embodiments, R₂₄ is hydrogen or
25 alkoxy, according to any of the respective embodiments described herein.

In some embodiments of any of the respective embodiments described herein, R₂₅ and R₂₆ are each independently hydrogen, alkyl, alkoxy, aryl, heteroaryl, or amido, or R₂₅ and R₂₆ together form an aromatic or alicyclic ring. In some embodiments, the heteroaryl is furanyl, for example, furan-2-yl. In some embodiments, the amido is a C-amido, for example, (CH₃)₂NC(=O)-. In some embodiments, the aromatic or alicyclic
30 ring is a six-membered ring. In some embodiments, the aromatic or alicyclic ring (optionally six-membered) is unsubstituted or substituted by fluoro.

In some embodiments of any of the respective embodiments described herein, R_{25} and R_{26} are each independently hydrogen, alkyl, alkoxy or amido, or R_{25} and R_{26} together form an aromatic ring, according to any of the respective embodiments described herein.

5 In some embodiments of any of the respective embodiments described herein, R_{25} and R_{26} are each independently hydrogen, alkyl or heteroaryl (e.g., furan-2-yl), or R_{25} and R_{26} together form an aromatic or alicyclic ring, according to any of the respective embodiments described herein.

10 In some embodiments of any of the respective embodiments described herein, Z_1 is S and Z_2 is N.

Examples of suitable X moieties include, without limitation, substituted or unsubstituted indol-2-yl (e.g., 5-methoxy-3-methylindol-2-yl); substituted or unsubstituted thiazol-2-yl, optionally a benzothiazol-2-yl (e.g., unsubstituted benzothiazol-2-yl); and substituted or unsubstituted 1,2,4-thiadiazol-5-yl (e.g., 3-methyl-
15 1,2,4-thiadiazol-5-yl).

In some embodiments of any of the respective embodiments described herein, L_1 is $-\text{CR}_1\text{R}_2-\text{A}-\text{C}(=\text{O})-\text{B}-$, $-\text{A}-\text{C}(=\text{O})-\text{B}-$, $-\text{CR}_5\text{R}_6-\text{NR}_7-\text{CR}_8\text{R}_9-$ or phenylene (e.g., p-phenylene); wherein A is NR_3 or absent; and B is NR_4 or absent. For each formula of L_1 described herein, either side of L_1 may be proximal or distal to X (as opposed to Y) in
20 formula I.

R_1-R_9 are each independently hydrogen, alkyl, alkenyl or alkynyl (e.g., C_{1-4} -alkyl, C_{2-4} -alkenyl or C_{2-4} -alkynyl), optionally unsubstituted. In some embodiments of any of the embodiments described herein, R_1-R_9 are each hydrogen or alkyl. In some embodiments, R_1-R_9 are each hydrogen or methyl. In some embodiments, R_1-R_9 are
25 each hydrogen.

In some embodiments of any of the respective embodiments described herein, L_1 is $-\text{CR}_1\text{R}_2-\text{A}-\text{C}(=\text{O})-\text{B}-$, $-\text{A}-\text{C}(=\text{O})-\text{B}-$, or $-\text{CR}_5\text{R}_6-\text{NR}_7-\text{CR}_8\text{R}_9-$. In some embodiments, L_1 is $-\text{NR}_4-\text{C}(=\text{O})-$, $-\text{NR}_4-\text{C}(=\text{O})-\text{CR}_1\text{R}_2-$, or $-\text{NR}_3-\text{C}(=\text{O})-\text{NR}_4-$. In some embodiments, L_1 is $-\text{NH}-\text{C}(=\text{O})-$, $-\text{NH}-\text{C}(=\text{O})-\text{CH}_2-$, or $-\text{NH}-\text{C}(=\text{O})-\text{NH}-$.

30 In some embodiments of any of the respective embodiments described herein, L_2 and L'_2 are each independently absent, or a linker of up to two atoms in length, or a

linker of 3-7 atoms in length which comprises a heteroalicyclic ring (the length being along the shortest route through the ring).

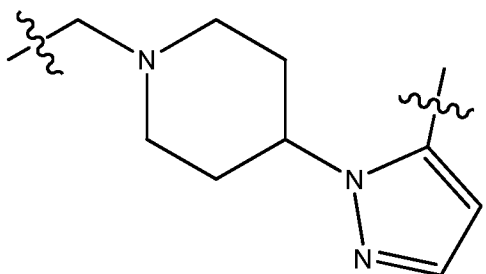
In some embodiments of any of the respective embodiments described herein, at least one of L_2 and L'_2 is absent. In some embodiments, L_2 and L'_2 are each absent.

5 Herein, the "linker of up to two atoms in length" encompasses one atom attached to each moiety attached to the linker (e.g., the L_2 moiety and X or Y moiety of formula I) as well as two atoms attached to each other (by a saturated or unsaturated bond) wherein each or the two atoms is further attached to a different moiety (e.g., the L_2 moiety, or X or Y moiety), thereby linking two moieties. The atoms in the linker may
10 optionally be any multivalent atom, such as carbon and/or a heteroatom (e.g., oxygen, sulfur and/or nitrogen). Atoms in the linker which have at least 3 valence bonds (e.g., carbon and nitrogen) may be further attached to hydrogen and/or to a substituent (e.g., any substituent of described herein for a carbon atom of an alkyl or nitrogen atom of an amine). An examples of a linker of two atoms is $-\text{CH}_2=\text{CH}_2-$.

15 Herein, the "linker of 3-7 atoms in length which comprises a heteroalicyclic ring" is as defined herein for a linker of up to two atoms, except that a greater number (3-7) of atoms are arranged in a chain between two moieties, and at least a portion of the atoms in the chain (e.g., 2-4, optionally 2-4) are part of a heteroalicyclic ring. The heteroalicyclic ring may optionally be a piperidine ring, optionally an N-linked
20 piperidine ring.

Examples of suitable linker of 3-7 atoms in length include, without limitation, piperidin-1-ylethyl (e.g., attached to L_1 at the 3-position of the piperidine ring) and (pyrazol-1-yl)piperidin-1-ylmethyl (e.g., attached to L_1 at the 5-position of the pyrazole ring).

25 In some embodiments of any of the respective embodiments described herein, L_2 has the formula:

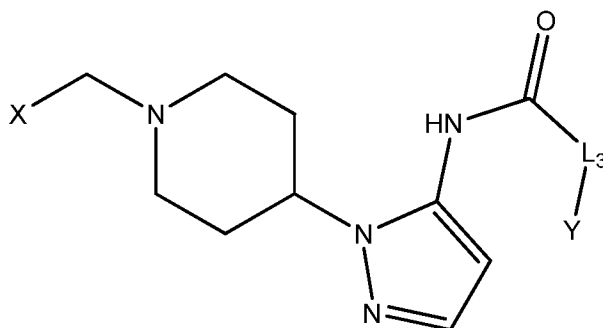


In some embodiments of any of the embodiments wherein L_2 has the above formula, L_1 is $-\text{NH}-\text{C}(=\text{O})-$, $-\text{NH}-\text{C}(=\text{O})-\text{CH}_2-$, or $-\text{NH}-\text{C}(=\text{O})-\text{NH}-$.

In some embodiments of any of the embodiments wherein L_2 has the above formula, Y is optionally substituted phenyl or optionally substituted cycloalkyl,
5 according to any of the respective embodiments described herein.

In some embodiments of any of the respective embodiments described herein, the compound capable of inhibiting dimerization (according to any of the respective embodiments described herein) is a compound presented in Table 1 and/or in Table 2 herein.

10 In some embodiments of any of the respective embodiments described herein relating to a compound of formula I, the compound is represented by formula Ia:



Formula Ia

15 wherein:

X is a heteroaryl or aryl (according to any of the respective embodiments described herein relating to formula I);

L_3 is CH_2 or NH , or is absent; and

Y is as defined for formula I (according to any of the respective embodiments described herein). In some embodiments, Y is substituted or unsubstituted phenyl or substituted or unsubstituted cycloalkyl (according to any of the respective embodiments described herein).

20 Non-limiting examples of compounds according to formula Ia are presented in Table 2 herein.

In some of any of the embodiments described herein, a compound as described in any of the embodiments herein, and any combination thereof, further comprises at least one moiety that enhances cell-permeability attached thereto.

Such moieties include, for example, positively charged groups and moieties such as, but not limited to, guanyl, guanidiny, amine, hydrazine, hydrazide, thiohydrazide, urea and thiourea groups (as defined herein).

In some embodiments, the compound comprises at least one guanyl and/or guanidiny. In some embodiments, the guanyl is $\text{H}_2\text{N}-\text{C}(=\text{NH})-$ and/or the guanidiny is $-\text{NH}-\text{C}(=\text{NH})-\text{NH}_2$.

For any of the embodiments described herein, and any combination thereof, the compound may be in a form of a salt, for example, a pharmaceutically acceptable salt.

As used herein, the phrase "pharmaceutically acceptable salt" refers to a charged species of the parent compound and its counter-ion, which is typically used to modify the solubility characteristics of the parent compound and/or to reduce any significant irritation to an organism by the parent compound, while not abrogating the biological activity and properties of the administered compound. A pharmaceutically acceptable salt of a compound as described herein can alternatively be formed during the synthesis of the compound, e.g., in the course of isolating the compound from a reaction mixture or re-crystallizing the compound.

In the context of some of the present embodiments, a pharmaceutically acceptable salt of the compounds described herein may optionally be an acid addition salt and/or a base addition salt.

An acid addition salt comprises at least one basic (e.g., amine and/or guanidiny) group of the compound which is in a positively charged form (e.g., wherein the basic group is protonated), in combination with at least one counter-ion, derived from the selected acid, that forms a pharmaceutically acceptable salt. The acid addition salts of the compounds described herein may therefore be complexes formed between one or more basic groups of the compound and one or more equivalents of an acid.

A base addition salt comprises at least one acidic (e.g., carboxylic acid) group of the compound which is in a negatively charged form (e.g., wherein the acidic group is deprotonated), in combination with at least one counter-ion, derived from the selected base, that forms a pharmaceutically acceptable salt. The base addition salts of the

compounds described herein may therefore be complexes formed between one or more acidic groups of the compound and one or more equivalents of a base.

Depending on the stoichiometric proportions between the charged group(s) in the compound and the counter-ion in the salt, the acid additions salts and/or base addition salts can be either mono-addition salts or poly-addition salts.

The phrase "mono-addition salt", as used herein, refers to a salt in which the stoichiometric ratio between the counter-ion and charged form of the compound is 1:1, such that the addition salt includes one molar equivalent of the counter-ion per one molar equivalent of the compound.

The phrase "poly-addition salt", as used herein, refers to a salt in which the stoichiometric ratio between the counter-ion and the charged form of the compound is greater than 1:1 and is, for example, 2:1, 3:1, 4:1 and so on, such that the addition salt includes two or more molar equivalents of the counter-ion per one molar equivalent of the compound.

An example, without limitation, of a pharmaceutically acceptable salt would be an ammonium cation or guanidinium cation and an acid addition salt thereof, and/or a carboxylate anion and a base addition salt thereof.

The base addition salts may include a cation counter-ion such as sodium, potassium, ammonium, calcium, magnesium and the like, that forms a pharmaceutically acceptable salt.

The acid addition salts may include a variety of organic and inorganic acids, such as, but not limited to, hydrochloric acid which affords a hydrochloric acid addition salt, hydrobromic acid which affords a hydrobromic acid addition salt, acetic acid which affords an acetic acid addition salt, ascorbic acid which affords an ascorbic acid addition salt, benzenesulfonic acid which affords a besylate addition salt, camphorsulfonic acid which affords a camphorsulfonic acid addition salt, citric acid which affords a citric acid addition salt, maleic acid which affords a maleic acid addition salt, malic acid which affords a malic acid addition salt, methanesulfonic acid which affords a methanesulfonic acid (mesylate) addition salt, naphthalenesulfonic acid which affords a naphthalenesulfonic acid addition salt, oxalic acid which affords an oxalic acid addition salt, phosphoric acid which affords a phosphoric acid addition salt, toluenesulfonic acid which affords a p-toluenesulfonic acid addition salt, succinic acid which affords a

succinic acid addition salt, sulfuric acid which affords a sulfuric acid addition salt, tartaric acid which affords a tartaric acid addition salt and trifluoroacetic acid which affords a trifluoroacetic acid addition salt. Each of these acid addition salts can be either a mono-addition salt or a poly-addition salt, as these terms are defined herein.

5 The present embodiments further encompass any enantiomers, diastereomers, prodrugs, solvates, hydrates and/or pharmaceutically acceptable salts of the compounds described herein.

As used herein, the term "enantiomer" refers to a stereoisomer of a compound that is superposable with respect to its counterpart only by a complete
10 inversion/reflection (mirror image) of each other. Enantiomers are said to have "handedness" since they refer to each other like the right and left hand. Enantiomers have identical chemical and physical properties except when present in an environment which by itself has handedness, such as all living systems. In the context of the present embodiments, a compound may exhibit one or more chiral centers, each of which
15 exhibiting an *R*- or an *S*-configuration and any combination, and compounds according to some embodiments of the present invention, can have any their chiral centers exhibit an *R*- or an *S*-configuration.

The term "diastereomers", as used herein, refers to stereoisomers that are not enantiomers to one another. Diastereomerism occurs when two or more stereoisomers
20 of a compound have different configurations at one or more, but not all of the equivalent (related) stereocenters and are not mirror images of each other. When two diastereomers differ from each other at only one stereocenter they are epimers. Each stereo-center (chiral center) gives rise to two different configurations and thus to two different stereoisomers. In the context of the present invention, embodiments of the
25 present invention encompass compounds with multiple chiral centers that occur in any combination of stereo-configuration, namely any diastereomer.

The term "prodrug" refers to an agent, which is converted into the active compound (the active parent drug) *in vivo*. Prodrugs are typically useful for facilitating the administration of the parent drug. They may, for instance, be bioavailable by oral
30 administration whereas the parent drug is not. A prodrug may also have improved solubility as compared with the parent drug in pharmaceutical compositions. Prodrugs are also often used to achieve a sustained release of the active compound *in vivo*. An

example, without limitation, of a prodrug would be a compound of the present invention, having one or more carboxylic acid moieties, which is administered as an ester (the "prodrug"). Such a prodrug is hydrolyzed *in vivo*, to thereby provide the free compound (the parent drug). The selected ester may affect both the solubility characteristics and the hydrolysis rate of the prodrug.

The term "solvate" refers to a complex of variable stoichiometry (e.g., di-, tri-, tetra-, penta-, hexa-, and so on), which is formed by a solute (the compound of the present invention) and a solvent, whereby the solvent does not interfere with the biological activity of the solute. Suitable solvents include, for example, ethanol, acetic acid and the like.

The term "hydrate" refers to a solvate, as defined hereinabove, where the solvent is water.

Hydrophobic core disruption:

According to some embodiments of any of the invention described herein, the compound capable of inhibiting dimerization of NF- κ B (e.g., NF- κ B comprising p65) according to any of the embodiments described herein is capable of disrupting a hydrophobic core domain of an NF- κ B. In some embodiments, the compound is capable of disrupting a hydrophobic core domain of a p65 unit of NF- κ B.

Withaferin A ((4 β ,5 β ,6 β ,22 R)-4,27-dihydroxy-5,6:22,26-diepoxyergosta-2,24-diene-1,26-dione) is an example of a compound (capable of disrupting a p65 hydrophobic core domain) which is excluded from preferred embodiments of the invention.

According some embodiments of any of the embodiments described herein, all withanolides and/or all compounds comprising an ergostane skeleton (of which withaferin A is one) are excluded from the invention.

According some embodiments of any of the embodiments described herein, steroids are excluded from the invention.

As used herein, a "hydrophobic core domain" comprises F228, W233, T254, Y257, V268, F286 and Y288 amino acid residues of p65 (e.g., according to SEQ ID NO: 1) or fragment thereof (e.g., according to SEQ ID NO: 2), or corresponding amino acid residues of a homolog of p65 (e.g., according to SEQ ID NO: 1) or fragment thereof (e.g., according to SEQ ID NO: 2), for example, a mutant p65 and/or a cRel, RelB, p50

or p52, or fragment thereof. The hydrophobic core domain optionally further comprises any other amino acids residues whose side chain is in proximity (e.g., 3 Å or less) to a side chain of any one or more of the aforementioned amino acid residues.

Herein, "disrupting" a hydrophobic core domain refers to changing a tertiary
5 structure of the domain, for example, by causing a pair of amino acid side chains which are in proximity to each other (e.g., 3 Å or less) in the non-disrupted core domain to become no longer in proximity to each other.

As shown in FIG. 5A, examples of (conserved) amino acid residues
10 corresponding to F228 of p65 include, without limitation, F220 of RelB, F327 of cRel, F284 of p50 and F262 of p52.

As further shown in FIG. 5A, examples of (conserved) amino acid residues corresponding to W233 of p65 include, without limitation, W225 of RelB, W332 of cRel, W294 of p50 and W270 of p52.

As further shown in FIG. 5A, examples of (conserved) amino acid residues
15 corresponding to T254 of p65 include, without limitation, T246 of RelB, T363 of cRel, T315 of p50 and T291 of p52.

As further shown in FIG. 5A, examples of (conserved) amino acid residues corresponding to Y257 of p65 include, without limitation, Y249 of RelB, Y366 of cRel, Y318 of p50 and Y294 of p52.

As further shown in FIG. 5A, examples of (conserved) amino acid residues
20 corresponding to V268 of p65 include, without limitation, V259 of RelB, V377 of cRel, V329 of p50 and V305 of p52.

As further shown in FIG. 5A, examples of (conserved) amino acid residues corresponding to F286 of p65 include, without limitation, F277 of RelB, F395 of cRel,
25 F347 of p50 and F323 of p52.

As further shown in FIG. 5A, examples of (conserved) amino acid residues corresponding to Y288 of p65 include, without limitation, Y279 of RelB, Y397 of cRel, Y349 of p50 and Y325 of p52.

In some embodiments, disrupting the hydrophobic core domain is by binding to
30 an amino acid residue within the hydrophobic core domain (e.g., an amino acid residue described hereinabove).

In some embodiments, disrupting the hydrophobic core domain is by binding to an amino acid residue that causes disrupting of the hydrophobic core domain. In some such embodiments, amino acid residue is not within the hydrophobic core domain, for example, the amino acid residue is adjacent to an amino acid residue within the hydrophobic core domain.

In some embodiments, the compound is capable of binding to an amino acid residue adjacent to an amino acid residue comprised by the hydrophobic core domain, for example, E285 and/or Q287 in p65, or a corresponding amino acid in a homologous sequence.

As shown in FIG. 5A, examples of amino acid residues corresponding to E285 of p65 include, without limitation, D276 of RelB, P394 of cRel, P346 of p50 and Q322 of p52; and examples of amino acid residues corresponding to Q287 of p65 include, without limitation, R278 of RelB, T396 of cRel, L348 of p50 and T324 of p52.

In some embodiments of any of the embodiments described herein, determining whether a compound is capable of binding a given site (e.g., a hydrophobic core domain and/or a given amino acid residue) is effected by computational docking (e.g., according to procedures described in the Examples section herein). Optionally molecular docking is performed computationally using Schrödinger (Schrödinger, LLC, New York, NY, 2015), e.g., including the LigPrep application (LigPrep, version 3.4) and Induced Fit Docking applications of Schrödinger Suite 2015 (e.g., as described in the Examples section), using a three-dimensional crystal structure of the NF- κ B protein from RCBS PDB database (e.g., PDB ID: 1MY5, resolution 1.80 Å). The structure is optionally optimized prior to docking using the Protein Preparation Wizard in Schrödinger Maestro Suite 2015.

Treatment:

The compound according to any of the respective embodiments described herein may optionally be for use in treating a medical condition in which an onset and/or progression of the condition is associated with NF- κ B dimerization and/or activity.

According to an aspect of some embodiments of the invention, there is provided a use of a compound according to any of the respective embodiments described herein in the manufacture of a medicament for treating a medical condition in which an onset

and/or progression of the condition is associated with NF- κ B dimerization and/or activity.

According to an aspect of some embodiments of the invention, there is provided a method of treating a medical condition in which an onset and/or progression of the condition is associated with NF- κ B dimerization and/or activity, the method comprising administering a therapeutically effective amount of a compound according to any of the respective embodiments described herein to a subject in need thereof, thereby treating the medical condition.

As used herein, the terms "treat", "treating", "treatment" and any derivatives thereof, in the context of a condition, includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

The term "therapeutically effective amount" denotes that dose of an active ingredient or a composition comprising the active ingredient that will provide the therapeutic effect for which the active ingredient is indicated, e.g., inhibiting dimerization and/or activity of NF- κ B.

In some embodiments of any of the embodiments described herein (according to any of the respective aspects described herein), onset and/or progression of the condition is associated with dimerization and/or activity of NF- κ B which comprises p65, for example, a p65-p65 dimer and/or a p65-p50 heterodimer.

Examples of medical conditions which may be treated by compounds described herein (e.g., in which an onset and/or progression of the condition is associated with NF- κ B dimerization and/or activity) include, without limitation, inflammatory diseases or disorders, autoimmune disease or disorders, cancers, adult respiratory distress syndrome, Alzheimer's disease, ataxia telangiectasia, atherosclerosis, cachexia (e.g., in a subject afflicted with cancer), diabetes, glomerulonephritis, restenosis, and substance abuse (e.g., associated with addiction).

Examples of inflammatory diseases and disorders which may be treated by compounds described herein include, without limitation, arthritis (e.g., rheumatoid arthritis), asthma, atopic dermatitis, chronic obstructive pulmonary disease (COPD), gastritis, hepatitis, inflammatory bowel disease, nephritis, osteoarthritis, osteoporosis,

radiation-induced skin damage (e.g., ultraviolet radiation-induced skin damage), renal failure (acute and/or chronic renal failure), sepsis, fibrotic diseases or disorders, and inflammation associated with infection, for example, viral infection (e.g., AIDS).

Examples of autoimmune diseases and disorders which may be treated by compounds described herein include, without limitation, lupus erythematosus (e.g., systemic lupus erythematosus), rheumatism, multiple sclerosis, psoriasis, psoriatic arthritis, ankylosing spondylitis, Hashimoto's thyroiditis, and tissue and/or organ rejection (e.g., upon tissue and/or organ transplant).

Examples of cancers which may be treated by compounds described herein include, without limitation, lymphoid cancers such as lymphomas (e.g., Hodgkin's disease, diffuse large B-cell lymphoma and multiple myeloma), leukemias (e.g., lymphocytic leukemias), breast cancer, colorectal cancer (e.g., KRAS-induced colorectal cancer and colitis-associated colon cancer), glioma (e.g., proneural glioma), head and neck cancer, lung cancer (e.g., KRAS-induced lung adenocarcinoma), pancreatic cancer (e.g., KRAS-induced pancreatic cancer), prostate cancer, and thyroid cancer (e.g., follicular thyroid carcinoma).

Pharmaceutical compositions:

The compounds described herein according to any of the aspects of embodiments of the invention described herein can be utilized (e.g., administered to a subject) *per se* or in a pharmaceutical composition where the compound is mixed with suitable carriers or excipients.

As used herein a "pharmaceutical composition" refers to a preparation of one or more compound according to any of the embodiments described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable
5 oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences", Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal,
10 transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intracardiac, e.g., into the right or left ventricular cavity, into the common coronary artery, intravenous, intraperitoneal, intranasal, or intraocular injections.

15 Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient.

The term "tissue" refers to part of an organism consisting of cells designed to perform a function or functions. Examples include, but are not limited to, brain tissue,
20 retina, skin tissue, hepatic tissue, pancreatic tissue, breast tissue, bone, cartilage, connective tissue, blood tissue, muscle tissue, cardiac tissue brain tissue, vascular tissue, renal tissue, pulmonary tissue, gonadal tissue, hematopoietic tissue.

Pharmaceutical compositions of some embodiments of the invention may be manufactured by processes well known in the art, e.g., by means of conventional
25 mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with some embodiments of the invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which
30 facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or

liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

5 For administration by nasal inhalation, the active ingredients for use according to some embodiments of the invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-
10 tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the active compound and a suitable powder base such as lactose or starch.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations
15 for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous
20 solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of
25 the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with
30 a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The pharmaceutical composition of some embodiments of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in context of some embodiments of the invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of the active ingredient(s) effective to prevent, alleviate or ameliorate symptoms of a deleterious medical condition (e.g., according to any of the respective embodiments described herein) or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from *in vitro* and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures or experimental animals. The data obtained from these *in vitro* and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, e.g., Fingl et al. (1975), in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide protein (e.g., p65 and/or p50 dimerization) inhibitory levels of the active ingredient which are sufficient to induce or suppress the biological effect (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from *in vitro* data, e.g., dimerization inhibition assay described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

5 The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of some embodiments of the invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may
10 contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of
15 pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared,
20 placed in an appropriate container, and labeled for treatment of an indicated condition, as is further detailed herein.

Identification of inhibitors:

According to an aspect of some embodiments of the invention, there is provided a method of identifying an inhibitor of NF- κ B dimerization (e.g., dimerization of an NF-
25 κ B according to any of the respective embodiments described herein).

The method according to this aspect comprises contacting at least one compound (which is being tested for inhibitory activity) with a first NF- κ B monomer and a second NF- κ B monomer, wherein the first NF- κ B monomer is attached to a first fragment of a luciferase protein and the second NF- κ B monomer is attached to a second fragment of a
30 luciferase protein, and wherein dimerization of the first NF- κ B monomer and the second NF- κ B monomer increases luciferase activity (e.g., luminescence); and identifying a

compound which inhibits the aforementioned increase in luciferase activity (e.g., relative to luciferase activity in the absence of the compound).

The method optionally comprises further contacting the luciferase with a substrate (e.g., coelenterazine) which results in luminescence upon contact with the luciferase.

In some embodiments of any of the embodiments described herein, the method comprises measuring luminescence at a wavelength suitable for detecting an activity of the luciferase, the luminescence being indicative of the luciferase activity.

The skilled person will be readily capable of selecting a suitable wavelength and (if needed) a suitable substrate for any given luciferase.

The luciferase is optionally *Renilla* luciferase (e.g., SEQ ID NO: 4), wherein the first fragment and second fragment together (if fused) form the *Renilla* luciferase.

In some embodiments of any of the embodiments described herein, the first fragment of luciferase comprises residues 1-229 of SEQ ID NO: 4 and/or the second fragment of luciferase comprises residues 230-311 of SEQ ID NO: 4, e.g., as described by Jiang et al. [*J Biol Chem* 2010, 285:21023-21036].

Herein, an "NF- κ B monomer" encompasses any unit of NF- κ B (e.g., p65/RelA, cRel, RelB, p50 and p52) or homolog thereof, and/or fragment thereof capable of forming a dimer with another NF- κ B monomer (e.g., p65/RelA, cRel, RelB, p50 and p52).

In some embodiments of any of the embodiments described herein, a fragment of a unit of NF- κ B comprises an N-terminal portion of the unit.

In some embodiments of any of the embodiments described herein, the first NF- κ B monomer and/or said second first NF- κ B monomer is a p65 (e.g., SEQ ID NO: 1) or a fragment of p65. An exemplary fragment of p65 (which comprises an N-terminal portion thereof) is represented by SEQ ID NO: 2.

Attachment of a fragment of a luciferase protein to an NF- κ B monomer may be effected by any suitable technique known in the art for attaching polypeptides to one another.

In some embodiments of any of the embodiments described herein, attachment is effected by incorporating the fragment of a luciferase protein to the NF- κ B monomer in a fusion protein. The fusion protein may comprise the fragment of a luciferase

protein attached directly to the NF- κ B monomer, or separated by a linker. SEQ ID NO: 3 is an exemplary linker (as described in the Examples section herein).

In some embodiments of any of the embodiments described herein, the first fragment of a luciferase protein is attached to an N-terminus of the first NF- κ B monomer (according to any of the respective embodiments described herein) and/or said second fragment of a luciferase protein is attached to an N-terminus of said second NF- κ B monomer (according to any of the respective embodiments described herein). In exemplary embodiment, both the first fragment of a luciferase protein and the second fragment of a luciferase protein are each attached to an N-terminus of the respective NF- κ B monomer.

In exemplary embodiments, a sequence comprising residues 1-229 of SEQ ID NO: 4 (a luciferase fragment) is attached via the linker SEQ ID NO: 3 to the N-terminus of SEQ ID NO: 2 (a p65 fragment); and a sequence comprising residues 230-311 of SEQ ID NO: 4 (a second luciferase fragment) is attached via the linker SEQ ID NO: 3 to the N-terminus of SEQ ID NO: 2. In such embodiments, the two obtained polypeptides may be used to identify inhibition of p65-p65 homodimerization.

Exemplary procedures for identifying an inhibitor (according to any of the respective embodiments described herein) are described in the Examples section below.

In some embodiments of any of the embodiments described herein, the method comprises screening a library of compounds, e.g., according to procedures described herein below. The library of compounds (e.g., a commercially available library) may optionally comprise at least 100 compounds, optionally at least 1,000 compounds, optionally at least 10,000 compounds, and optionally at least 100,000 compounds.

In some embodiments of any of the embodiments described herein, the method further comprises contacting the compound which inhibits an increase in luciferase activity in the presence of a first NF- κ B monomer and a second NF- κ B monomer (according to any of the respective embodiments described herein) with an active (e.g., intact) luciferase protein (e.g., SEQ ID NO: 4) having a luciferase activity, and determining an effect of said compound on said luciferase activity of said active luciferase protein (e.g., by measuring luminescence according to any of the respective embodiments described herein). Inhibition of a luciferase activity of the active luciferase protein by the compound is indicative of the compound not being an inhibitor

of NF- κ B dimerization (e.g., indicating that the inhibition of increase in luciferase activity in the presence of a first NF- κ B monomer and a second NF- κ B monomer represents a false positive).

Additional definitions and information:

5 As used herein throughout, the term “alkyl” refers to any saturated aliphatic hydrocarbon including straight chain and branched chain groups. Preferably, the alkyl group has 1 to 20 carbon atoms. Whenever a numerical range; e.g., “1-20”, is stated herein, it implies that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 20 carbon atoms. More
10 preferably, the alkyl is a medium size alkyl having 1 to 10 carbon atoms. Most preferably, unless otherwise indicated, the alkyl is a lower alkyl having 1 to 4 carbon atoms. The alkyl group may be substituted or non-substituted. When substituted, the substituent group can be, for example, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl,
15 cyano, nitro, azide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, guanyl, guanidinyl, hydrazine, hydrazide, thiohydrazide, and amino, as these terms are defined herein.

Herein, the term “alkenyl” describes an unsaturated aliphatic hydrocarbon
20 comprise at least one carbon-carbon double bond, including straight chain and branched chain groups. Preferably, the alkenyl group has 2 to 20 carbon atoms. More preferably, the alkenyl is a medium size alkenyl having 2 to 10 carbon atoms. Most preferably, unless otherwise indicated, the alkenyl is a lower alkenyl having 2 to 4 carbon atoms. The alkenyl group may be substituted or non-substituted. Substituted alkenyl may have
25 one or more substituents, whereby each substituent group can independently be, for example, cycloalkyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy,
30 sulfonamido, guanyl, guanidinyl, hydrazine, hydrazide, thiohydrazide, and amino.

Herein, the term “alkynyl” describes an unsaturated aliphatic hydrocarbon comprise at least one carbon-carbon triple bond, including straight chain and branched

chain groups. Preferably, the alkynyl group has 2 to 20 carbon atoms. More preferably, the alkynyl is a medium size alkynyl having 2 to 10 carbon atoms. Most preferably, unless otherwise indicated, the alkynyl is a lower alkynyl having 2 to 4 carbon atoms. The alkynyl group may be substituted or non-substituted. Substituted alkynyl may have one or more substituents, whereby each substituent group can independently be, for example, cycloalkyl, alkenyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, guanyl, guanidinyl, hydrazine, hydrazide, thiohydrazide, and amino.

A “cycloalkyl” group refers to a saturated or unsaturated all-carbon monocyclic or fused ring (i.e., rings which share an adjacent pair of carbon atoms) group wherein one or more of the rings does not have a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, cycloheptane, cycloheptatriene, and adamantane. A cycloalkyl group may be substituted or non-substituted. When substituted, the substituent group can be, for example, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, guanyl, guanidinyl, hydrazine, hydrazide, thiohydrazide, and amino, as these terms are defined herein. When a cycloalkyl group is unsaturated, it may comprise at least one carbon-carbon double bond and/or at least one carbon-carbon triple bond.

An “aryl” group refers to an all-carbon monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or non-substituted. When substituted, the substituent group can be, for example, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide,

phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, guanyl, guanidinyl, hydrazine, hydrazide, thiohydrazide, and amino, as these terms are defined herein.

5 A “heteroaryl” group refers to a monocyclic or fused ring (i.e., rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms, such as, for example, nitrogen, oxygen and sulfur and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups include pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine,
10 quinoline, isoquinoline and purine. The heteroaryl group may be substituted or non-substituted. When substituted, the substituent group can be, for example, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy,
15 sulfonamido, guanyl, guanidinyl, hydrazine, hydrazide, thiohydrazide, and amino, as these terms are defined herein.

 A “heteroalicyclic” group refers to a monocyclic or fused ring group having in the ring(s) one or more atoms such as nitrogen, oxygen and sulfur. The rings may also
20 have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic may be substituted or non-substituted. When substituted, the substituted group can be, for example, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide,
25 phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, guanyl, guanidinyl, hydrazine, hydrazide, thiohydrazide, and amino, as these terms are defined herein. Representative examples are piperidine, piperazine, tetrahydrofuran, tetrahydropyran, morpholine and the like.

30 Herein, the terms “amine” and “amino” each refer to either a $-NR'R''$ group or a $-N^+R'R''R'''$ group, wherein R' , R'' and R''' are each hydrogen or a saturated or unsaturated hydrocarbon moiety (as defined herein), the hydrocarbon moiety being

substituted or non-substituted. Optionally, R', R'' and R''' are hydrogen or alkyl comprising 1 to 4 carbon atoms. Optionally, R' and R'' (and R''', if present) are hydrogen. When substituted, the carbon atom of an R', R'' or R''' hydrocarbon moiety which is bound to the nitrogen atom of the amine is preferably not substituted by oxo, such that R', R'' and R''' are not (for example) carbonyl, C-carboxy or amide, as these groups are defined herein, except where indicated otherwise.

An "azide" group refers to a $-N=N^+=N^-$ group.

An "alkoxy" group refers to both an -O-alkyl and an -O-cycloalkyl group, as defined herein.

An "aryloxy" group refers to both an -O-aryl and an -O-heteroaryl group, as defined herein.

A "hydroxy" group refers to a -OH group.

A "thiohydroxy" or "thiol" group refers to a -SH group.

A "thioalkoxy" group refers to both an -S-alkyl group, and an -S-cycloalkyl group, as defined herein.

A "thioaryloxy" group refers to both an -S-aryl and an -S-heteroaryl group, as defined herein.

A "carbonyl" group refers to a $-C(=O)-R'$ group, where R' is defined as hereinabove.

A "thiocarbonyl" group refers to a $-C(=S)-R'$ group, where R' is as defined herein.

A "carboxyl", "carboxylic" or "carboxylate" refers to both "C-carboxy" and "O-carboxy".

A "C-carboxy" group refers to a $-C(=O)-O-R'$ groups, where R' is as defined herein.

An "O-carboxy" group refers to an $R'C(=O)-O-$ group, where R' is as defined herein.

A "carboxylic acid" refers to a $-C(=O)OH$ group, including the deprotonated ionic form and salts thereof.

An "oxo" group refers to a $=O$ group.

A "thiocarboxy" or "thiocarboxylate" group refers to both $-C(=S)-O-R'$ and $-O-C(=S)R'$ groups.

A “halo” group refers to fluorine, chlorine, bromine or iodine.

A “sulfinyl” group refers to an $-S(=O)-R'$ group, where R' is as defined herein.

A “sulfonyl” group refers to an $-S(=O)_2-R'$ group, where R' is as defined herein.

5 A “sulfonate” group refers to an $-S(=O)_2-O-R'$ group, where R' is as defined herein.

A “sulfate” group refers to an $-O-S(=O)_2-O-R'$ group, where R' is as defined as herein.

A “sulfonamide” or “sulfonamido” group encompasses both S-sulfonamido and N-sulfonamido groups, as defined herein.

10 An “S-sulfonamido” group refers to a $-S(=O)_2-NR'R''$ group, with each of R' and R'' as defined herein.

An “N-sulfonamido” group refers to an $R'S(=O)_2-NR''$ group, where each of R' and R'' is as defined herein.

15 An “O-carbamyl” group refers to an $-OC(=O)-NR'R''$ group, where each of R' and R'' is as defined herein.

An “N-carbamyl” group refers to an $R'OC(=O)-NR''$ group, where each of R' and R'' is as defined herein.

A “carbamyl” or “carbamate” group encompasses O-carbamyl and N-carbamyl groups.

20 An “O-thiocarbamyl” group refers to an $-OC(=S)-NR'R''$ group, where each of R' and R'' is as defined herein.

An “N-thiocarbamyl” group refers to an $R'OC(=S)NR''$ group, where each of R' and R'' is as defined herein.

25 A “thiocarbamyl” or “thiocarbamate” group encompasses O-thiocarbamyl and N-thiocarbamyl groups.

A “C-amido” group refers to a $-C(=O)-NR'R''$ group, where each of R' and R'' is as defined herein.

An “N-amido” group refers to an $R'C(=O)-NR''$ group, where each of R' and R'' is as defined herein.

30 A “urea” group refers to an $-N(R')-C(=O)-NR''R'''$ group, where each of R' , R'' and R''' is as defined herein.

A “nitro” group refers to an $-NO_2$ group.

A "cyano" group refers to a $-C\equiv N$ group.

The term "phosphonyl" or "phosphonate" describes a $-P(=O)(OR')(OR'')$ group, with R' and R'' as defined hereinabove.

The term "phosphate" describes an $-O-P(=O)(OR')(OR'')$ group, with each of
5 R' and R'' as defined hereinabove.

The term "phosphinyl" describes a $-PR'R''$ group, with each of R' and R'' as defined hereinabove.

The term "thiourea" describes a $-N(R')-C(=S)-NR''R'''$ group, where each of R' , R'' and R''' is as defined herein.

10 The term "hydrazine" describes a $-NR'-NR''R'''$ group, with R' , R'' , and R''' as defined herein.

As used herein, the term "hydrazide" describes a $-C(=O)-NR'-NR''R'''$ group, where R' , R'' and R''' are as defined herein.

As used herein, the term "thiohydrazide" describes a $-C(=S)-NR'-NR''R'''$
15 group, where R' , R'' and R''' are as defined herein.

A "guanidiny" group refers to an $-RaNC(=NRd)-NRbRc$ group, where each of Ra , Rb , Rc and Rd can be as defined herein for R' and R'' .

A "guanyl" or "guanine" group refers to an $RaRbNC(=NRd)-$ group, where Ra , Rb and Rd are as defined herein.

20 The polypeptides of some embodiments of the invention (e.g., an NF- κ B and/or luciferase sequence, according to any of the respective embodiments described herein) may be synthesized by any techniques that are known to those skilled in the art of peptide synthesis. For solid phase peptide synthesis, a summary of the many techniques may be found in J. M. Stewart and J. D. Young, Solid Phase Peptide Synthesis, W. H. Freeman Co. (San Francisco), 1963 and J. Meienhofer, Hormonal Proteins and Peptides,
25 vol. 2, p. 46, Academic Press (New York), 1973. For classical solution synthesis see G. Schroder and K. Lupke, The Peptides, vol. 1, Academic Press (New York), 1965.

In general, these methods comprise the sequential addition of one or more amino acids or suitably protected amino acids to a growing polypeptide chain. Normally, either
30 the amino or carboxyl group of the first amino acid is protected by a suitable protecting group. The protected or derivatized amino acid can then either be attached to an inert solid support or utilized in solution by adding the next amino acid in the sequence

having the complimentary (amino or carboxyl) group suitably protected, under conditions suitable for forming the amide linkage. The protecting group is then removed from this newly added amino acid residue and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining protecting groups (and any solid support) are removed sequentially or concurrently, to afford the final polypeptide compound. By simple modification of this general procedure, it is possible to add more than one amino acid at a time to a growing chain, for example, by coupling (under conditions which do not racemize chiral centers) a protected tripeptide with a properly protected dipeptide to form, after deprotection, a pentapeptide and so forth. Further description of peptide synthesis is disclosed in U.S. Pat. No. 6,472,505.

A preferred method of preparing the polypeptide compounds of some embodiments of the invention (e.g., a therapeutically active agent and/or a protease inhibitor described herein) involves solid phase peptide synthesis.

Large scale polypeptide synthesis is described by Andersson et al. [*Biopolymers* 2000; 55:227-250].

Herein, a "homolog" of a given polypeptide refers to a polypeptide that exhibits at least 80 % homology, preferably at least 90 % homology, and more preferably at least 95 % homology, and more preferably at least 98 % homology to the given polypeptide.

In some embodiments, a homolog of a given polypeptide further shares a therapeutic activity with the given polypeptide. The percentage of homology refers to the percentage of amino acid residues in a first polypeptide sequence which match a corresponding residue of a second polypeptide sequence to which the first polypeptide is being compared. Generally, the polypeptides are aligned to give maximum homology. A variety of strategies are known in the art for performing comparisons of amino acid or nucleotide sequences in order to assess degrees of identity, including, for example, manual alignment, computer assisted sequence alignment and combinations thereof. A number of algorithms (which are generally computer implemented) for performing sequence alignment are widely available, or can be produced by one of skill in the art. Representative algorithms include, e.g., the local homology algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2: 482); the homology alignment algorithm of Needleman and Wunsch (J. Mol. Biol., 1970, 48: 443); the search for

similarity method of Pearson and Lipman (Proc. Natl. Acad. Sci. (USA), 1988, 85: 2444); and/or by computerized implementations of these algorithms (e.g., GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, Wis.). Readily available
5 computer programs incorporating such algorithms include, for example, BLASTN, BLASTP, Gapped BLAST, PILEUP, CLUSTALW etc. When utilizing BLAST and Gapped BLAST programs, default parameters of the respective programs may be used. Alternatively, the practitioner may use non-default parameters depending on his or her experimental and/or other requirements (see for example, the Web site having URL
10 [www\(dot\)ncbi\(dot\)nml\(dot\)nih\(dot\)gov](http://www.ncbi.nlm.nih.gov)).

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of" means "including and limited to".

The term "consisting essentially of" means that the composition, method or
15 structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

The word "exemplary" is used herein to mean "serving as an example, instance or illustration". Any embodiment described as "exemplary" is not necessarily to be
20 construed as preferred or advantageous over other embodiments and/or to exclude the incorporation of features from other embodiments.

The word "optionally" is used herein to mean "is provided in some embodiments and not provided in other embodiments". Any particular embodiment of the invention may include a plurality of "optional" features unless such features conflict.

25 As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be
30 presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should

be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non-limiting fashion.

EXAMPLE 1

High Throughput Screening Assay

Materials and methods

High throughput drug screening:

5 The assay was performed using GNF liquid handling system (San Diego, CA, USA). The chemical compounds were added by Echo 550 liquid handler (Labcyte Inc., Sunnyvale, CA, USA). Luminescence signal was detected by luminescence module of PheraStar FS plate reader (BMG Labtech, Ortenberg, Germany). For the primary screen 10 nl of about 46000 bioactive compounds from the G-INCPM (Weizmann
10 Institute of Science) chemical libraries were transferred into 1536-well plates (#264712, Nunc) and kept frozen in -30 °C before the screen. p65-split RL expressing bacterial cells were lysed in 20 mM Tris pH 8, 100 mM NaCl, 10 % glycerol, 2 mM EDTA, 0.5 % NP-40, 1 mM DTT, 1 % protease inhibitor cocktail. 5µl of p65-split RL were dispensed into the assay plates. Full length RL and lysis buffer without RL served as
15 positive and negative controls, respectively. For inhibitory control, p65-split RL was incubated with p65 (competitor) for 25 minutes prior the screen at RT, and 5 µl of the solution were added into the assay plates as well. Plates were incubated for 15 minutes at room temperature (RT) and 5 µl of 5 µg/ml CTZ reagent (Gold Biotechnology, Olivette, MO, USA) in 80 mM K₂HPO₄, 20 mM KH₂PO₄ were added into each well.
20 The signal was detected 10 minutes after incubation at RT in the dark. 380 hits selected from the primary screen were further analyzed in a dose response assay (0.3, 1, 3, 10, 30 µM) in duplicates. The compounds that inhibited full-length Renilla signal were considered false positives.

Computational modeling:

25 Molecular docking was performed using different modules of Schrödinger (Schrödinger, LLC, New York, NY, 2015). The three-dimensional crystal structure of NF-κB protein was retrieved (PDB ID: 1MY5, resolution 1.80 Å) from RCBS PDB database [Huxford et al. 2002. J Mol Biol 324:587-597]. The structure was optimized prior to docking using the Protein Preparation Wizard in Schrödinger Maestro Suite
30 2015. Inconsistencies in the structure such as missing hydrogen, incorrect bond orders, orientation of the different functional groups of the amino acids were rectified during

the optimization process [Sastry et al. 2013. Journal of Computer-Aided Molecular Design 27:221-234].

The prepared protein was then used for Induced Fit Docking. Withaferin A (WFA) was prepared prior to docking using the LigPrep application in Schrödinger Maestro Suite 2015 (LigPrep, version 3.4, Schrödinger, LLC, New York, NY, 2015). The Induced Fit Docking (IFD) was performed using the Induced Fit Docking application of Schrödinger Suite 2015. The IFD application in Schrödinger Suite 2015 combines Grid-based Ligand Docking with Energetics (GLIDE) and Prime refinement modules [Farid et al. 2006. Bioorganic & Medicinal Chemistry 14:3160-3173; Sherman et al. 2006. Chemical Biology & Drug Design 67:83-84; Sherman et al. 2006. Journal of Medicinal Chemistry 49:534-553]. During IFD, the application was set so as to retain the default 20 poses for the initial GLIDE docking stage. Standard-precision (SP) was selected for Glide Redocking stage with the default cut-off of redocking poses within 30 kcal/mol of the best-docked conformation. The poses were ordered based on Glide score (kcal/mol) and IFD score (kcal/mol). Glide score (GScore) is calculated by the software as $GScore = 0.065 \cdot vdW + 0.130 \cdot Coul + Lipo + Hbond + Metal + BuryP + RotB + Site$, wherein vdW: van der Waals energy; Coul: Coulomb energy; Lipo: Lipophilic term; Hbond: Hydrogen-bonding; Metal: Metal-binding term; BuryP: Buried Polar groups' penalty; RotB: Penalty for rotatable bonds that have been frozen; Site: active site polar interactions. IFD score is calculated by the software as $IFD\ Score = 1.0 \cdot Glide\ Score + 0.05 \cdot Prime_Energy$. Emodel has a more significant weighting of the force field components (electrostatic and van der Waals energies), which makes it well-suited for comparing conformers, but much less so for comparing chemically-distinct species. The best scoring conformation of the ligand was screened and selected by re-ranking according to Emodel GLIDE score.

Plasmids construction:

A20 luciferase, RSV-Renilla and miR-22-luciferase were previously described [Amir-Zilberstein et al. 2007. Mol Cell Biol 27:5246-5259; Marbach-Bar et al. 2013. Nat Commun 4:2118]. IL-2-luciferase (NFAT/AP-1 3xluc) is a gift from Orly Avni (Bar Ilan University). p65 expression plasmid in pCDNA3 was previously described [Yamit-Hezi A et al. 2000. J Biol Chem 275:18180-18187]. pCMV-SPORT6-mNFAT2 was obtained as a gift. GFP-RelA [Chen et al. 2001. Science 293:1653-1657] was

obtained from Addgene (#23255). H2B-RFP was obtained as a gift. Point mutations were generated by site directed mutagenesis using the tPCR method [Erijman et al. 2011. J Struct Biol 175:171-177; Unger et al. 2010. J Struct Biol 172:34-44]. All mutants were verified by sequencing. The Split-Renilla fusion plasmids were constructed by two steps PCR using the RSV-Renilla (pRL-null) and pCDNA3-p65 as backbones. As proposed by Jiang et al [2010, J Biol Chem 285:21023-21036], the N' of the Renilla luciferase contains positions 1-229, the C' contains positions 230-311, of the luciferase sequence (SEQ ID NO: 4). Each fragment was followed by a linker GGTGGCGGAGGGAGC (SEQ ID NO: 5), corresponding to amino acids GGGGS (SEQ ID NO: 3), and then positions 1-298 of p65 (SEQ ID NO: 2). Mutations were introduced by site directed mutagenesis as described above. Primer sequences can be provided upon request.

Cells, transfections, extract preparations, Western Blot, and antibodies:

HEK293T and HeLa cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal calf serum. Transfection into HEK293T cells and A20-luciferase reporter gene analysis were carried out as previously described [[Amir-Zilberstein et al. 2007, *supra*]. Whole cell extract was prepared by lysing cells in 50 mM Tris pH 8, 250 mM NaCl, 5 mM EDTA, 0.5 % NP-40 and 1 % protease inhibitor cocktail or by commercial lysis buffer (Promega). Samples were then separated by SDS-PAGE and subjected to Western blot as previously described [Elfakess and Dikstein R. 2008. PLoS ONE 3:e3094]. Antibodies for p65 were purchased from *Santa Cruz Biotechnology*; I κ B α antibody is from *BD-transductions*; A20 antibody is from *eBioscience* and the GFP monoclonal antibody is from Abcam.

Expression and purification of His-p65 and Microscale Thermophoresis analyses:

p65 WT and E285A/Q287A mutants were cloned into pRSFDuet with an N-terminal 6xHis tag. Transformed BL21 (DE3) bacteria were grown at 20°C following induction with 200 μ M of isopropyl-1-thio- β -D-galactopyranoside (IPTG) over night. Bacteria were lysed by a cooled cell disrupter in lysis buffer containing: 50 mM Tris-HCl pH 8, 150 mM NaCl, 1 mM PMSF, protease inhibitor cocktail (Calbiochem) in the presence of DNase (1 μ g/ml) and lysosyme (40U/ml culture). Soluble protein was

captured on a HiTrap-Chelating_HP_5ml (GE Healthcare) and eluted with the same buffer containing 0.5M imidazole. Fractions containing p65 were applied to a size exclusion column (HiLoad 16/60 Superdex 75, GE Healthcare) equilibrated with PBS. Fractions containing p65 were loaded onto a Tricorn Q 10/100 GL column (GE
5 Healthcare) equilibrated with 20mM sodium phosphate pH 7.2, 1mM DTT. p65 eluted from the column with a linear gradient of the same buffer containing 1M NaCl and 1mM DTT. Fractions containing pure p65 were pooled and frozen with liquid nitrogen.

For the non-labeled Microscale Thermophoresis assays, purified WT or mutant p65 protein (3.375µM) in 20 mM Hepes pH 7.8, 140 mM NaCl, 0.1 % pluronic was
10 centrifuged at 10,000 g for 10 minutes and then incubated with increasing concentrations of WFA (97.65 nM to 200 µM) for 5 minutes at room temperature. Hydrophilic capillaries were loaded with the samples and measurements were done in Monolith NT.LabelFree instrument (NanoTemper) using laser intensities of 20 % and 40 %. Data evaluation was performed with the Monolith software NT.Analysis
15 (NanoTemper).

For the labeled Microscale Thermophoresis 15 µM short length p65, (1-298), was freshly labeled with Maleimide (#MO-L004, NanoTemper Technologies GmbH, Germany) according to the manufacturer's protocol, including optional buffer exchange. Labeling reaction yielded 600 µl of 2.5 µM labeled protein in 20 mM Hepes, 140 mM
20 NaCl, pH=7.8. Protein was then centrifuged at 10,000 g for 5 minutes.

For assessment of SA788 binding to p65, 18 µl of a 50 nM stock solution of p65 were mixed with 2 µl of serial dilution of SA788 (initial 250 µM). Samples were loaded onto premium coated capillaries, (MO-K005) and measured at 10 % LED power and 60 % MST power with a laser on time of 30 seconds and laser off time of 5
25 seconds, using the Monolith Nt.115 Pico instrument (#MO-G006). Analysis was performed using the MO.Affinity Analysis software.

RNA analysis:

Total RNA was extracted from HEK293T and MEF using Tri-reagent. cDNA was synthesized from 1µg of total RNA using ABI reverse transcription kit. cDNA
30 samples were analyzed by qPCR in an ABI 7300 Real Time PCR system using Power SYBR PCR reaction mix (ABI) as previously described [Diamant et al. 2012. Cell Rep 2:722-731].

Chromatin immunoprecipitation:

HEK293T cells were transfected either with p65 WT, Y288A mutant or mock constructs (0.5 µg), or treated with Withaferin A (WFA; 10 µM, 60 minutes) followed by TNFα induction (20 ng/ml, 30 minutes) in 100 mm plates. The cells were then cross-linked with 1 % formaldehyde for 10 minutes at room temperature and fixing was terminated by adding 1/20 volume of 2.5 M glycine. Chromatin extraction and immunoprecipitations were carried out as previously described [Diamant et al. 2012. Cell Rep 2:722-731]. For immunoprecipitation, 6 µl of anti-p65 or anti-Oct4 (Santa Cruz) was added to 1ml of the soluble chromatin DNA. Samples were analyzed by qPCR in an ABI 7300 Real Time PCR system using Power SYBR PCR reaction mix (ABI).

Electrophoresis mobility shift assay (EMSA):

WT and mutant p65 were transcribed and translated *in vitro* using T7 polymerase and rabbit reticulocyte lysate kit (Promega). Binding reactions were assembled on ice with 3µl translated lysate in a buffer containing 10 mM Tris pH8, 100 mM KCl, 0.5 mM EDTA, 0.1 % triton-X 100, 12.5 % glycerol, 1mM DTT; 1 µg poly dIdC and 300 fmole of double stranded NF-κB binding site probe, which was fluorescently labeled with 6FAM. Samples were transferred to room temperature for 30 minutes and then loaded onto 6% non-denaturing TBE-polyacrylamide gel.

GFP-p65 localization:

Sub-confluent HEK293T cells were transfected with 25 ng/ml WT or mutant GFP-p65 and 40 ng/ml H2B-RFP. Cells were visualized 48 hours after transfection with either Fluorescent microscope (Nikon ECLIPSE TiS).

Software:

Computational modeling was carried out using Schrödinger suite 2015 and Emodel scores. Structural illustrations were done using the *PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC*. Amino acid sequence alignment was done using Praline (www.ibi.vu.nl/programs/pralinewww/). Microscopic photos were processed with Nikon NIS-Element F 3.0; ImageJ version 1.45 k software.

In all figures the asterisks denote statistically significance difference (p<0.05).

Results

A high-throughput screening for direct inhibitors of p65/RelA dimerization was designed and conducted.

As discussed hereinabove, dimerization of NF- κ B is crucial for its ability to bind DNA. Consistent with that, mutation of four dimerization interface residues of p65 R198A, E211A, F213A and R246A, as shown in FIG. 1A, dramatically diminished its transcriptional activity. The potential of NF- κ B dimerization activity as a drug target was therefore explored. A protein-protein interaction approach that is based on the split *Renilla luciferase* (RL) complementation assay was applied [Paulmurugan and Gambhir SS. 2003. Anal Chem **75**:1584-1589]. In this assay RL is split into two inactive N- and C-terminal fragments and fused to target proteins. As shown in FIG. 1B, interaction of the target proteins brings the RL N- and C-termini in close proximity, restoring the enzymatic activity. p65 (amino acids 1-298, without the NLS and the activation domain) was fused to RL N- and C-termini, and then co-transfected into cells together with a *Firefly luciferase* reporter as normalizing control. The p65-RL fusion proteins were also transfected with the empty counterparts to determine the background levels of the RL activity. As shown in FIG. 1C the strongest activity is conferred by the p65-RL N and C pair. To validate that this activity is dependent on dimerization p65-split-RL with an E211A dimerization site mutation was constructed. As further shown in FIG. 1C, with this mutation the RL activity is diminished, indicating that the assay is a faithful readout of dimerization.

Next the p65-split-RL luminescence assay was established in a cell-free system to avoid indirect effects on dimerization. A plasmid that directs the expression of the two parts of p65-split-RL in bacteria, each with a different tag, His or Flag, was engineered. As shown in FIG. 1D, bacterial cells transformed with this plasmid and induced to express the two parts of the p65-RL were found to have a strong RL enzymatic activity compared to the control bacteria. Expression of the two fusion proteins was validated by western blot with antibodies against the His and the Flag tags (see, FIG. 1E). Western blot with p65 antibody indicate that the level of expression of the p65 fusion proteins is comparable (see, FIG. 1E).

The luminescence assay was then used in a 1536-well plate format to screen a library of about 46,000 compounds of diverse chemical nature, for inhibitors of the p65-

split-RL activity, as schematically depicted in FIG. 2A. 380 inhibitors (0.8 %) were identified and these were further validated in a dose-response assay using the p65-split-RL and the full-length RL that served as a control for inhibitors of the enzymatic activity rather than dimerization. The vast majority of the compounds inhibited both
5 reporters, leaving 14 compounds that specifically inhibited the activity of the p65-split-RL, one of which being Withaferin A (WFA), the chemical structure of which is presented in FIG. 2B. WFA was previously characterized as having an anti-inflammatory and anti-cancer activity and was postulated to interfere with the signaling pathway of NF- κ B [Rai et al., *Pharm Biol* 2016, 54:189-197]. As shown in FIG. 2C,
10 WFA inhibited p65-split-RL effectively with an IC₅₀ of about 10 μ M whereas the RL activity directed by the empty RL pair was unaffected by the drug.

EXAMPLE 2

The Effect of WFA on NF- κ B dimerization and activity

15 To gain further support for the effect of WFA on dimerization co-immunoprecipitation assays were performed. Cells were co-transfected with plasmids directing the expression of p65 and GFP-p65 fusion and then subjected to immunoprecipitation using GFP antibodies. The immune complexes were then treated either with vehicle or with 10 and 30 μ M of WFA. Western blot with p65 antibodies,
20 presented in FIG. 2D, shows that p65 co-precipitated with GFP-p65 and the addition of WFA released the p65 from its GFP-p65 counterpart, confirming its direct effect on p65 dimerization.

One of the major functional NF- κ B complexes consists of a heterodimer of p65-p50. To determine the effect of WFA on this complex p50 (amino acids 1-361) fusion
25 with the N- and C- RL domains was constructed. These plasmids were transfected into cells and analyzed for p50-p50 homodimer and p65-p50 heterodimer formation by promoting RL enzymatic activity. As shown in FIG. 2E, both p50-p50 and p65-p50 pairs directed RL activity that is significantly above background. Next, the effect of WFA on the RL activity directed by p50-p50 and p65-p50 was determined and found
30 that both were inhibited with an IC₅₀ of about 30 and about 10 μ M, respectively, as shown in FIG. 2F. Thus the extent of WFA inhibition of p65-p50 is comparable to that of p65-p65 while the inhibition of p50-p50 is smaller.

Next, the effect of WFA on the activity of the endogenous NF- κ B was examined and found that the drug diminished the mRNA induction of the NF- κ B target genes A20 and I κ B α by TNF α , as shown in FIG. 3A. Likewise the induced A20 protein levels were severely diminished, as shown in FIG.3B. The occupancy of the A20 promoter by p65 following TNF α treatment in the presence or absence of WFA was analyzed and it was found that WFA abolished the strong induction of p65 binding to the A20 promoter, as shown in FIG. 3C, consistent with the importance of dimerization for DNA binding.

To explore the mechanism by which WFA inhibits NF- κ B dimerization a computational modeling for molecular docking of WFA on p65 dimerization domain was applied, as described hereinabove. As shown in FIG. 4A, left, the model with the best docking score suggests one primary binding site, spanning both subunits. WFA interacts with dimerization site residues (e.g. E211) in one monomer and with surface residues E285 and Q287 in the other, as shown in FIG. 4A, right. (

Modeling of WFA interaction with p65-p50 heterodimer suggested a highly similar docking site with a comparable score that involves the E267 dimerization residue of p50 and E285 and Q287 of p65 (data not shown). The predicted contacts of WFA with the dimerization residues, E211 in p65 and E267 in p50, are consistent with its effect on dimerization. E285 and Q287 on the other hand, are distant from the dimerization interface, raising the question whether they contribute to the effect of WFA on dimerization.

To test this possibility, these amino acids were substituted with alanine either individually or together (E285A, Q287A and E285A/Q287A) in the context of the p65-split-RL and the full-length protein. The obtained data, shown in FIG. 4B, show that each of these mutants decreased p65 dimerization activity and the double mutant reduced it even further, suggesting that these residues indeed contribute to dimerization. As shown in FIG.4C, these mutants also diminished the transcriptional activity to a similar extent (FIG. 4C; upper bar graph), and equivalent expression levels of the WT and mutants was confirmed by Western blot analysis (FIG. 4C; lower).

To test whether the effect of WFA on p65 dimerization is mediated in part by E285 and Q287, its inhibitory effect in their absence was assayed. Cells were transfected with p65-split-RL either WT or E285A/Q287A mutant pairs. Cell lysates were then incubated with increasing amounts of WFA. As shown in FIG. 4D, the

dimerization activity of the WT p65 is clearly more sensitive than the E285A/Q287A mutant to WFA inhibition.

To investigate further WFA interaction with the WT and the E285A/Q287A p65 proteins, the MicroScale Thermophoresis (MST) method which allows precise analysis of binding by monitoring the directed movement of the intrinsic fluorescence of p65 through tiny temperature gradients [Jerabek-Willemsen et al. 2011. Assay Drug Dev Technol 9:342-353; Seidel et al. 2013. Methods 59:301-315], was used. WT and E285A/Q287A mutants (aa 1-298) were expressed in *E. Coli* and purified to homogeneity. As shown in FIG. 4E, both WT and E285A/Q287A mutant display significant intrinsic fluorescence, directed by a single tryptophan (W233). WFA decreased the intrinsic fluorescence of the WT p65 in a dose dependent manner while it has no significant effect on the E285A/Q287A mutant. These findings provide strong support to the computational docking model in which WFA disrupts dimerization by contacting not only the dimerization site but also E285 and Q287. Moreover E285 and Q287 residues emerge as allosteric modulators of p65 dimerization.

This study establishes that NF- κ B dimerization activity is usable as a drug target. WFA was identified as a direct inhibitor of p65 dimerization. By combining computer-assisted molecular docking with mutagenesis of specific residues, it was uncovered that the mechanism by which WFA inhibits p65 dimerization is both direct and allosteric.

EXAMPLE 3

A highly conserved hydrophobic core domain (HCD) as a dimerization scaffold in NF- κ B and NFAT

To elucidate the underlying basis for the unexpected role of E285 and Q287 in dimerization, their conservation among RHR proteins was examined and found to be non-conserved (see, FIG. 5A, asterisks). These residues however, are adjacent to the highly conserved F286 and Y288 amino acids (see, FIG. 5A, arrowheads).

It was therefore tested whether the F286 and Y288 are also involved in dimerization using the split-RL assay. As shown in FIG. 5B, substituting F286 or Y288 to alanine (F286A and Y288A) caused complete loss of dimerization.

To gain further support for the effect of Y288 on dimerization co-immunoprecipitation assays were applied using p65 and GFP-p65 fusion proteins (WT

and mutant) as described above. The results, shown in FIG. 5C, clearly show that WT p65 was co-precipitated with WT GFP-p65 whereas GFP-p65-Y288A failed to efficiently co-precipitate the p65-Y288A, confirming the dimerization defect associated with Y288A. The transcriptional activity of Y288A was then analyzed and it was found
5 that this mutant is inactive while a mutation to phenylalanine (Y288F) retained partial activity, as shown in FIG. 5D. Consistently the Y288A mutant failed to activate the mRNA levels of the NF- κ B target genes A20 and I κ B α , as shown in FIG. 5E.

To test whether the Y288A mutation affects nuclear localization the GFP-p65 fusion proteins and the nuclear marker H2B-RFP were used to trace sub-cellular
10 localization and it was found that GFP-p65-Y288A can translocate into the nucleus similar to the WT GFP-p65 and the partially active GFP-p65-Y288F (data not shown).

The DNA binding activity of Y288 mutants was tested using *in vitro* synthesized p65 variants that were subjected to electrophoresis mobility shift assay (EMSA) with a fluorescently labeled DNA probe. As shown in FIG. 5F, while the WT and Y288F
15 proteins display high and moderate DNA binding activity respectively, the Y288A mutant lacks any detectable DNA binding. Likewise, as presented in FIG. 5G, chromatin immunoprecipitation assays show that WT p65 was efficiently associated with the A20 promoter but Y288A binding was undetected.

According to the various three-dimensional structures of NF- κ B, it appears that
20 F286 and Y288 are part of a structural domain consisting a hydrophobic core (HCD), as depicted in FIG. 6A.

To examine whether this HCD is linked to the function of Y288, 4 additional residues in this core F228, W233, T254 and Y257 were substituted to alanine and their function was analyzed. As shown in FIG. 6B, each of these HCD mutations caused loss
25 of function towards the A20 reporter gene. Analysis of the expression of the endogenous target gene I κ B α showed that it was stimulated by the WT p65, but its activation by all HCD mutants was severely impaired (see, FIG. 6C). Similar to Y288A, these HCD mutants are also defective in DNA binding activity (see, FIG. 6D, lanes 3, 4, 7 and 10). These findings uncovered the importance of the HCD structural arrangement
30 for p65 activities.

Among the HCD residues examined, T254 has been previously reported to induce NF- κ B activity through phosphorylation, and substitution of this residue to

alanine diminished p65 activity [Ryo et al. 2003. Mol Cell **12**:1413-1426]. As T254 is buried inside the HCD (see, FIG. 6A), its accessibility to protein kinases is expected to be limited. Accordingly, the effect of T254A substitution previously reported may be the consequence of HCD rearrangement, similar to the other alanine substitutions described above.

To address this issue further T254 was substituted either with serine (S) and valine (V) which are similar to threonine; with isoleucine (I) and with the phospho-mimetic residues aspartate and glutamate (D and E respectively). The results, shown in FIG. 6E, revealed that the phospho-mimetic substitution of T254 caused complete loss of transcriptional activity whereas T254S and T254V substitutions retained partial activity. In addition, the I substitution that has a relatively big side chain, is also inactive. The induction levels of the endogenous I κ B α recapitulate the effect of the various T254 mutants on the A20 reporter (see, FIG. 6C). Likewise, DNA binding assays support the importance of T254 for this activity as any substitution of this residues diminished DNA binding (see, FIG. 6D).

To validate the role of HCD residues in dimerization the split-RL assay was employed. As shown in FIG. 6F, the HCD mutants F228A, W233A, T254A, Y257A failed to interact and restore RL enzymatic activity. T254S and T254V displayed partial dimerization activity, but the phospho-mimetic T254D and T254E as well as T254I had no significant dimerization activity. These results confirm that the HCD is a scaffold for p65 dimerization. Moreover, these findings suggest that an active conformation of p65 requires T254 to be within the HCD to support dimerization and transcriptional activity.

Comparing the HCD amino acid sequence between NF- κ B and NFAT families, it is clear that the residues that constitute the hydrophobic core are among the most conserved (see, FIG. 5A), suggesting that NFAT HCD is also essential for function.

To test this, mutations were introduced in several homologous conserved HCD residues in NFAT2 and analyzed for their ability to induce a reporter gene driven by the NFAT target promoter IL-2. As shown in FIG. 6G, mutations in NFAT2 HCD residues I661A, F664A and F692A (corresponding to I642, Y645 and F673 in NFAT1) abolished its transcriptional activity, reminiscent of the effect of HCD mutation on p65 activity.

The WFA-p65 complex discussed in Example 2 hereinabove therefore led to the discovery of a region consisting of the HCD and adjacent surface residues, as an allosteric modulator (AM) of dimerization and DNA binding. The critical residues of this domain are the most highly conserved among RHR proteins suggesting that these residues were optimized during evolution to maintain the proper structure. This is in line with the observation that even conservative substitutions of HCD residues such as Y288F or T254S/V, resulted in significant loss of activity. While this domain is not part of the interfaces of dimerization and DNA binding, its integrity is nevertheless critical for these activities. The data shown herein therefore suggest that the AM serves as a critical structural scaffold that supports the dimerization and DNA binding surfaces.

As the DNA binding activity of NF- κ B is substantially stabilized by dimerization, it is possible that the effect of the HCD on NF- κ B DNA binding is a consequence of its primary effect on dimerization. NFAT DNA binding and transcriptional activity are not strictly dependent on its ability to dimerize [Stroud and Chen 2003. J Mol Biol 334:1009-1022; Bates et al. 2008. Structure 16:684-694; Hogan et al. 2003. Genes Dev 17:2205-2232], yet mutating HCD in NFAT2 completely abolished its activity, suggesting that the effect of the HCD is not limited to dimerization.

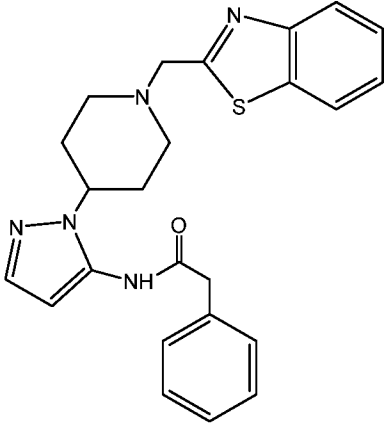
The findings presented herein clearly show that p65/RelA T254 is an integral part of the HCD, in accordance with the various three dimensional structures of NF- κ B complexes. Furthermore it is suggested that phosphorylation of this residue, if occurring, requires a significant structural rearrangement, as T254 is buried under a loop constituting residues 206-210 which is adjacent to dimerization residues 211-213 [See, FIG. 6A and Hogan et al. 2003. Genes Dev 17:2205-2232; Huxford et al. 1998. Cell 95:759-770; and Jacobs and Harrison 1998. Cell 95:749-758]. Therefore the phosphorylation of this residue, which requires its exposure to the surface, would shift the spatial location of the dimerization interface and this is expected to inhibit rather than activate NF- κ B. This is supported by the biochemical and functional evidence presented herein. For example T254V, which has a similar chemical nature, but cannot be phosphorylated, resulted only in a partial loss of activity. On the other hand substituting T254 to phospho-mimetic negatively charged D and E residues resulted in a complete loss of DNA binding, dimerization and transactivation.

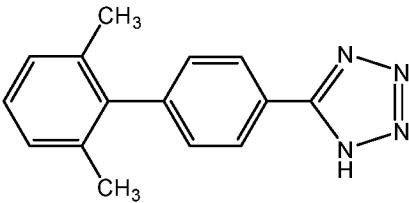
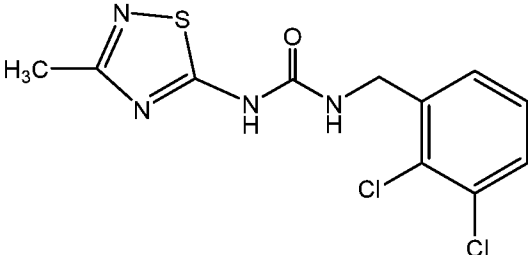
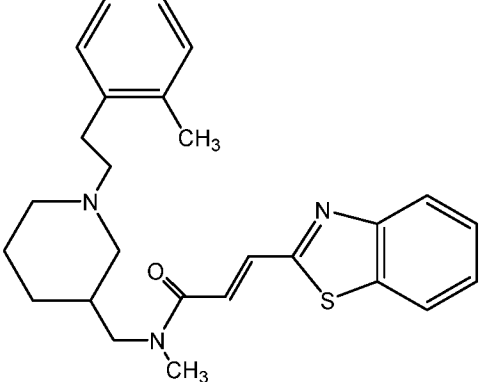
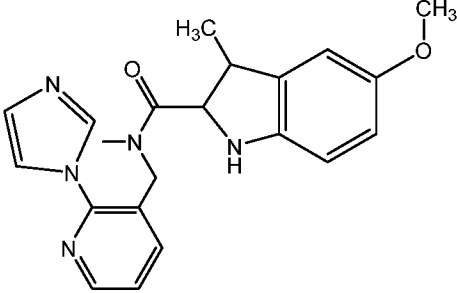
EXAMPLE 4***Effect of SA788 and related compounds on NF- κ B activity***

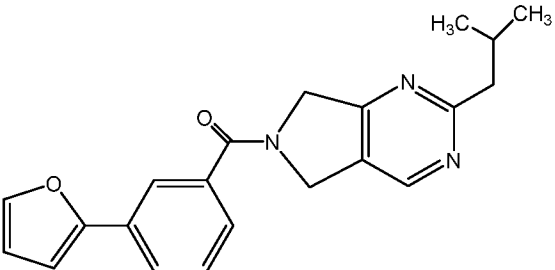
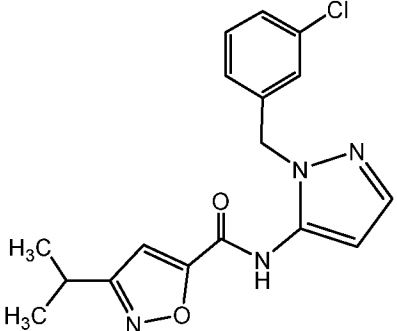
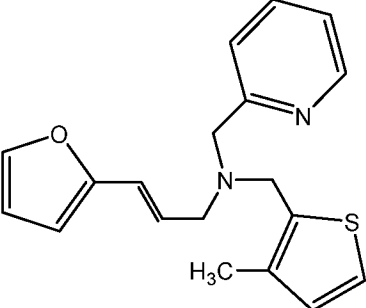
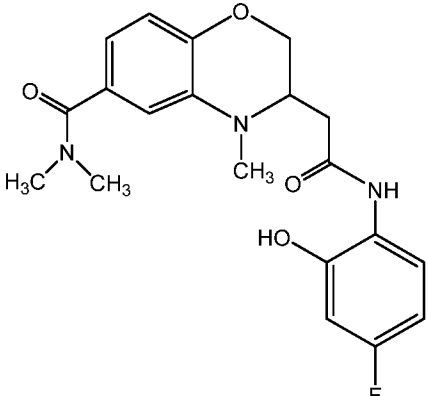
Encouraged by the findings that established targeting NF- κ B dimerization by drugs as highly potential for modulating the activities of RHR proteins, the p65-split-RL system described hereinabove was used to screen another library of about 50,000 small molecule compounds from Chembridge. The luminescence assay was used in a 1536-well plate format for inhibitors of the p65-split-RL activity, as depicted in FIG. 7.

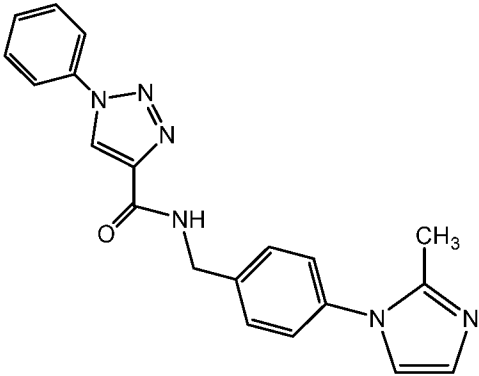
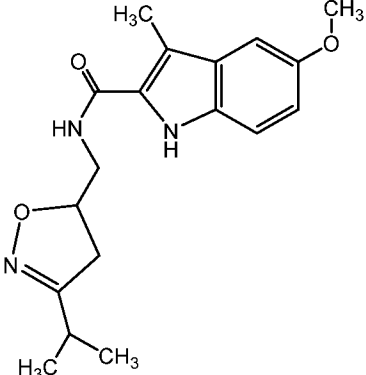
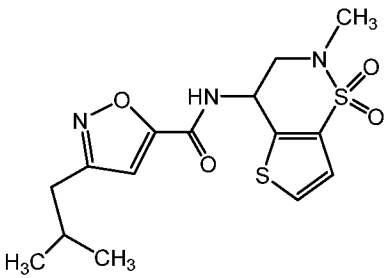
119 inhibitors (0.23 %) were identified and these were further validated in a dose-response assay using the p65-split-RL and the full-length RL that served as a control for inhibitors of the enzymatic activity rather than dimerization. The vast majority of the compounds inhibited both reporters, leaving 15 compounds. 12 of these compounds were purchased and analyzed for their ability to inhibit dimerization *in vitro* and NF- κ B transcriptional activity in cell-based reporter gene assay. The obtained data is presented in Table 1 below.

Table 1

No.	Compound	In vitro IC ₅₀ of inhibition of NF- κ B (p65) dimerization (μ M)	Cell-based NF- κ B transcriptional activity (μ M)
1	 SA788	30	25

No.	Compound	In vitro IC ₅₀ of inhibition of NF-κB (p65) dimerization (μM)	Cell-based NF-κB transcriptional activity (μM)
2		20	(-)
3	 SA321	30	25
4		30	(-)
5		10	(-)

No.	Compound	In vitro IC ₅₀ of inhibition of NF-κB (p65) dimerization (μM)	Cell-based NF-κB transcriptional activity (μM)
6		>30	(-)
7		30	(-)
8		>30	(-)
9		20	(-)

No.	Compound	In vitro IC ₅₀ of inhibition of NF- κ B (p65) dimerization (μ M)	Cell-based NF- κ B transcriptional activity (μ M)
10		20	25
11		20	(-)
12		30	(-)

(Table 1 Cont.)

The first and third compounds shown in Table 1, termed SA788 and SA321, respectively, were subjected to further studies as follows.

- 5 To examine further the inhibitory effect of SA788 on NF- κ B dimers, its effect on p65-p65 homodimers was compared with that on p65-p50 heterodimer, which is the major form of NF- κ B, using the split-RL system, as described hereinabove.

As shown in FIG. 8A, SA788 similarly inhibited both forms of NF- κ B complexes with an IC₅₀ of 10 μ M – 25 μ M. The full length intact Renilla enzyme was not inhibited by SA788.

The ability of SA788 to inhibit the expression of a reporter gene driven by the NF- κ B target promoter A20 was also tested. Cells were co-transfected with a p65-p65 homodimer or with a p65-p50 heterodimer together with A20-luciferase and RSV-Renilla which served as an internal control. Cells were treated with increasing concentrations of SA788 4 hours after the transfection and harvested 20 hours later. As shown in FIG. 8B, luminescence was significantly decreased in a dose dependent manner, both in the context of p65-p65 and in the context of p65-p50. Both NF- κ B dimers were inhibited to a similar extent which is in line the in vitro inhibition shown in FIG. 8A.

To confirm that SA788 directly binds the NF- κ B subunit p65 the MicroScale Thermophoresis (MST) assay which allows precise analysis of binding by monitoring changes in the thermophoresis of p65 through tiny temperature gradients was performed. p65 (aa 1-298) was expressed in *E. Coli*, purified to homogeneity, labeled with the fluorescent dye Maleimide and incubated with increasing concentrations of SA788. As shown in FIG. 9, the thermophoresis of the complex of p65 and SA788 was changed in a dose dependent manner with an average calculated K_d of 50.5 μ M, indicating for direct binding.

To test whether SA788 can inhibit endogenous NF- κ B the induction of endogenous NF- κ B target genes by TNF α in the presence of SA788 was assayed. Mouse embryonic fibroblasts (MEF) were incubated with 25 μ M of SA788 for 1 hour and then induced with TNF α for 24 hours. The obtained data is shown in FIG. 10, and show that treatment with TNF α resulted in induction of all the target genes analyzed, including the pro-inflammatory cytokine TNF α . The treatment with the SA788 diminished the induced levels of TNF α , CXCL-10 and CXCL-11 and ICAM-1. The pro-inflammatory cytokine CXCL-1 was unaffected by the SA788 treatment.

The effect SA788 on the growth of RPMI8226, a multiple myeloma cell line was also tested. Cells were supplemented with fresh SA788 and counted daily. It was found that control cells replicate regularly every second day while SA788 treated cells

were strikingly stalled. These results imply that SA788 is an inhibitor of multiple myeloma cell proliferation.

SA321 was examined according to procedures similar to those used described for S788 hereinabove.

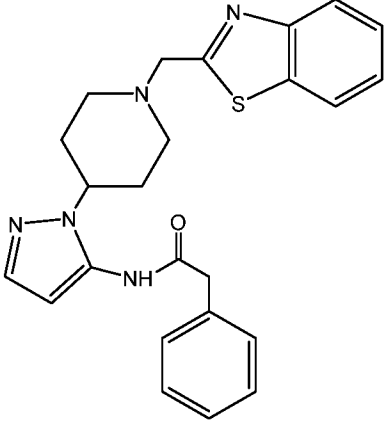
5 As shown in FIG. 11A, SA321 inhibited the p65-p65 form of NF- κ B with an IC₅₀ of about 10 μ M. The full length intact Renilla enzyme was not inhibited by SA321.

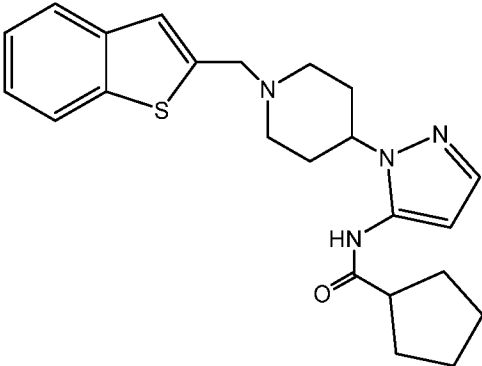
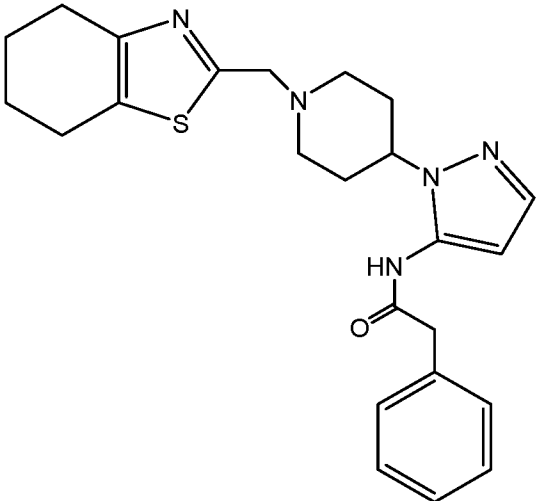
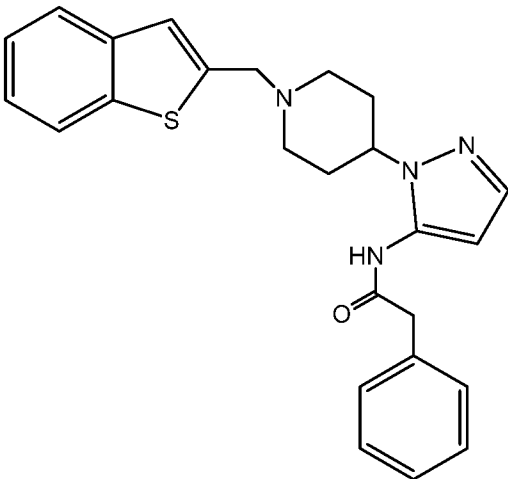
The ability of SA321 to inhibit the expression of a reporter gene driven by the NF- κ B target promoter A20 was also tested, as described hereinabove for SA788. Cells
10 were co-transfected with a p65-p65 homodimer together with A20-luciferase and RSV-Renilla which served as an internal control.

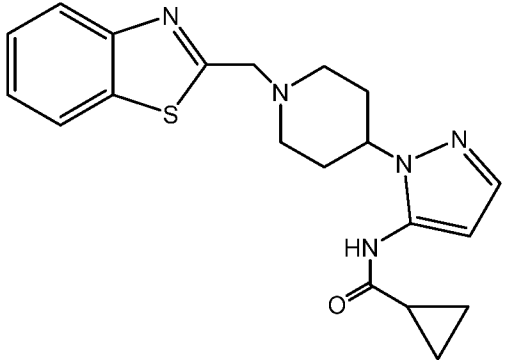
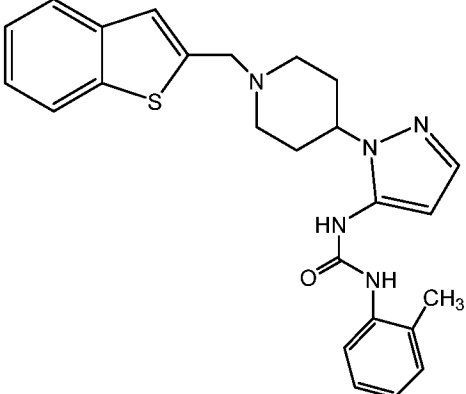
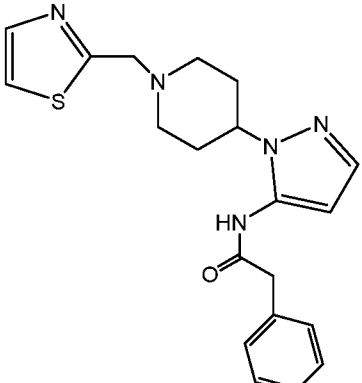
As shown in FIG. 11B, luminescence was significantly decreased in a dose dependent manner, indicating inhibition of the NF- κ B dimer.

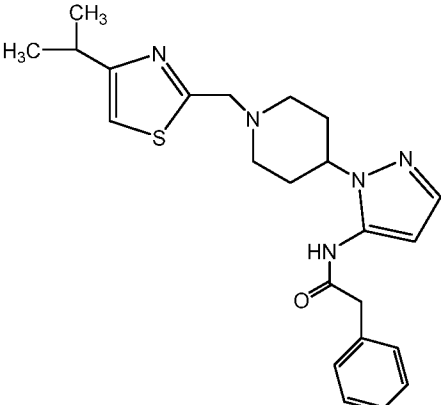
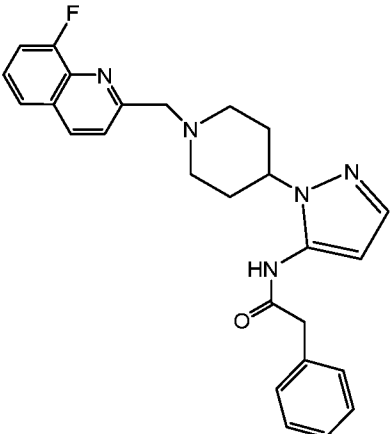
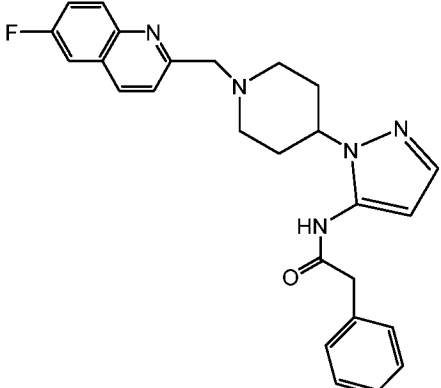
Structure-activity relationship (SAR) studies were conducted by catalog analysis
15 in which the effect of increasing concentrations of commercially available analog of SA788 on NF- κ B dimerization was analyzed using the p65-split-RL system. Several analogs were further tested for their ability to induce the expression of a reporter gene driven by the promoter of the NF- κ B target gene A20, as described in FIG. 8B. The structures of the tested analogs are presented in Table 2 below. None of these
20 compounds were more active than SA788.

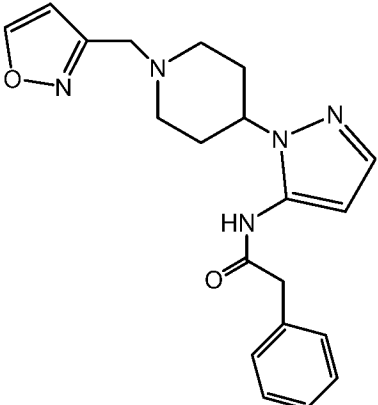
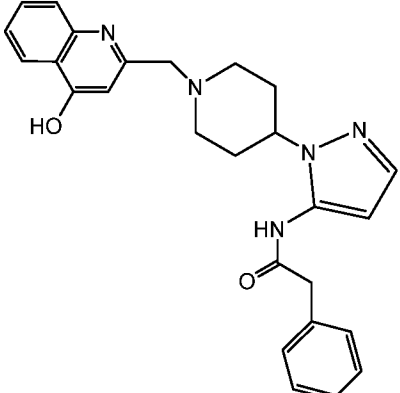
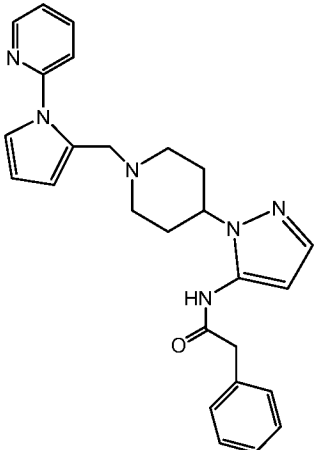
Table 2

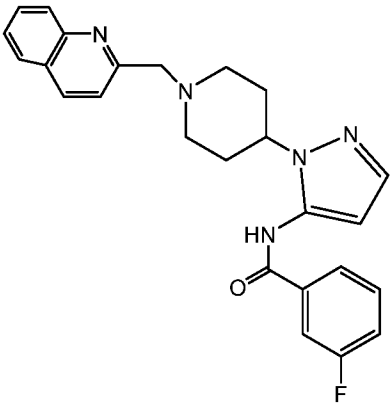
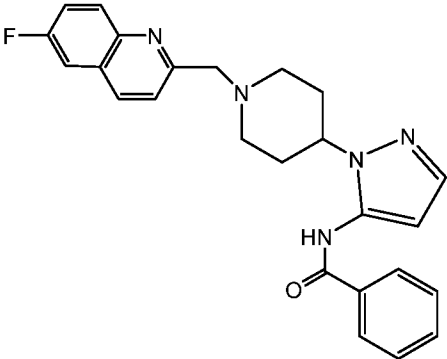
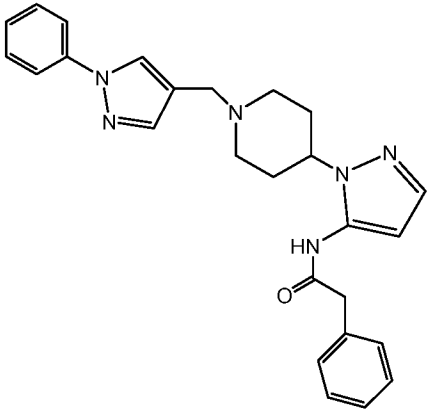
No.	Compound	Cambridge ID
1	 SA788	44377788

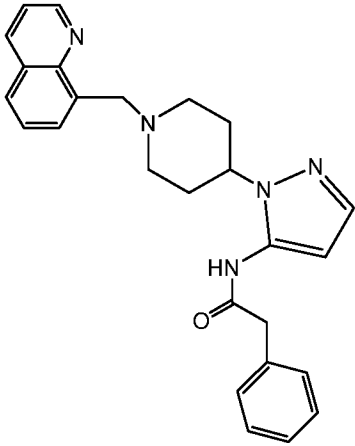
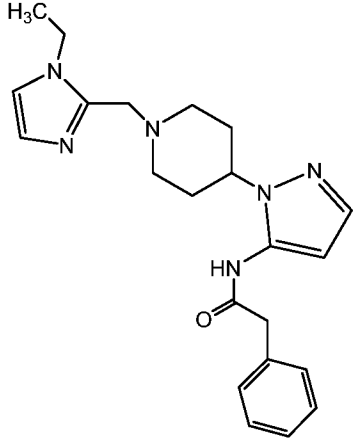
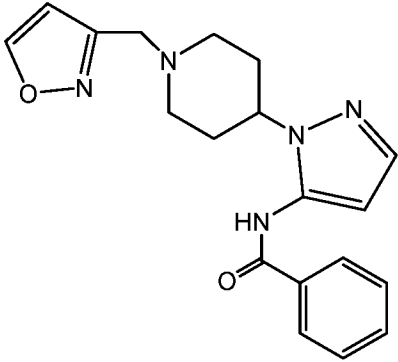
No.	Compound	Cambridge ID
2		33670742
3		46135656
4		47393992

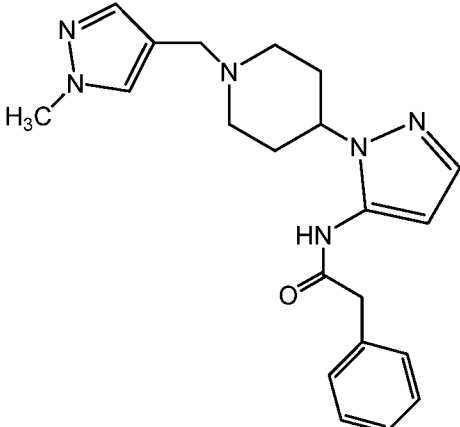
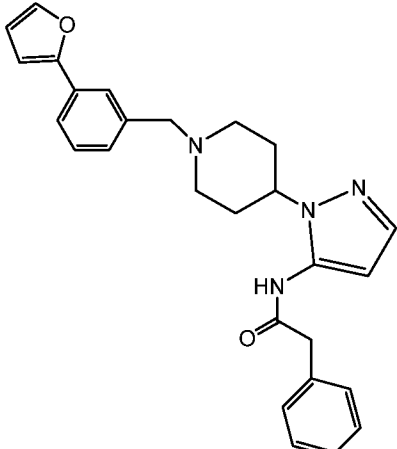
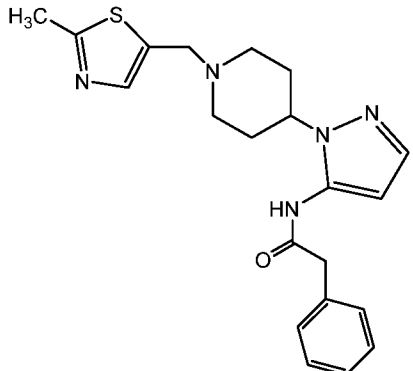
No.	Compound	Cambridge ID
5	 <chem>C1CC1C(=O)NC2=CN=C3C2N(CCN3Cc4nc5ccccc45S6)CC6</chem>	60488412
6	 <chem>Cc1ccccc1NC(=O)NC2=CN=C3C2N(CCN3Cc4c5ccccc4s5)CC6</chem>	69309224
7	 <chem>C1=CC=C(C=C1)CC(=O)NC2=CN=C3C2N(CCN3Cc4nc5ccccc45S6)CC6</chem>	27965142

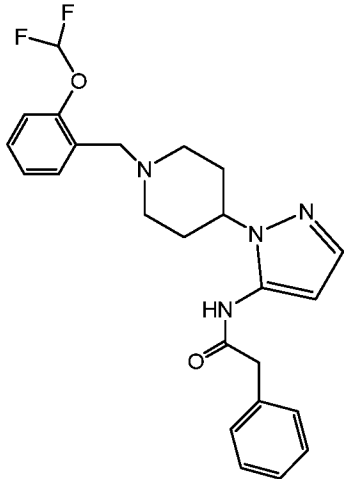
No.	Compound	Cambridge ID
8		75301979
9		13539126
10		16276984

No.	Compound	Cambridge ID
11		64645526
12		54213905
13		59291144

No.	Compound	Cambridge ID
14		20282330
15		10608342
16		35347615

No.	Compound	Cambridge ID
17		72409787
18		14852988
19		38734818

No.	Compound	Cambridge ID
20	 <chem>Cc1cc2nc(CCN3CCCCC3N4C=CN=C4CNC(=O)Cc5ccccc5)cc2n1</chem>	84330124
21	 <chem>c1cc2c(cc1)cc(CCN3CCCCC3N4C=CN=C4CNC(=O)Cc5ccccc5)cc2o</chem>	87190471
22	 <chem>Cc1cc2nc(CCN3CCCCC3N4C=CN=C4CNC(=O)Cc5ccccc5)cn2s1</chem>	47162607

No.	Compound	Cambridge ID
23		55729452

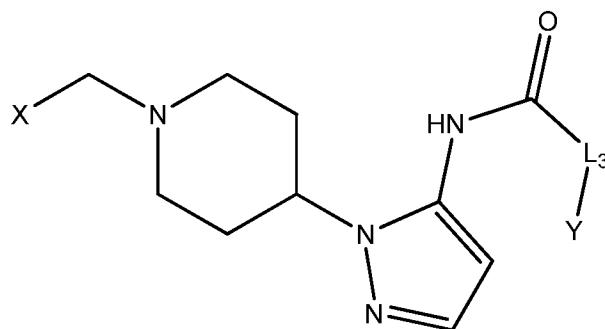
* N/T = not tested

Although the invention has been described in conjunction with specific
embodiments thereof, it is evident that many alternatives, modifications and variations
5 will be apparent to those skilled in the art. Accordingly, it is intended to embrace all
such alternatives, modifications and variations that fall within the spirit and broad scope
of the appended claims.

All publications, patents and patent applications mentioned in this specification
are herein incorporated in their entirety by reference into the specification, to the same
10 extent as if each individual publication, patent or patent application was specifically and
individually indicated to be incorporated herein by reference. In addition, citation or
identification of any reference in this application shall not be construed as an admission
that such reference is available as prior art to the present invention. To the extent that
section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. A compound represented by Formula Ia:



Formula Ia

wherein:

X is a heteroaryl or aryl;

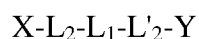
L₃ is absent or is selected from the group consisting of CH₂ and NH; and

Y is selected from the group consisting of optionally substituted phenyl and cycloalkyl,

for use in inhibiting dimerization of NF-κB.

2. A compound represented by Formula Ia as in claim 1, for use in treating a medical condition in which an onset and/or progression of the condition is associated with NF-κB dimerization and/or activity.

3. A compound capable of inhibiting p65-p65 NF-κB homodimerization and/or p50-p65 NF-κB heterodimerization, the compound being represented by formula I:



Formula I

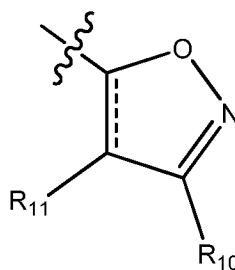
wherein:

X is a heteroaryl or aryl;

L_1 is selected from the group consisting of $-\text{CR}_1\text{R}_2-\text{A}-\text{C}(=\text{O})-\text{B}-$, $-\text{A}-\text{C}(=\text{O})-\text{B}-$, $-\text{CR}_5\text{R}_6-\text{NR}_7-\text{CR}_8\text{R}_9-$ and phenylene, wherein A is NR_3 or absent, and B is NR_4 or absent;

L_2 and L'_2 are each independently absent, a linker of up to two atoms in length, or a linker of 3-7 atoms in length which comprises a heteroalicyclic ring;

Y is selected from the group consisting of optionally substituted phenyl, optionally substituted pyridinyl, cycloalkyl, and a heterocyclic moiety having the general formula II:

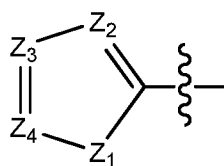


Formula II

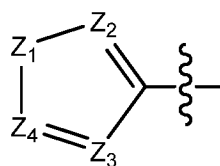
wherein the dashed line represents a saturated bond or an unsaturated bond; and

R_1 - R_{11} are each independently selected from the group consisting of hydrogen, alkyl, alkenyl and alkynyl.

4. The compound of claim 3, wherein X is represented by formula IIIa or formula IIIb:



Formula IIIa



Formula IIIb

wherein:

Z_1 is selected from the group consisting of O, S, NR_{21} , and $-\text{CR}_{22}=\text{CR}_{23}-$;

Z_2 is selected from the group consisting of N and CR_{24} ;

Z_3 is selected from the group consisting of N and CR_{25} ;

Z_4 is selected from the group consisting of N and C_{26} ; and

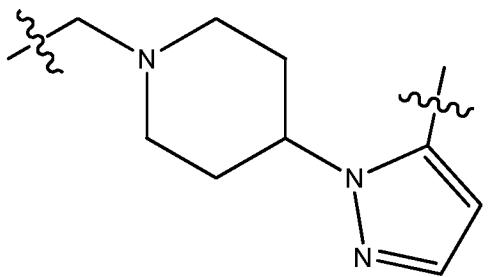
R_{21} - R_{26} are each independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, and hydroxy, or alternatively, R_{25} and R_{26} together form a six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring and/or any one of R_{21} - R_{26} together with L_1 or L_2 forms a five-membered or six-membered alicyclic or heteroalicyclic ring.

5. The compound of any one of claims 3 to 4, wherein Y is said phenyl or pyridinyl, each being unsubstituted or substituted by one or more substituent selected from the group consisting of methyl, hydroxy, halo, and heteroaryl.

6. The compound of any one of claims 3 to 5, wherein R_{10} is C_{1-5} -alkyl.

7. The compound of any one of claims 3 to 6, wherein R_{11} is hydrogen.

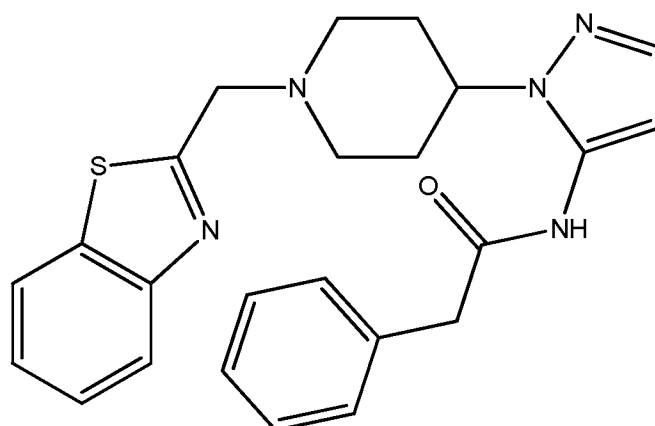
8. The compound of any one of claims 3 to 7, wherein L_2 has the formula:



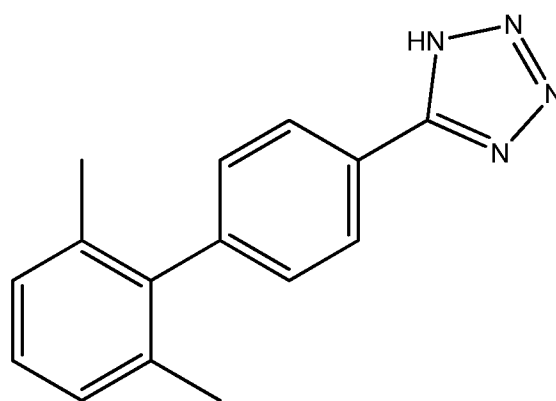
9. The compound of claim 8, wherein L_1 is selected from the group consisting of $-NH-C(=O)-$, $-NH-C(=O)-CH_2-$, and $-NH-C(=O)-NH-$.

10. The compound of any one of claims 8 to 9, wherein Y is selected from the group consisting of optionally substituted phenyl and cycloalkyl.

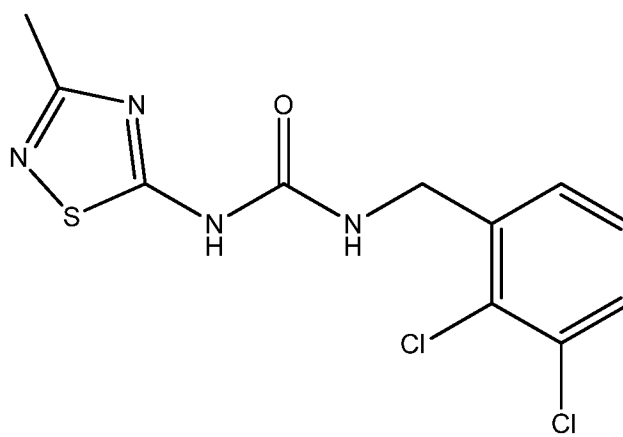
11. The compound for use according to claim 3, wherein the compound is selected from the group consisting of:



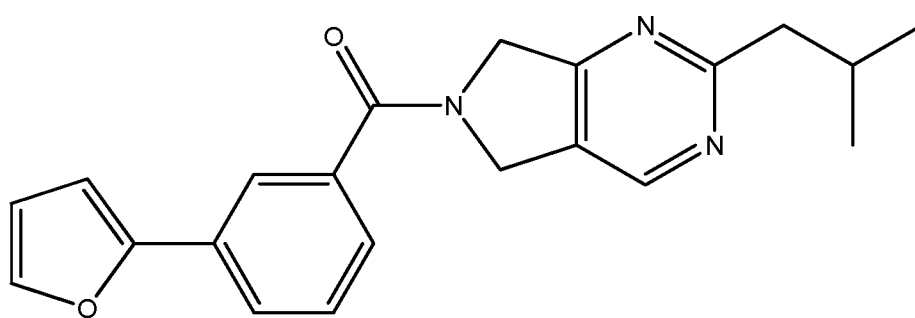
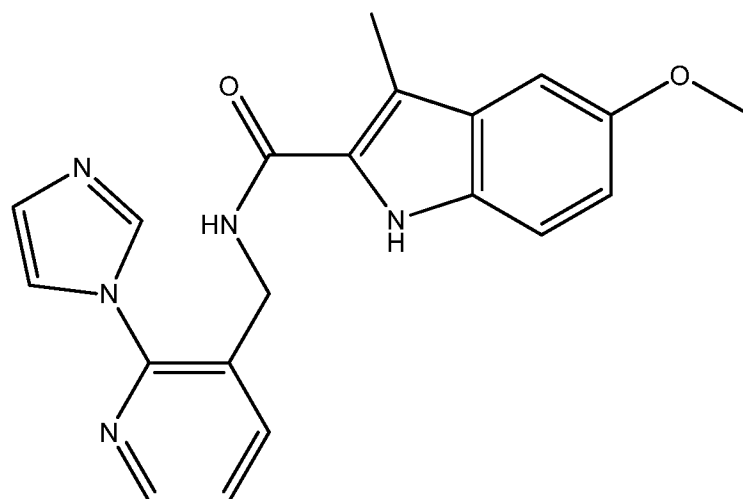
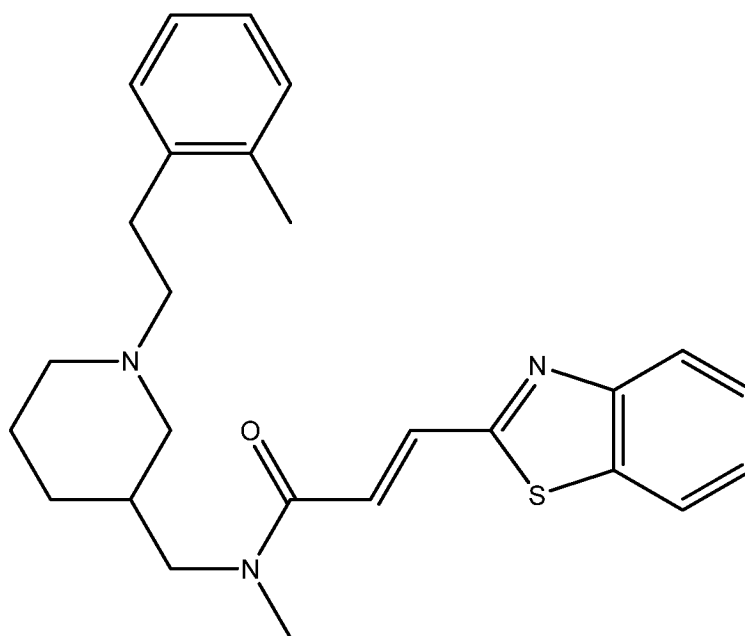
,

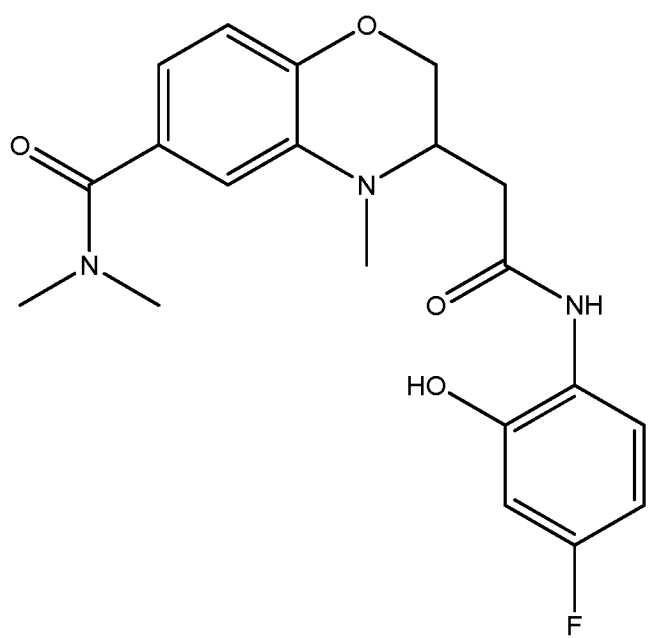
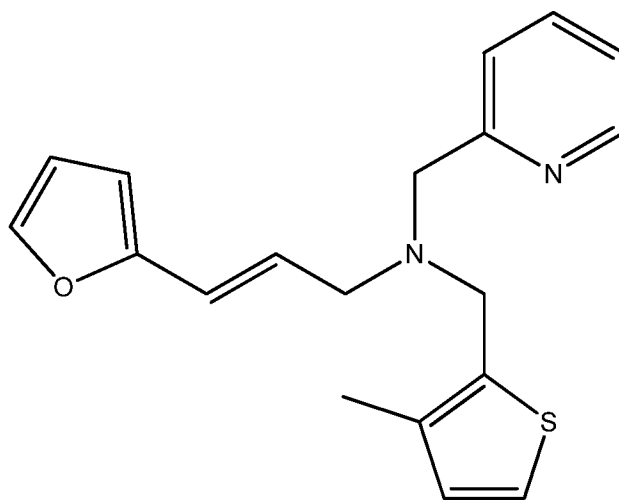
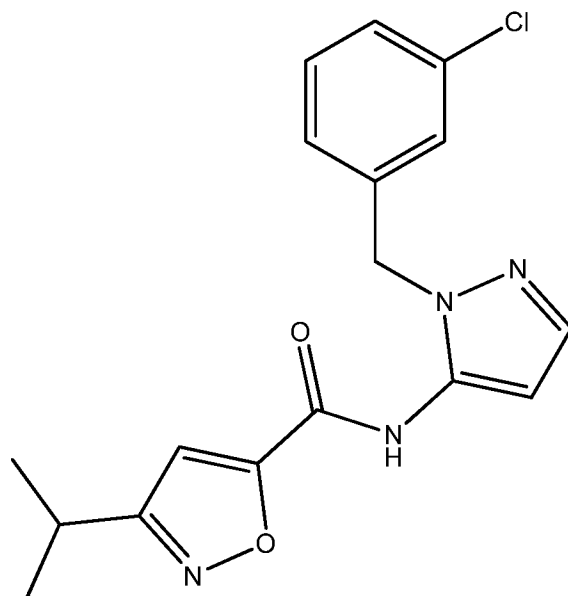


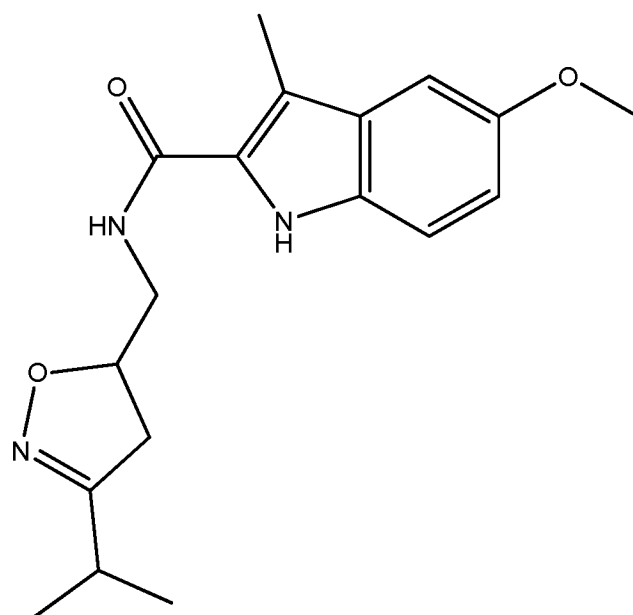
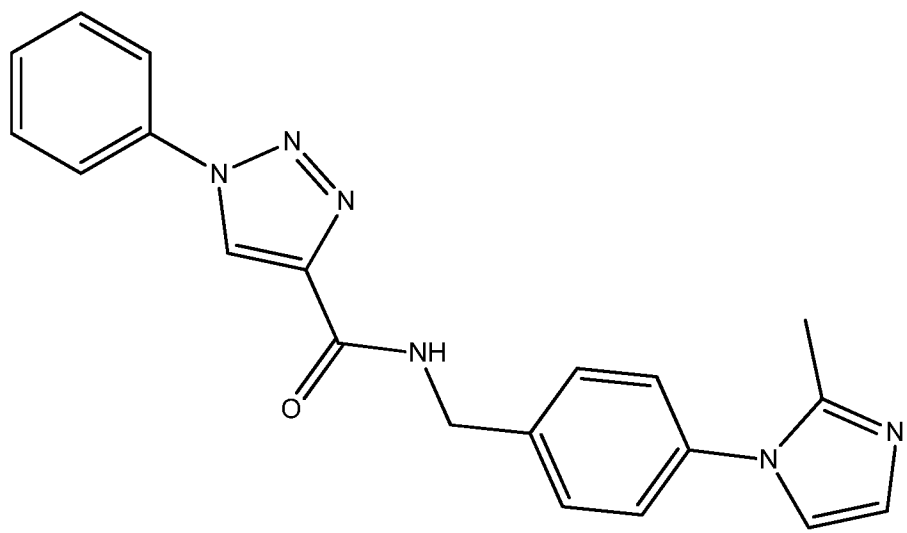
,



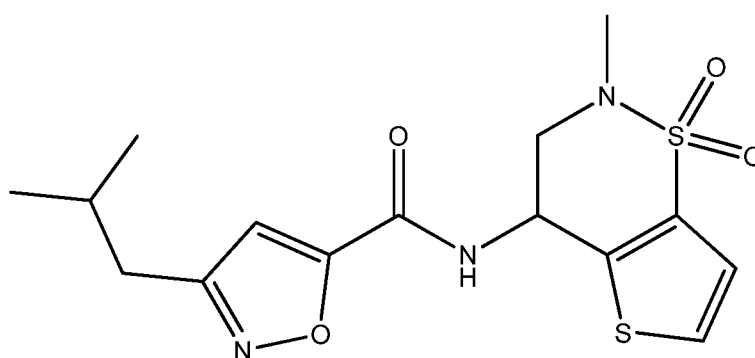
,







, and



12. The compound of any one of claims 3 to 11, for use in treating a medical condition in which an onset and/or progression of the condition is associated with NF- κ B dimerization and/or activity.

13. A compound capable of disrupting a hydrophobic core domain of a p65 unit of NF- κ B, for use in inhibiting dimerization of an NF- κ B comprising said p65 unit, with the proviso that the compound is not withaferin A.

14. The compound of claim 13, being capable of binding to an amino acid residue of said p65 unit, said amino acid residue being selected from the group consisting of F228, W233, T254, Y257, V268, F286, and Y288.

15. The compound of claim 13 or 14, being capable of binding to an E285 amino acid residue and/or Q287 amino acid residue of said p65 unit.

16. The compound of any one of claims 13 to 15, for use in treating a medical condition in which an onset and/or progression of the condition is associated with NF- κ B dimerization and/or activity.

17. The compound for use according to any one of claims 2, 12 and 16, wherein said medical condition is selected from the group consisting of an inflammatory diseases or disorder, an autoimmune disease or disorder, cancer, adult respiratory distress syndrome, Alzheimer's disease, ataxia telangiectasia, atherosclerosis, cachexia, diabetes, glomerulonephritis, restenosis, and substance abuse.

18. The compound for use according to claim 17, wherein said medical condition is a lymphoid cancer.

19. The compound for use according to claim 18, wherein said cancer is multiple myeloma.

20. A method of identifying an inhibitor of NF- κ B dimerization, the method comprising:

contacting at least one compound with a first NF- κ B monomer and a second NF- κ B monomer, wherein said first NF- κ B monomer is attached to a first fragment of a luciferase protein and said second NF- κ B monomer is attached to a second fragment of a

luciferase protein, and wherein dimerization of said first NF- κ B monomer and said second NF- κ B monomer increases luciferase activity, and

identifying a compound which inhibits said increase in luciferase activity.

21. The method of claim 20, wherein said at least one compound comprises a library comprising at least 100 compounds, the method comprising screening said library.

22. The method of any one of claims 20 to 21, further comprising measuring luminescence at a wavelength suitable for detecting an activity of luciferase, wherein luminescence is indicative of said luciferase activity.

23. The method of any one of claims 20 to 22, further comprising contacting said compound which inhibits said increase in luciferase activity with an active luciferase protein having a luciferase activity, and

determining an effect of said compound on said luciferase activity of said active luciferase protein,

wherein inhibition of a luciferase activity of said active luciferase protein by said compound is indicative of the compound not being an inhibitor of NF- κ B dimerization.

24. The method of any one of claims 20 to 23, wherein said first NF- κ B monomer and/or said second NF- κ B monomer is a fragment of p65.

25. The method of any one of claims 20 to 24, wherein said first fragment of a luciferase protein is attached to an N-terminus of said first NF- κ B monomer and/or said second fragment of a luciferase protein is attached to an N-terminus of said second NF- κ B monomer.

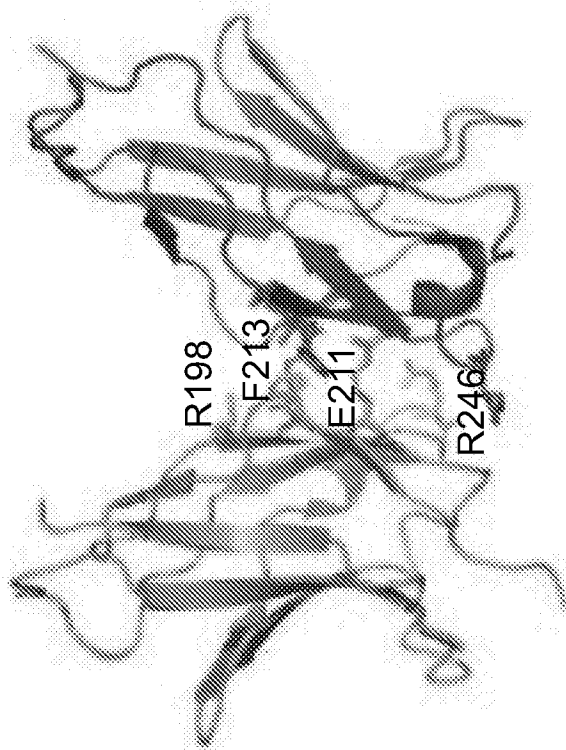
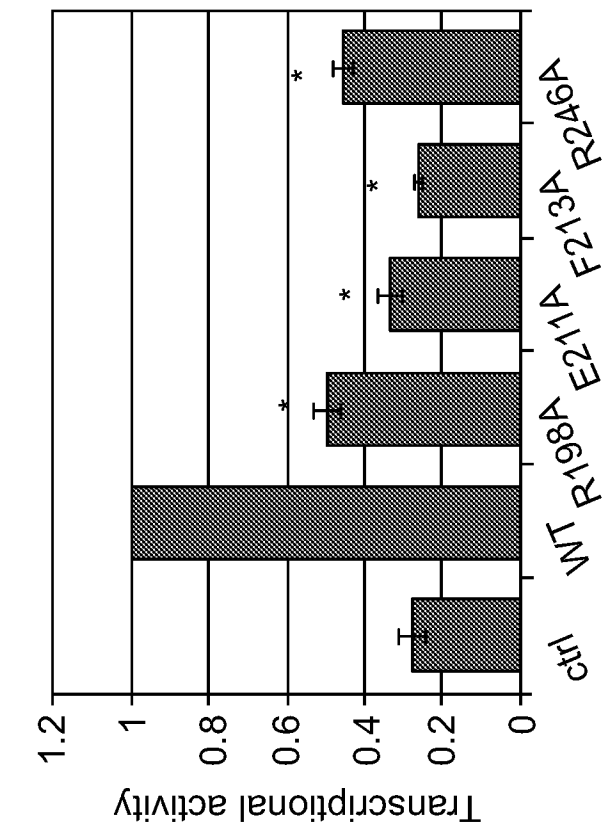


FIG. 1A

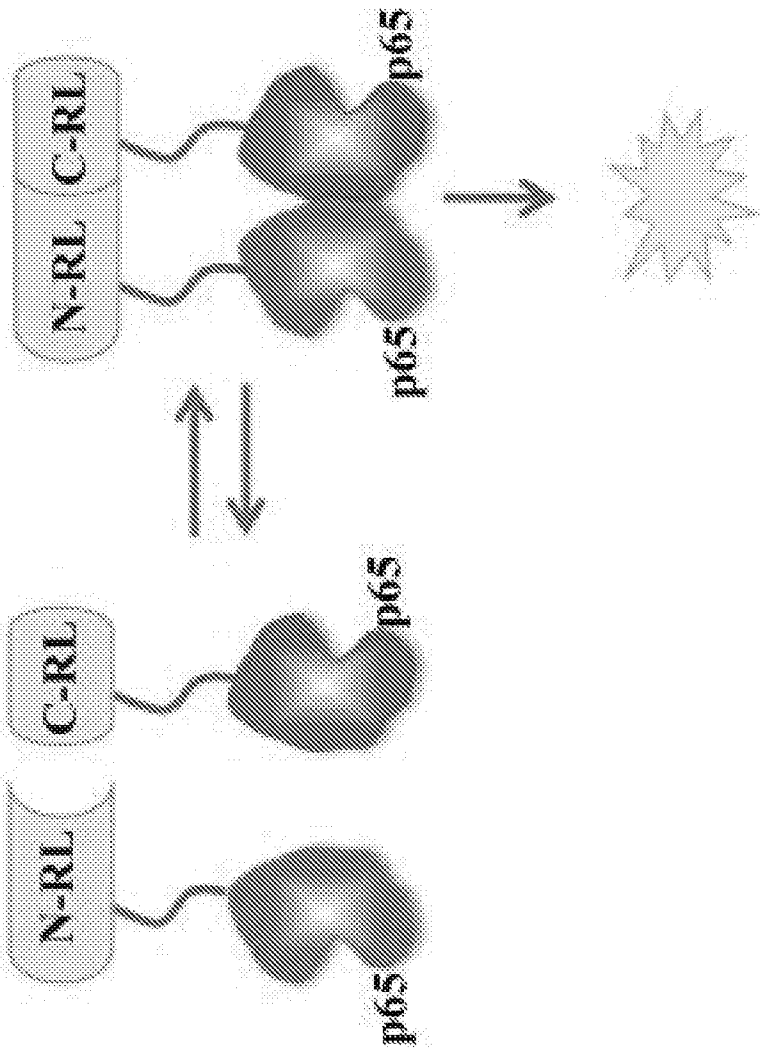


FIG. 1B

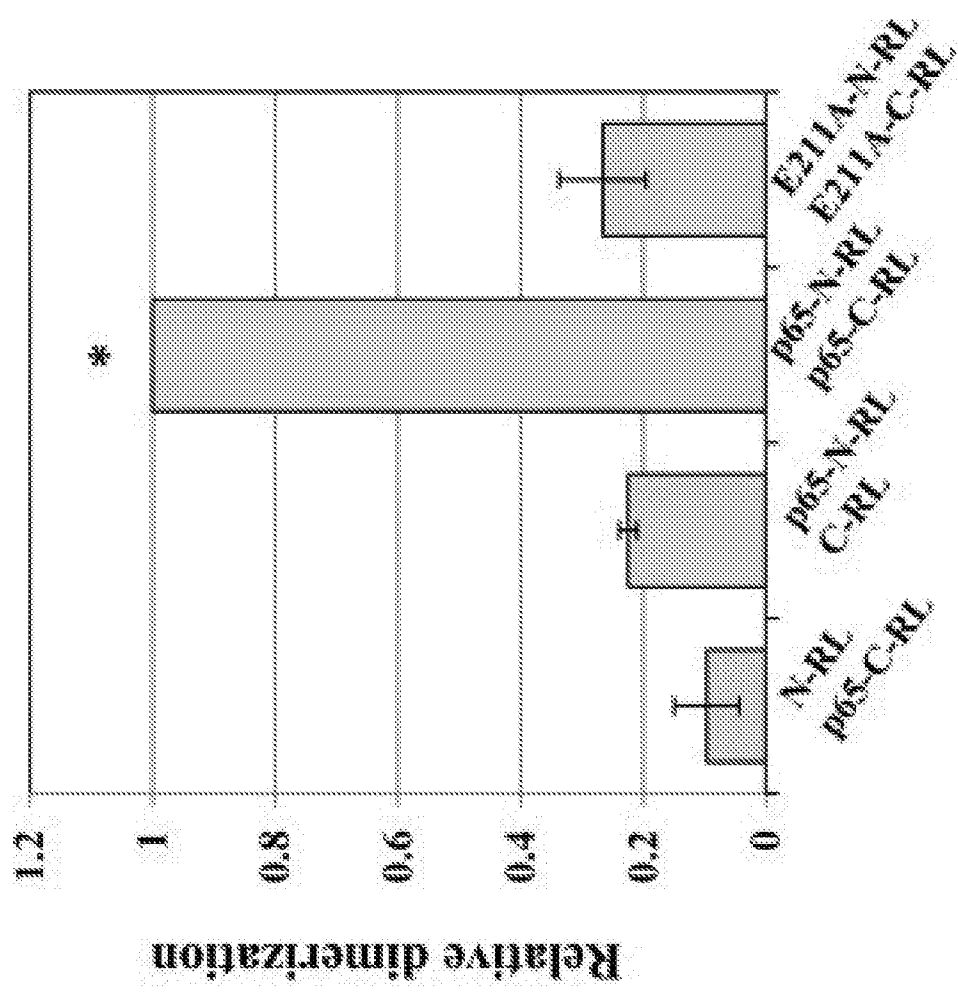


FIG. 1C

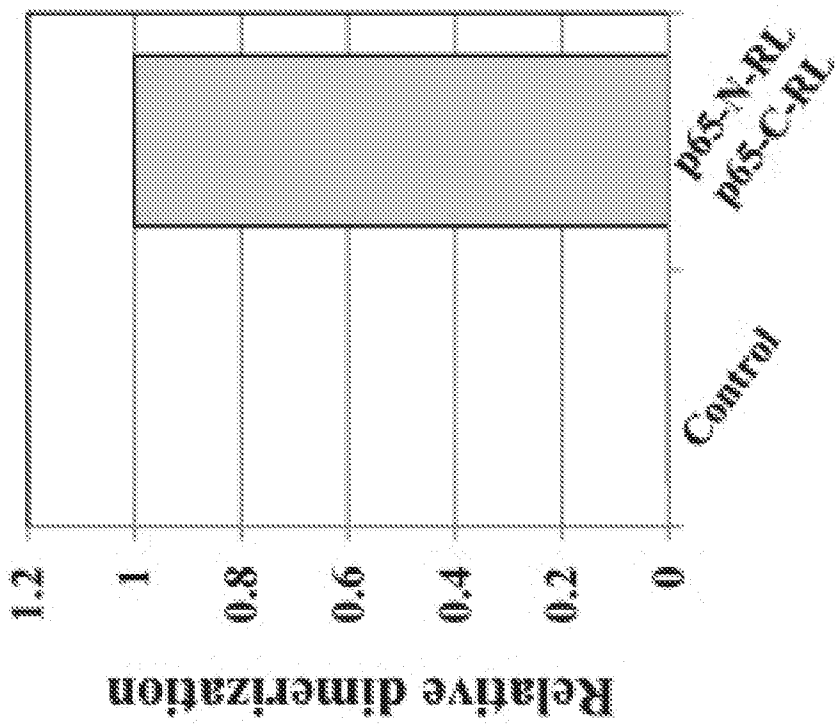


FIG. 1D

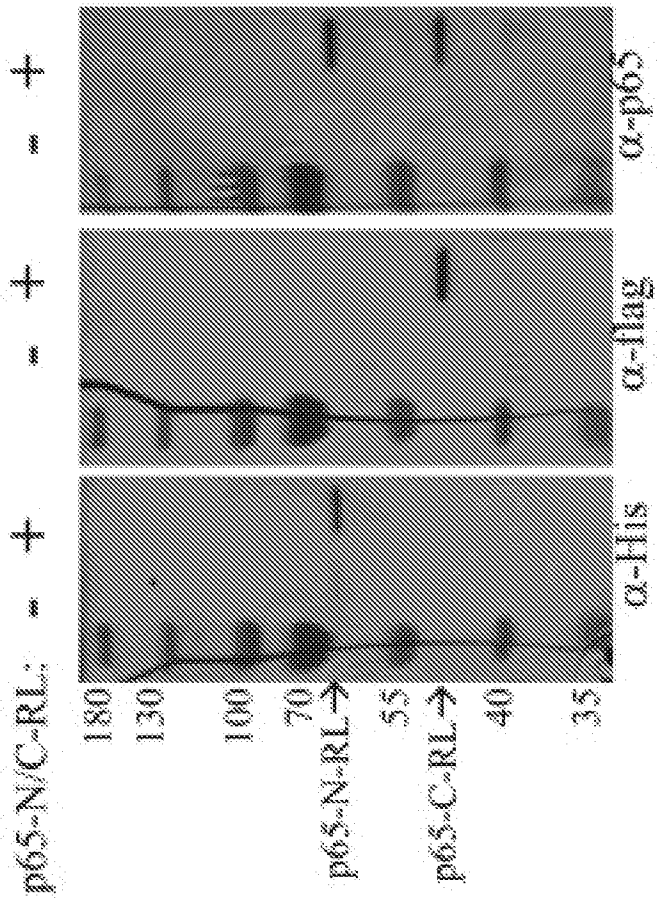


FIG. 1E

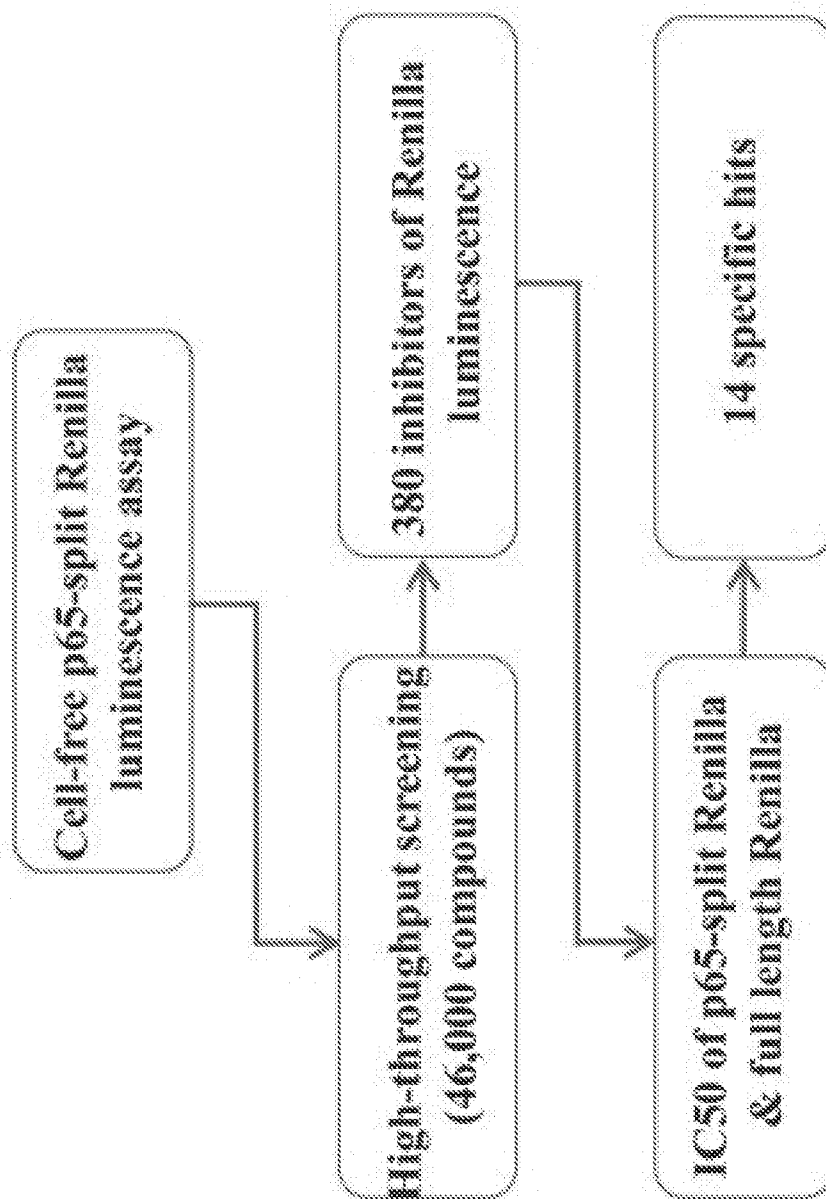


FIG. 2A

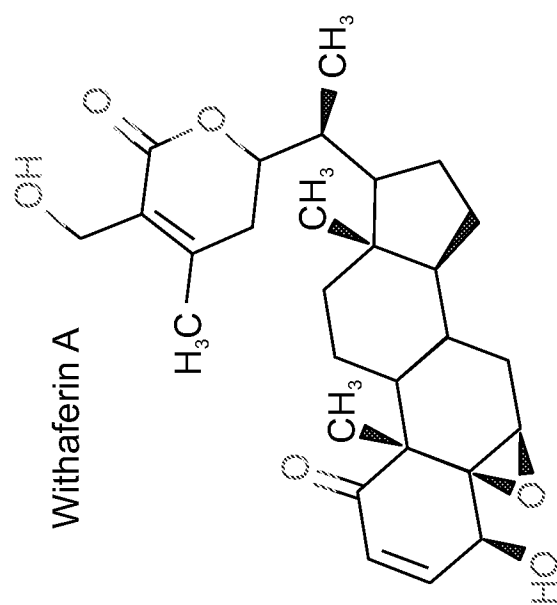


FIG. 2B

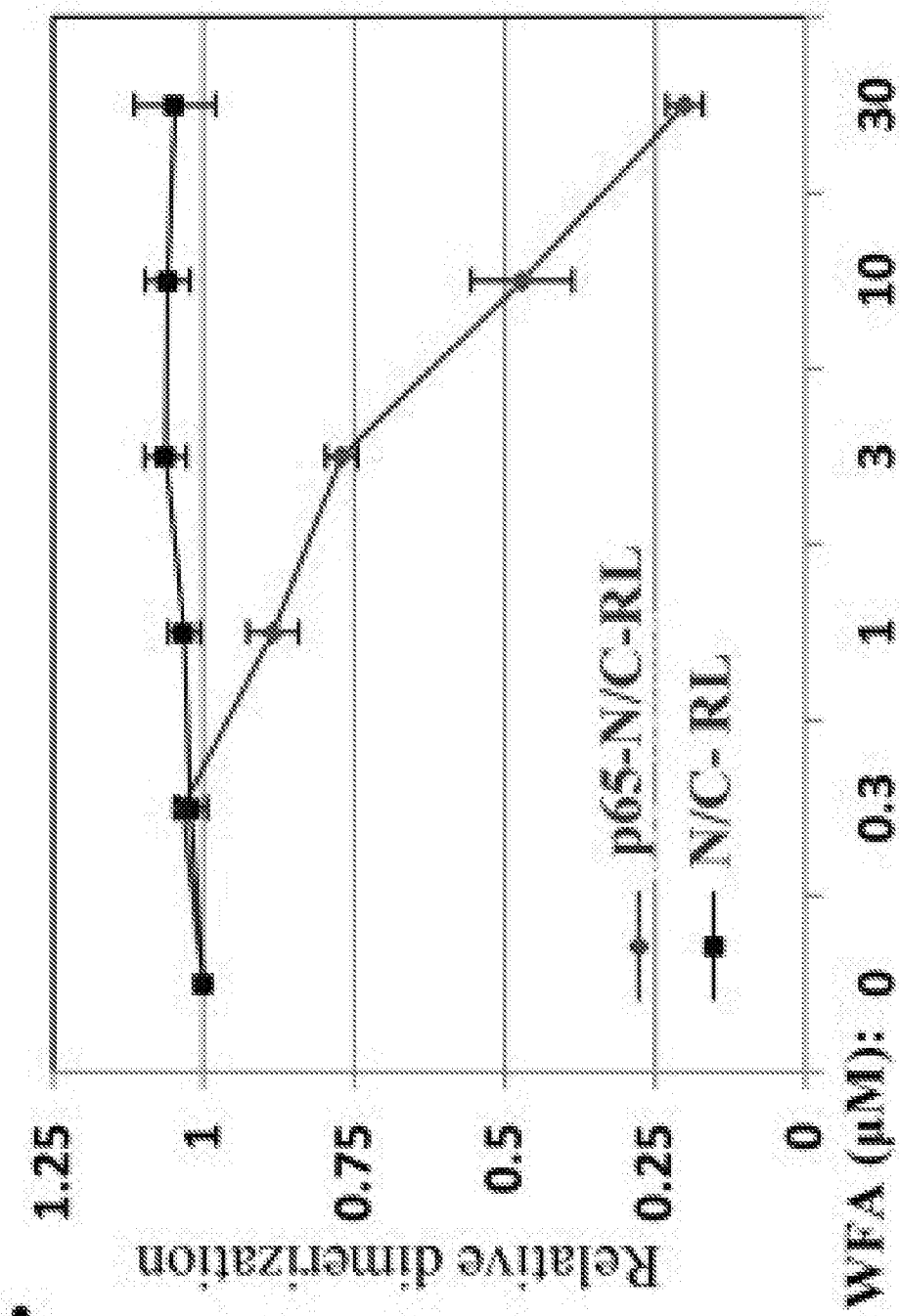


FIG. 2C

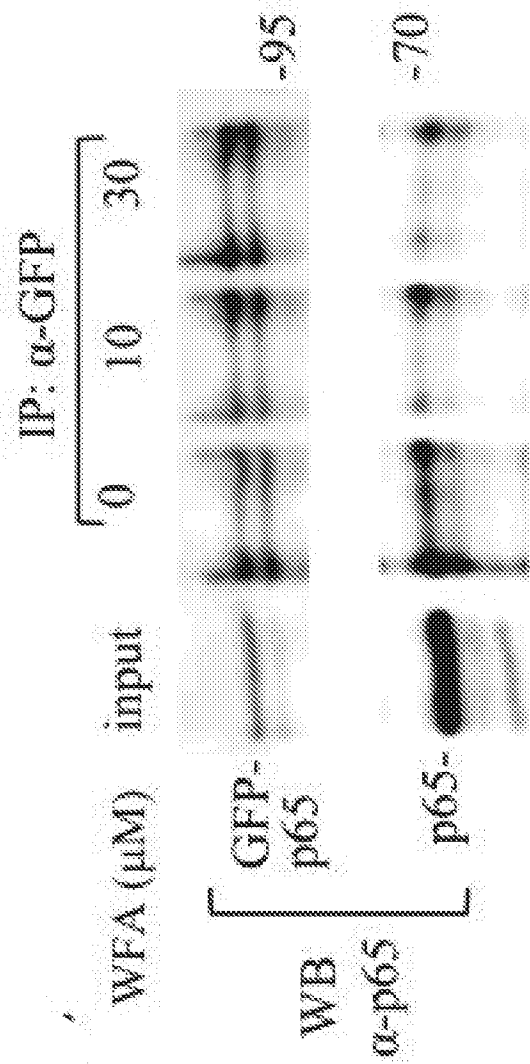


FIG. 2D

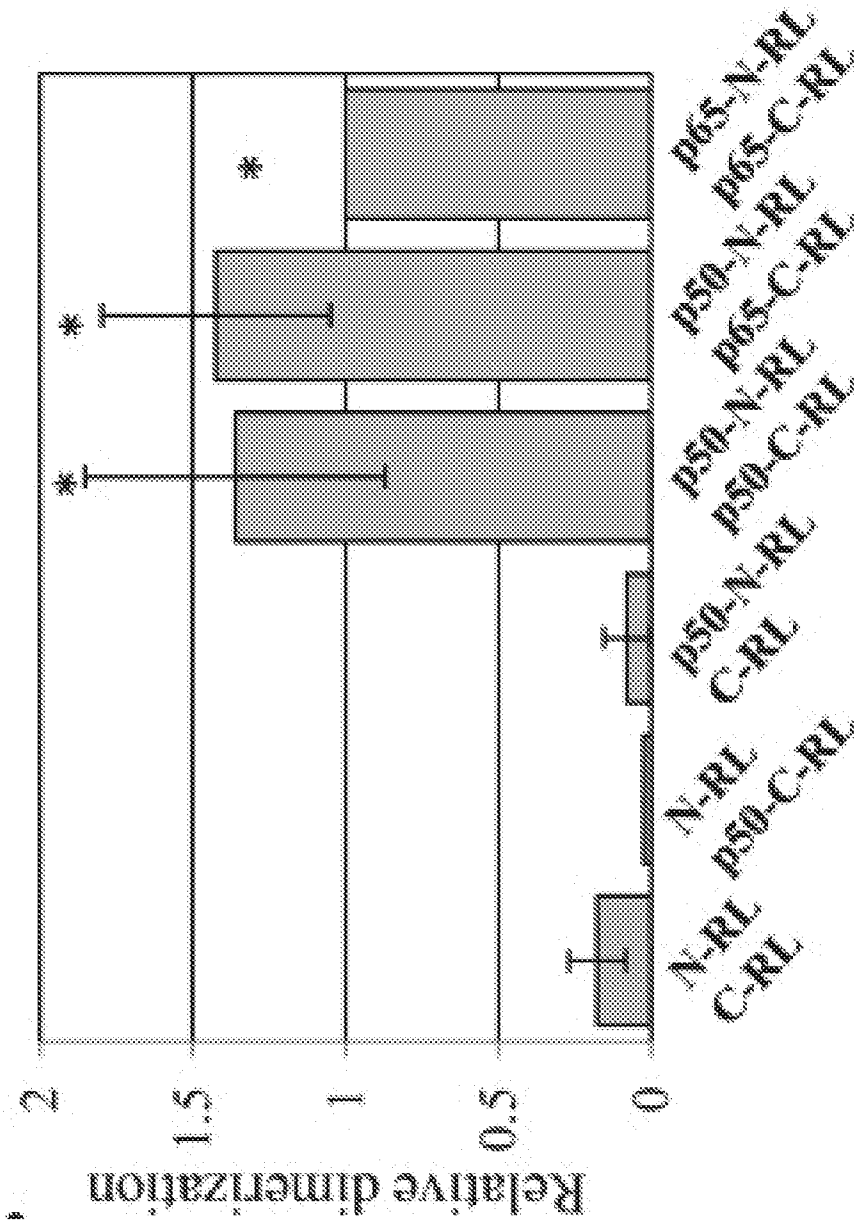


FIG. 2E

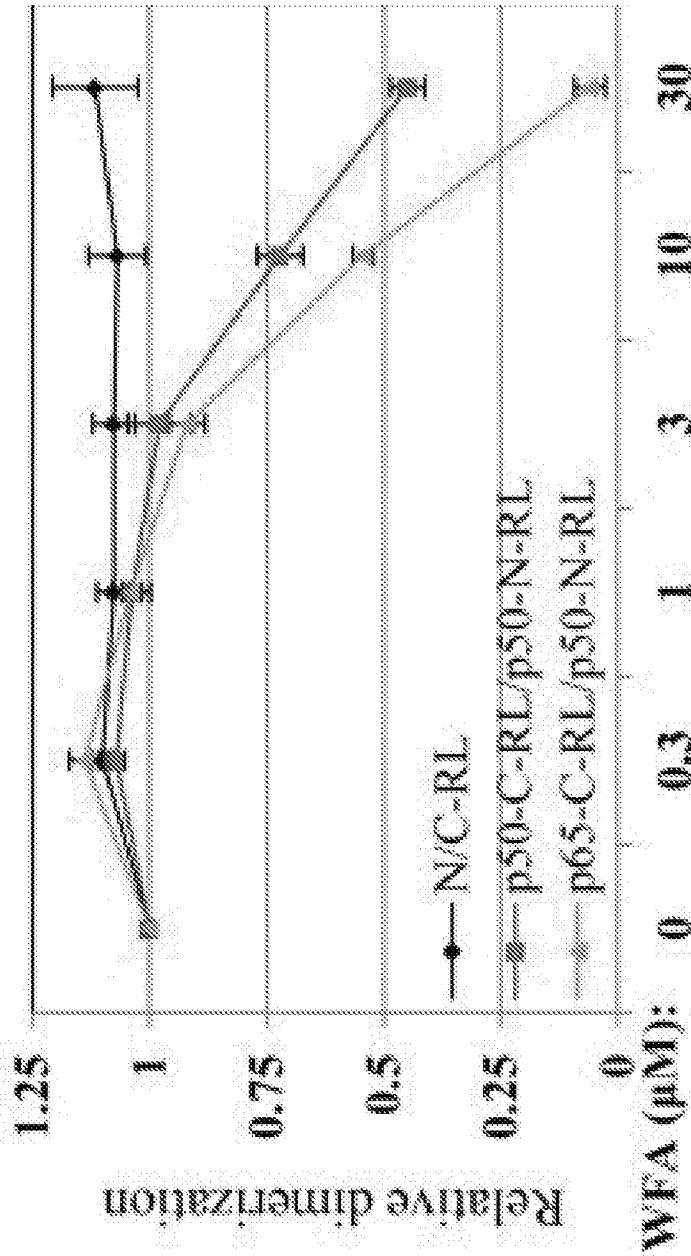


FIG. 2F

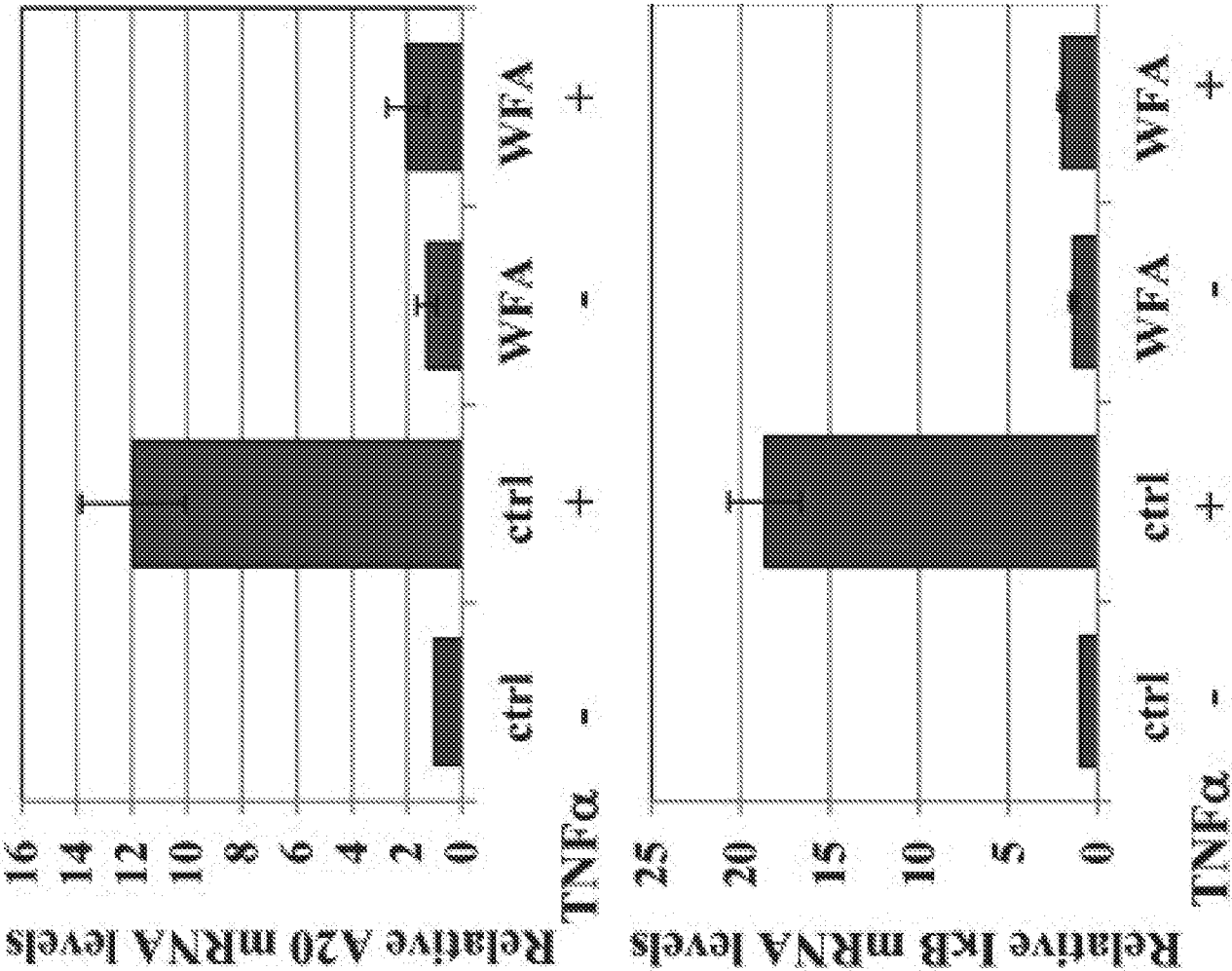


FIG. 3A

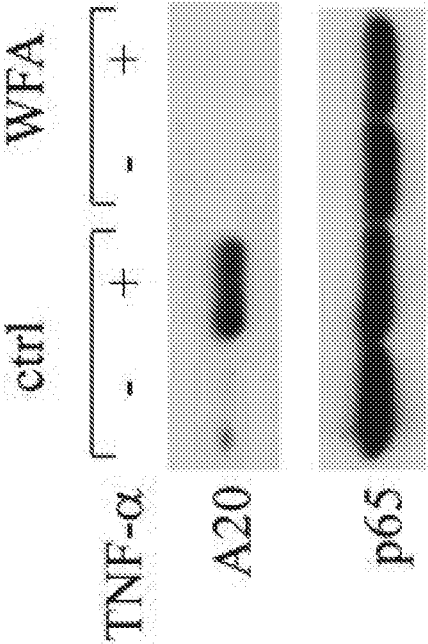


FIG. 3B

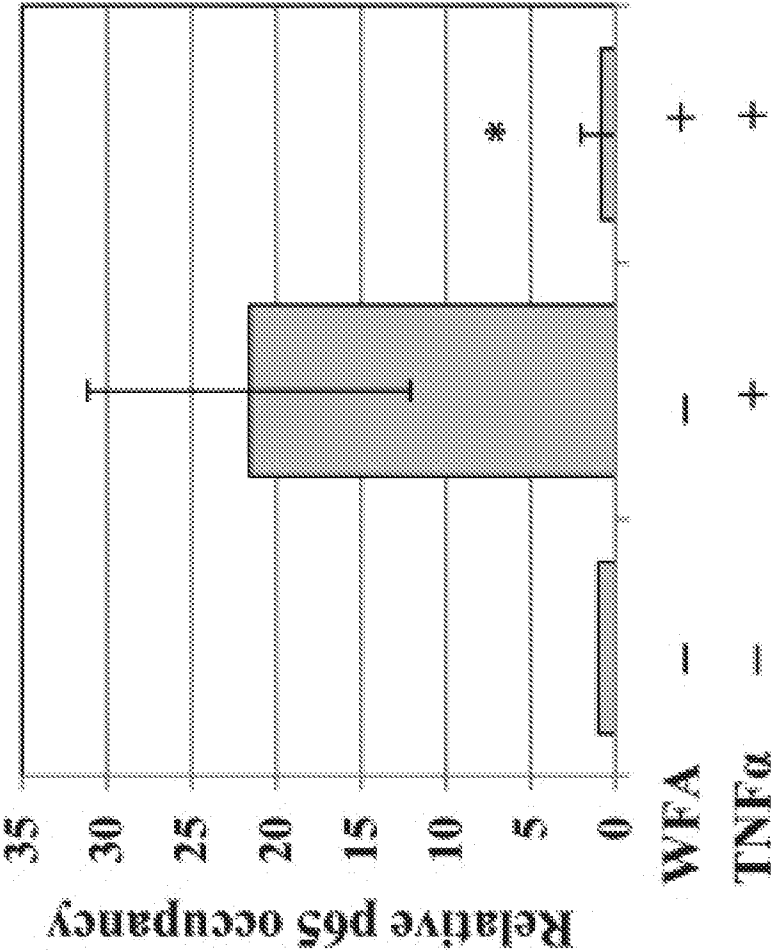


FIG. 3C

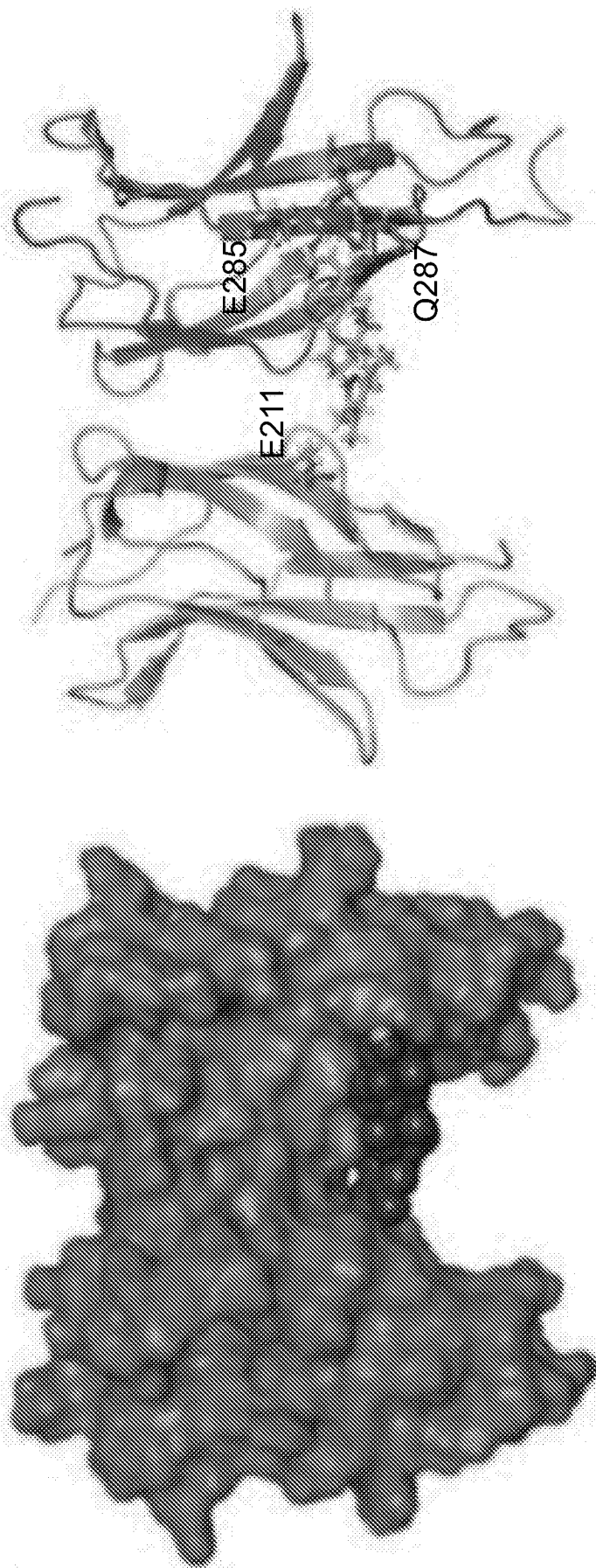


FIG. 4A

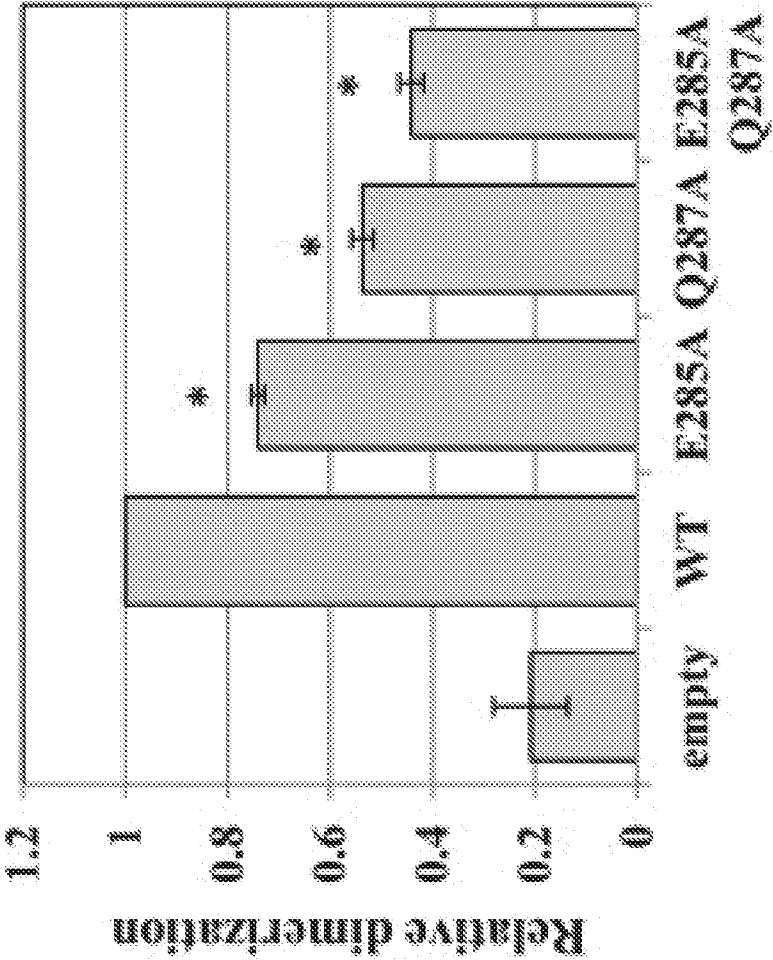


FIG. 4B

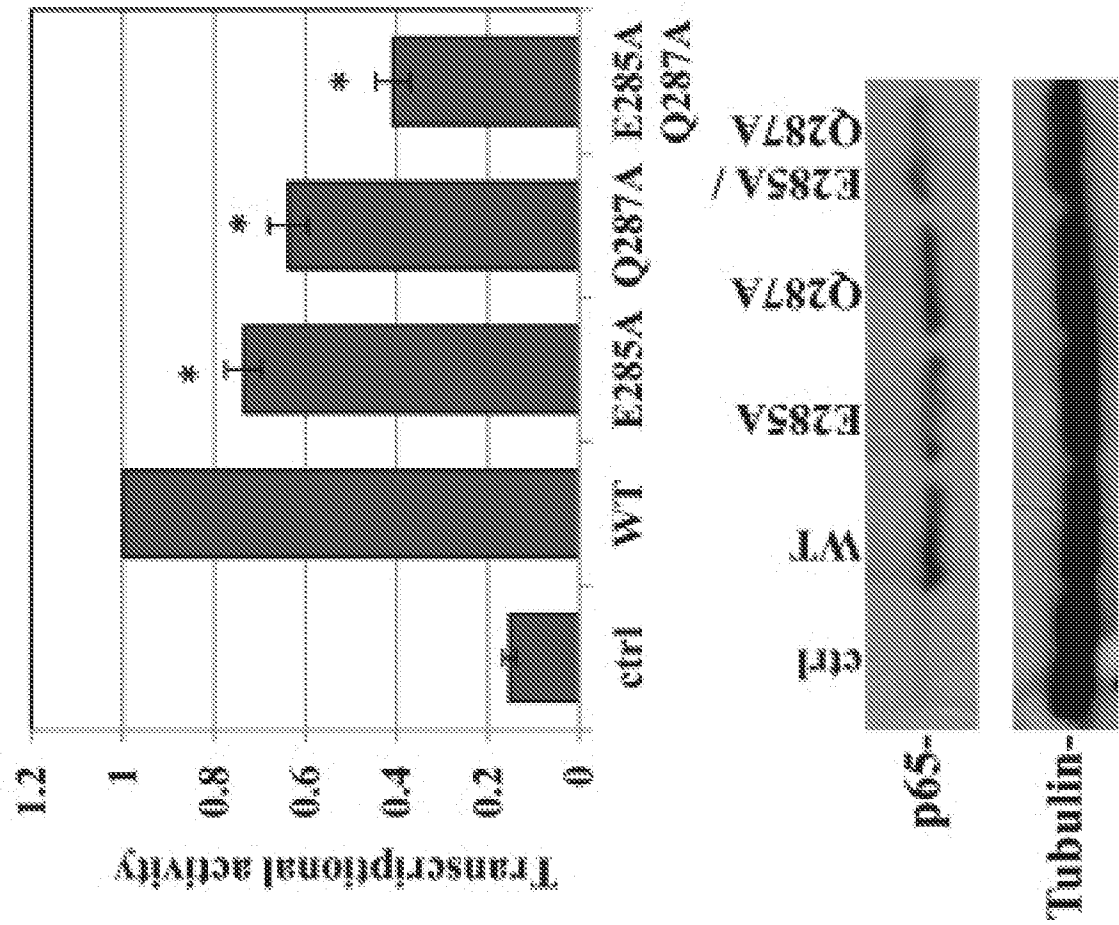


FIG. 4C

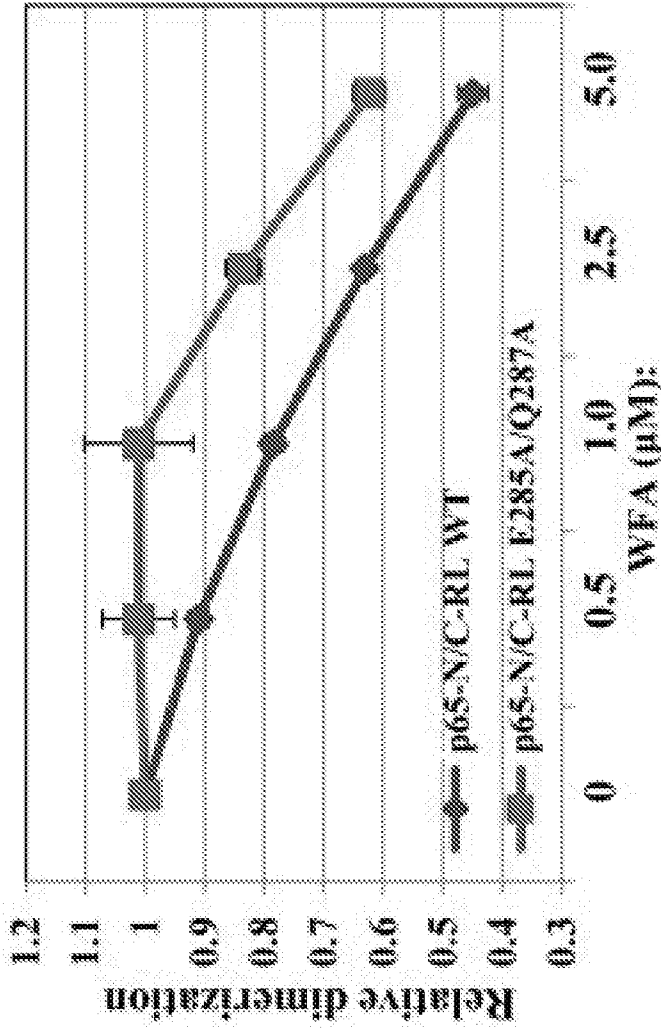


FIG. 4D

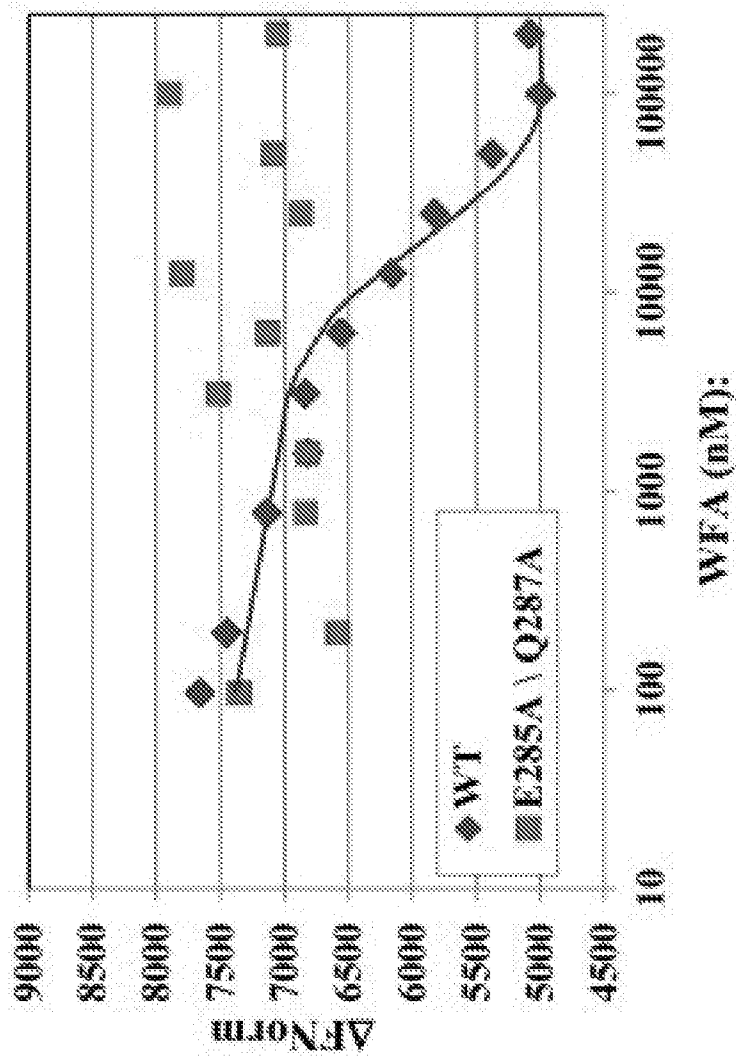


FIG. 4E

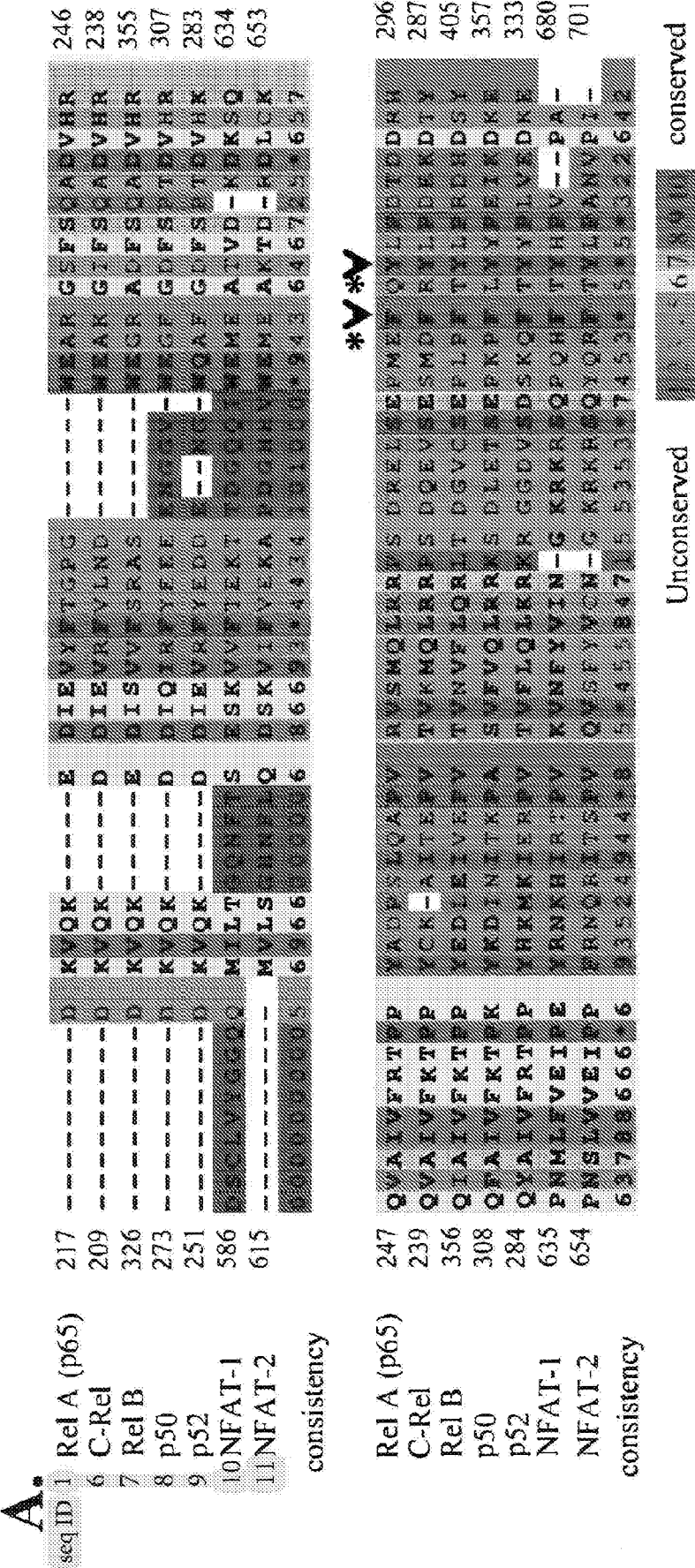


FIG. 5A

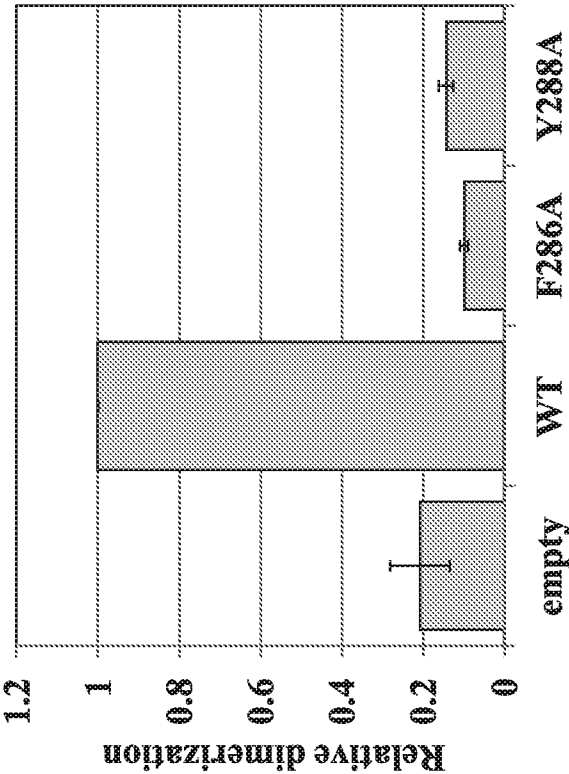


FIG. 5B

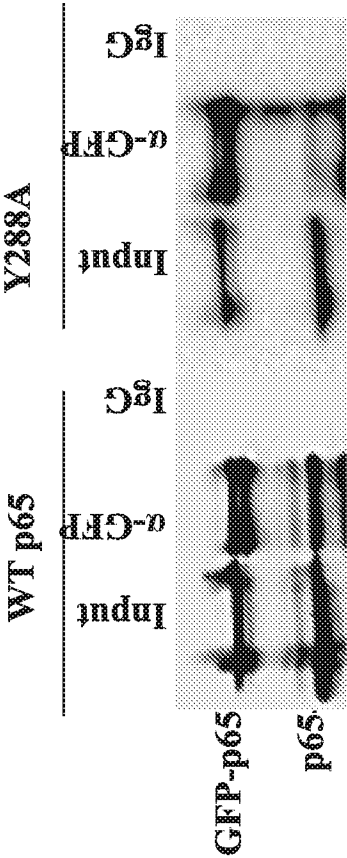
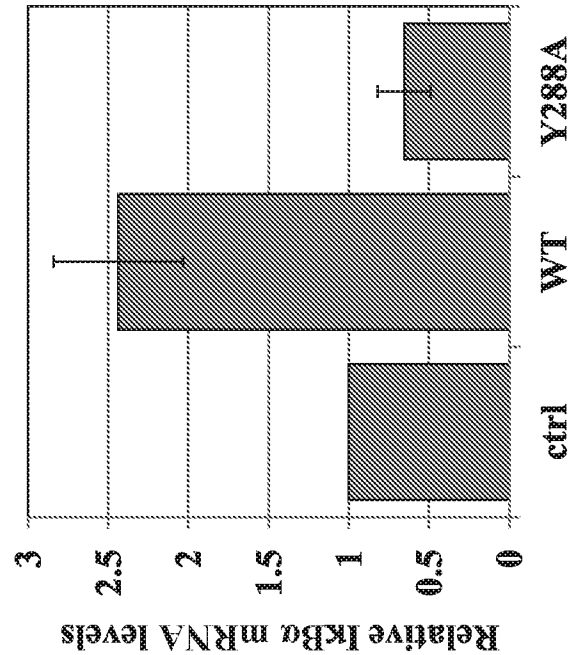
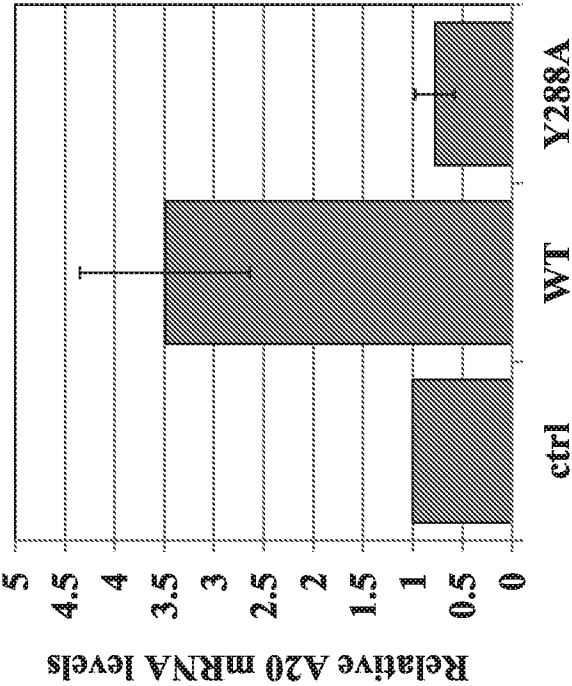
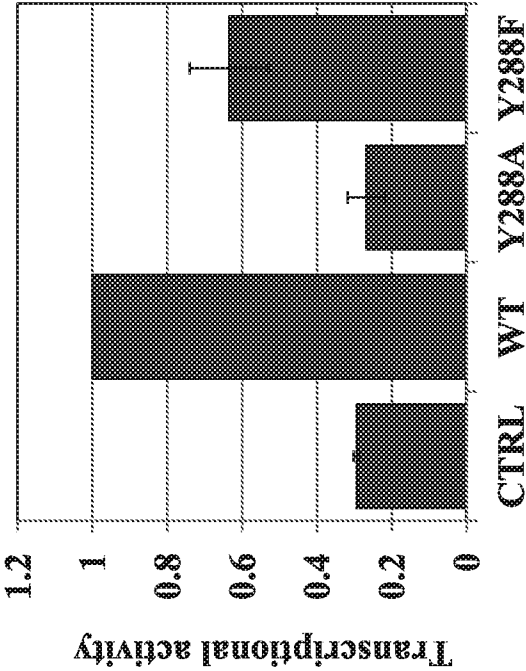


FIG. 5C



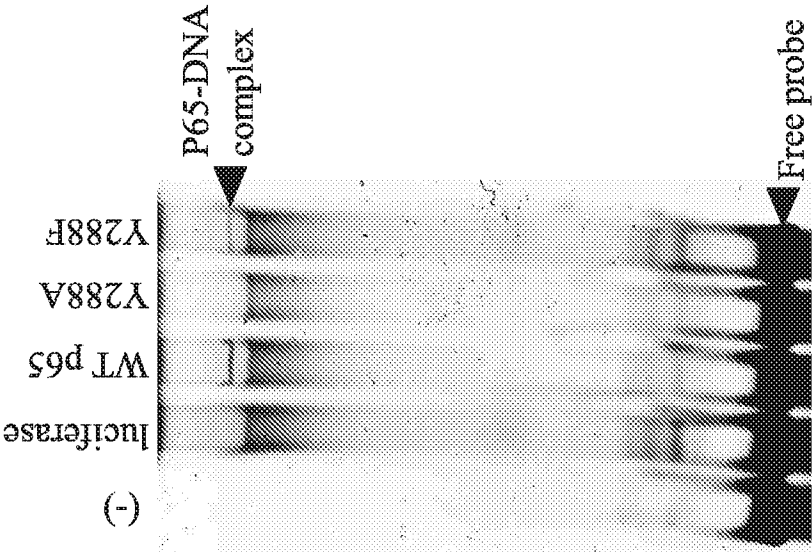


FIG. 5F

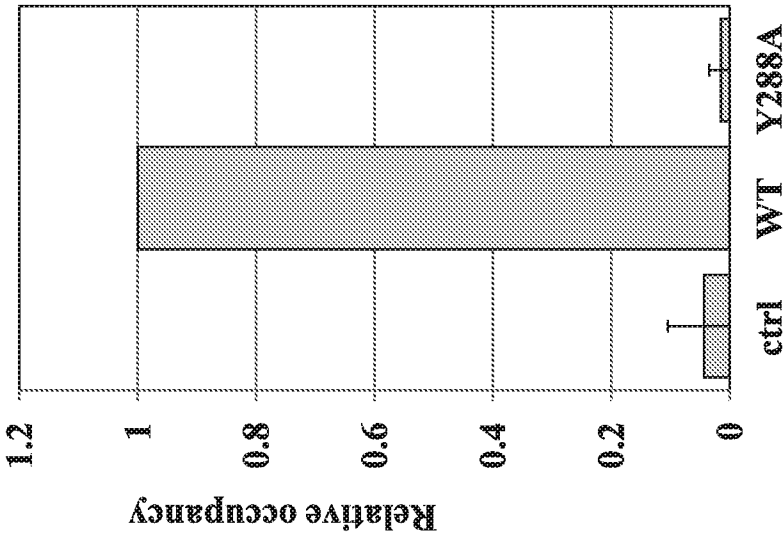


FIG. 5G

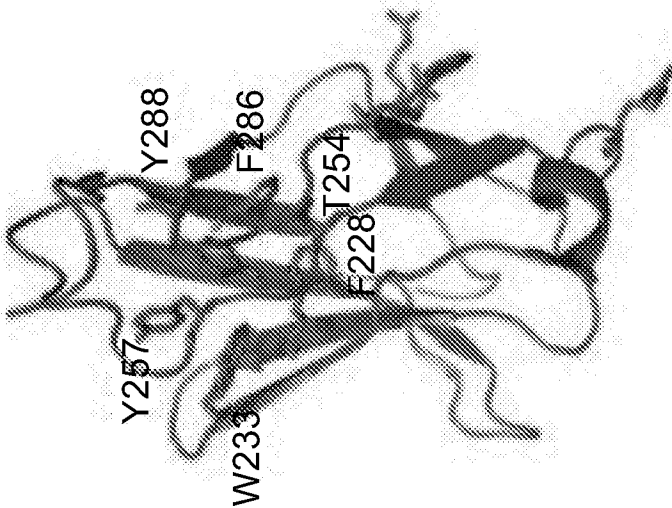


FIG. 6A

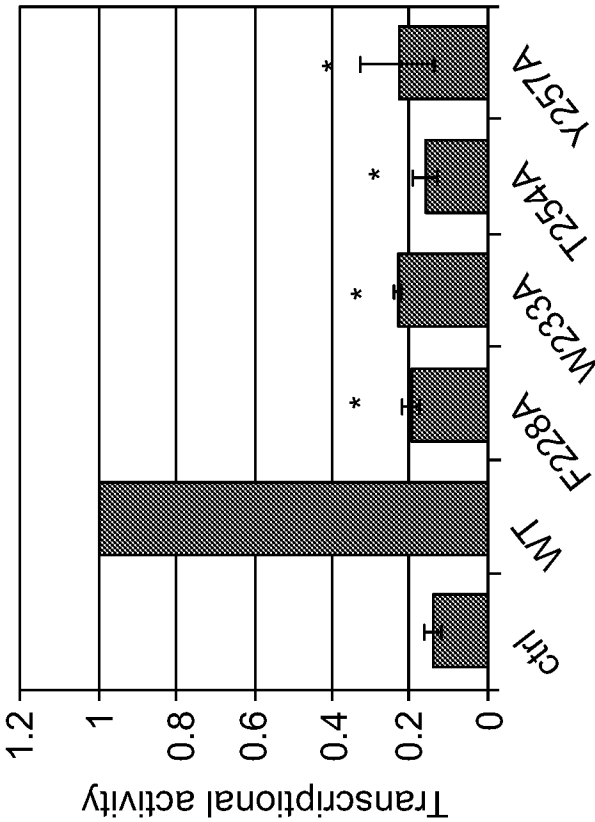
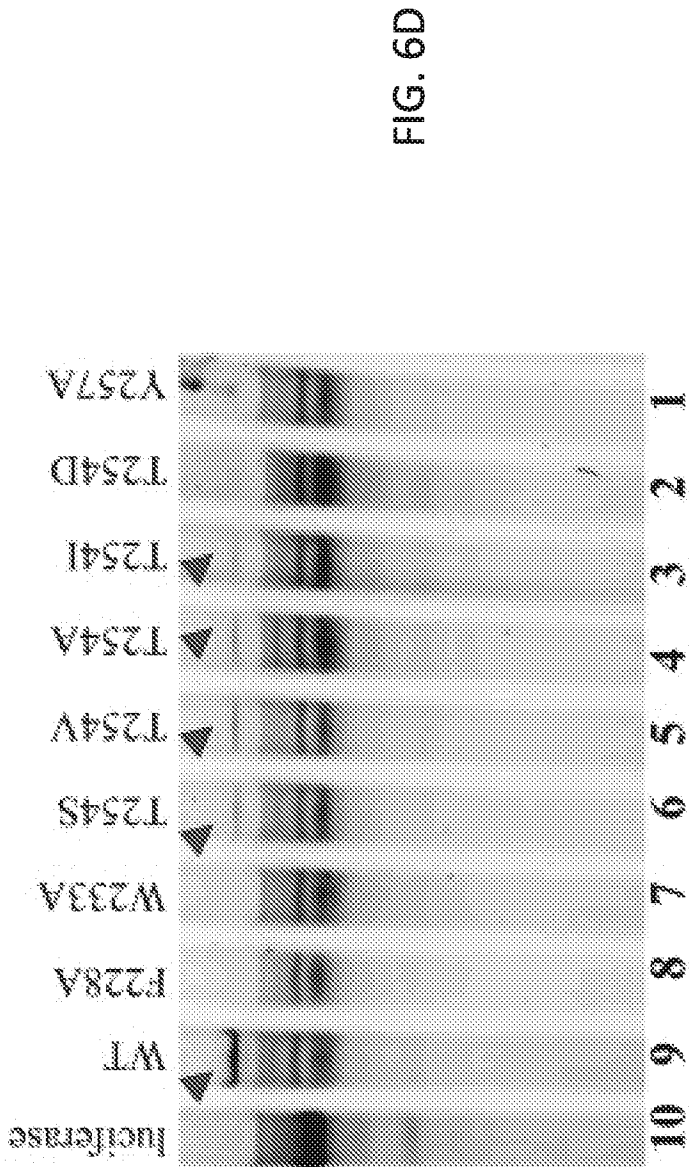
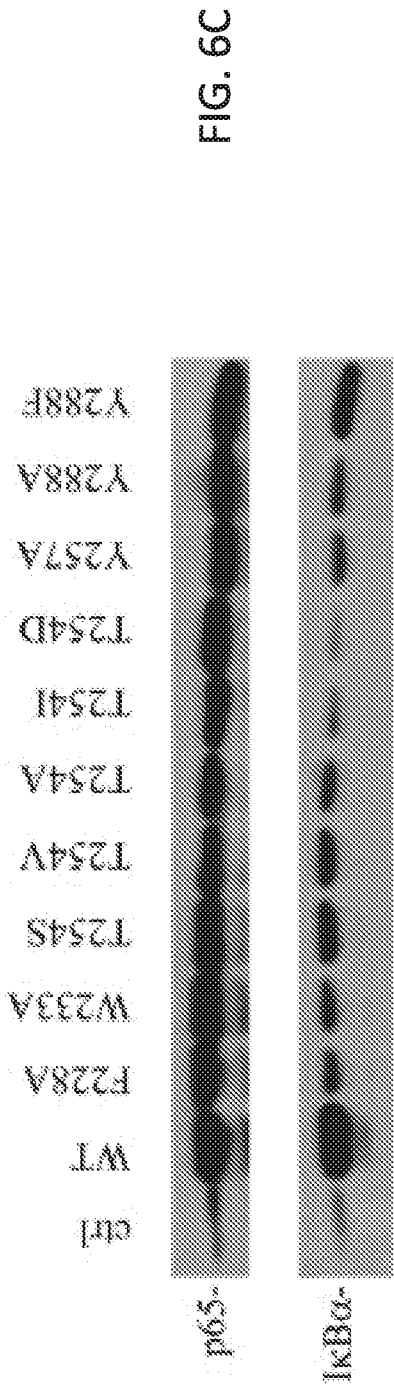
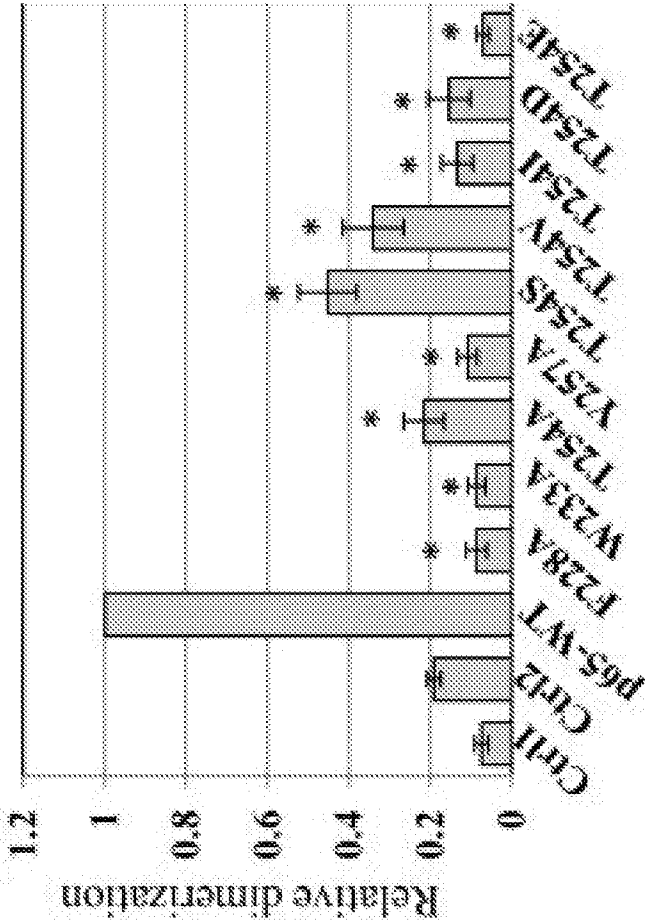
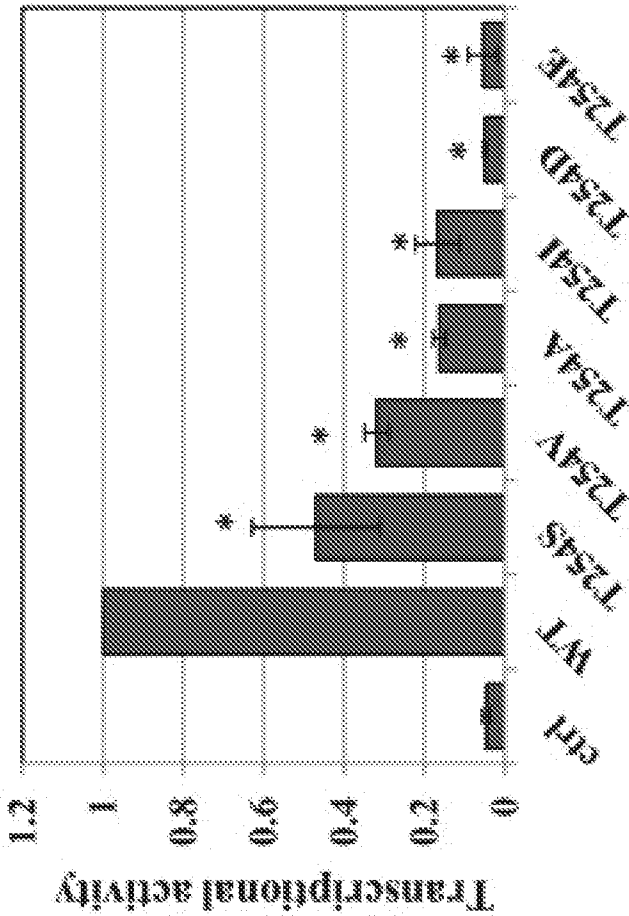


FIG. 6B





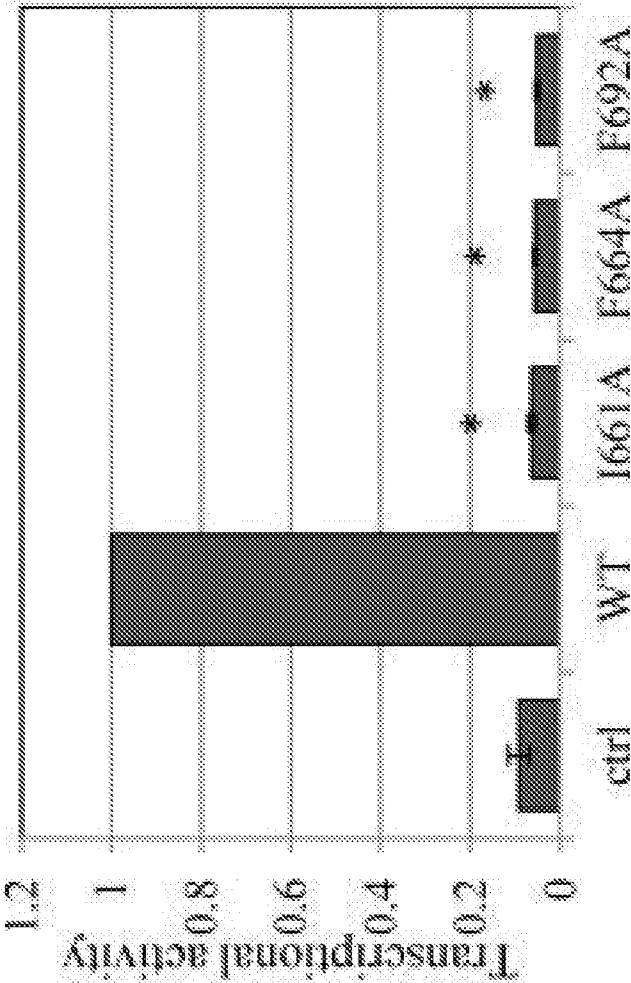


FIG. 6G

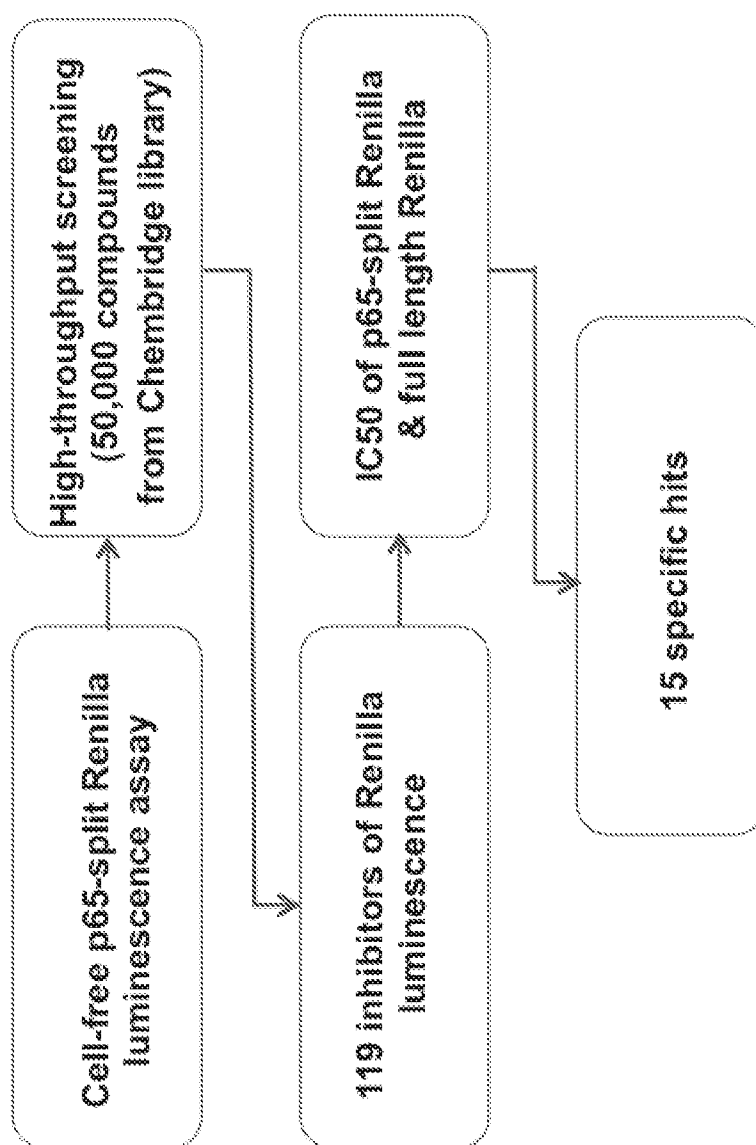


FIG. 7

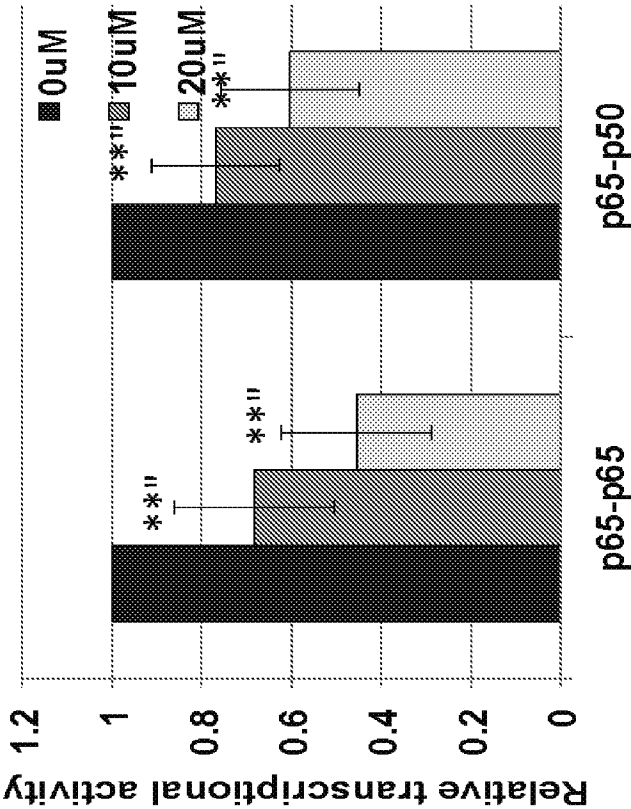


FIG. 8B

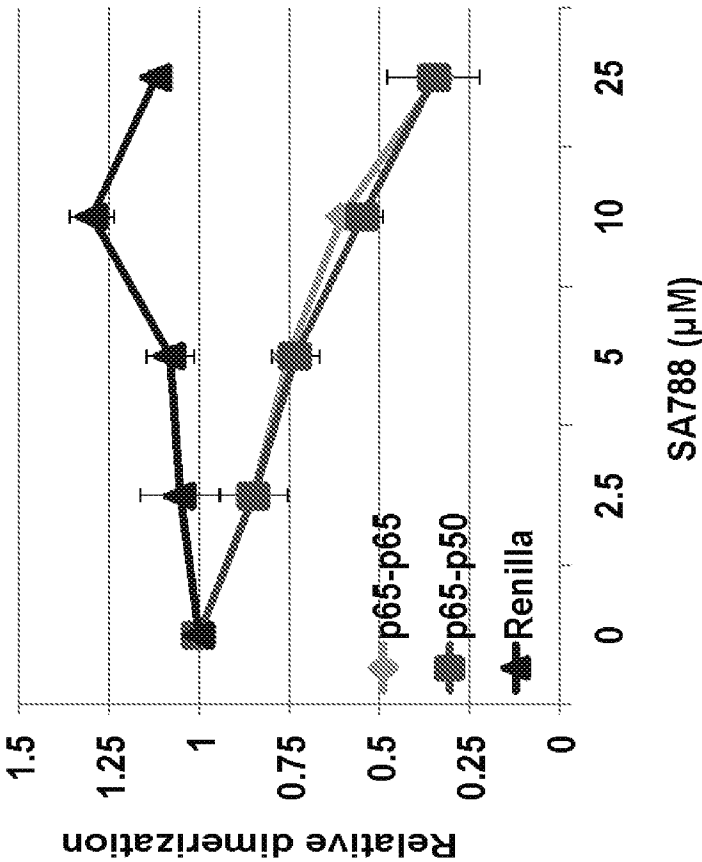


FIG. 8A

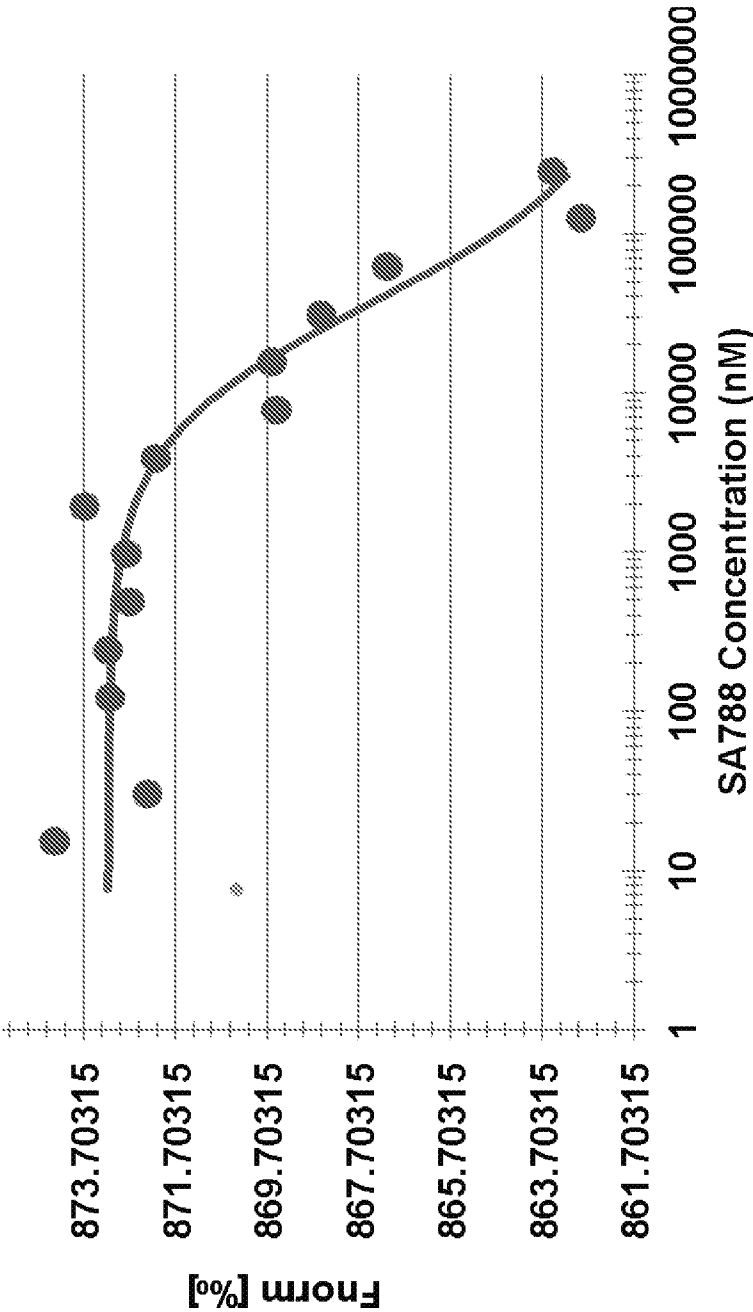


FIG. 9

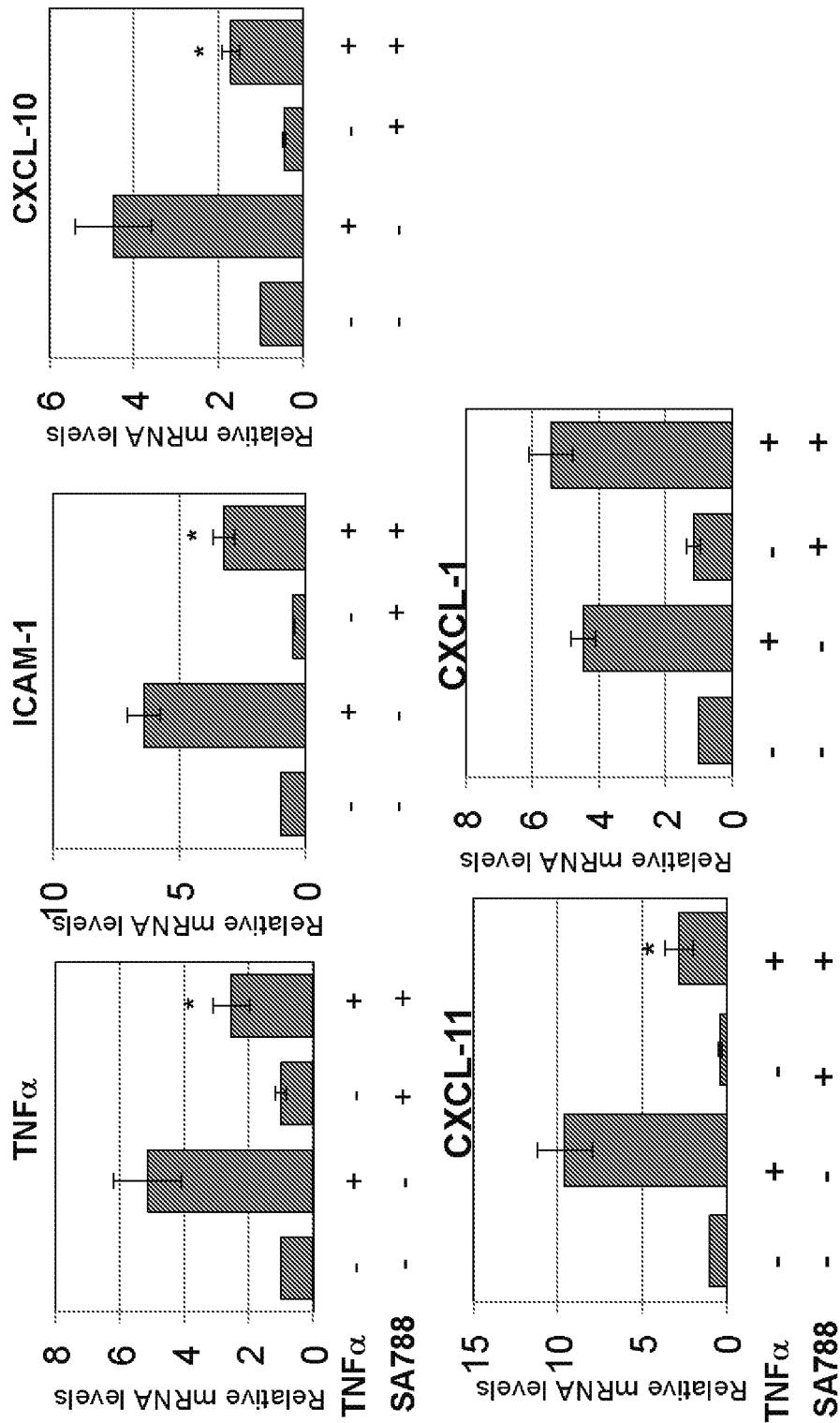


FIG. 10

