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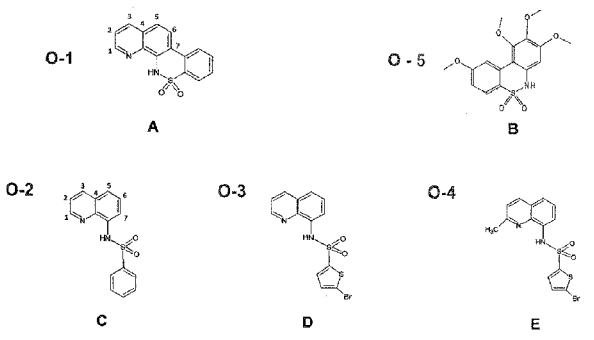

Figure 1

(57) **Abstract**: The present invention relates to the NQBS class of molecules. It is based, at least in part, on the discovery that a representative group of compounds have been observed to inhibit nuclear translocation of NF-κB subunits. Without being bound by any particular theory, this inhibition of nuclear translocation may be mediated by either (i) binding of the NQBS or related compound to the C-terminus of the RHD, which specifically mediates the nuclear internalization; or (ii) NQBS-mediated stabilization of the dimer/IκB complex, disallowing dissociation of the active NF~KB monomers, and thus, inhibiting the generation of the subunits necessary to enter the nucleus. The NQBS class of molecules, and related molecules, may be used in therapeutic applications where inhibition of NF-κB translocation is beneficial, including but not limited to the treatment of cancer, autoimmune disorders, and inflammatory states.

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N-QUINOLIN-BENZENSULFONAMIDES AND RELATED COMPOUNDS FOR THE TREATMENT OF CANCER, AUTOIMMUNE DISORDERS AND INFLAMMATION

PRIORITY CLAIM

This application claims priority to U.S. Provisional Application Serial No. 61/451,408, filed March 10, 2011, the contents of which are incorporated by reference in their entirety herein.

GRANT INFORMATION

This invention was made with government support under P50 AG08702 and 5RO1 AT001643 awarded by the National Institutes of Health. The government has certain rights in the invention.

1. INTRODUCTION

The present invention relates to N-(quinolin-8-yl) benzenesulfonamides ("NQBS") and related compounds and their use as agents for treating cancer, autoimmune disorders and inflammatory conditions.

2. BACKGROUND OF THE INVENTION

One of the most ubiquitously implicated transcription factors in all of carcinogenesis is nuclear factor-κB (NF-κB). NF-κB represents a family of five proteins (p105/50, p100/p52, c-Rel, RelA/p65 and RelB) that affect over 400 genes, many of which are important in the context of cancer cell growth and survival (Perkins, 2007, Nature Rev. Mol. Cell Biol. 8:40-62). Many of these gene activate genes that: promote cancer cell growth; inhibit those mechanisms responsible for cell cycle arrest; and promote resistance to cell death. All NF-κB members contain a Rel homology domain ("RHD"), an approximately 300 amino acid long residue, which is a highly conserved sequence (e.g. SEQ ID NO:1 from AF134870 and see López-Rodríguez et al., 1999, Proc. Natl. Acad. Sci. 96:7214-7219) near their N terminus of the RHD. This domain is the site that mediates binding to DNA, dimerization with other NF-κB subunits, and nuclear localization. SEQ ID NO: 1 is a human sequence as follows:

LSQLTTDNKGNSKAGNGTLENQKGTGVKKSPMLCGQYPVKSEGKELKIVVQPET
QHRARYLTEGSRGSVKDRTQQGFPTVKLEGHNEPVVLQVFVGNDSGRVKPHGF

YQACRVTGRNTTPCKEVDIEGTTVIEVGLDPSNNMTLAVDCVGILKLRNADVEA RIGIAGSKKKSTRARLVFRVNIMRKDGSTLTLQTPSSPILCTQPAGVPEILKKSLHS CSVKGEEEVFLIGKNFLKGTKVIFQENVSDENSWKSEAEIDMELFHQNHLIVKVPP YHDQHITLPVSVGIYV VTNAGRSHDVQPFTYTPD.

In normal cells, following an activation signal from the surface of the cell, NF-κB subunits translocate to the nucleus where they exert their effect on gene transcription by binding to DNA. The ability to transactivate specific genes in DNA is absolutely dependent on the ability of the NF-κB subunits to enter the nucleus, the site of all DNA replication and transcription.

There are at least three known NF-κB activation pathways: (1) the canonical or classical pathway, which is an IκB dependent pathway activated by extracellular signals such as TNFα, IL-1 and LPS; (2) the non-canonical or alternative pathway, which is an IκB independent pathway activated by CD40/CD40L interaction, and (3) the atypical pathway, which is stimulated by various signals including genotoxic stress, hypoxia and ROS (Perkins, 2007, Nature Rev. Mol. Cell Biol. 8:40-62). Within the classical pathway, NF-κB transcription factors are sequestered in the cytoplasm in their inactive state by the IκB family of inhibitory proteins (IκBa, IκBß, IκBe, p105/κ and p100/d). Upon an activation signal, IκB kinase (IKK) phosphorylates IκB, rendering it a substrate for ubiquitination and subsequent proteosome mediated degradation. Removal of the IκB allows for nuclear translocation of the NF-κB complex, and activation of its target genes (Perkins, 2007, Nature Rev. Mol. Cell Biol. 8:40-62).

In the alternative pathway, IKK directly phosphorylates p100 which in turn induces the processing of p100 to p52, which is then translocated to the nucleus with subsequent activation of the target genes. The atypical pathway can lead to NF-κB activation in a IKK independent way (hypoxia and ROS activate Tyr kinase) or an IKK dependent way (genotoxic stress). Over expression and constitutive activation of NF-κB is thought to be one of the central events leading to cancer. This biology, first described in normal lymphocytes, is thought to play a pivotal role in the formation of lymphomas. Identifying pharmacologic strategies to inhibit the activation of target NF-κB genes has been a major pursuit for cancer research laboratories over the past 2 decades.

Over the years, select agents indirectly affecting NF-κB biology have been identified, as discussed below. These agents affect NF-κB biology by inhibiting IκB kinase, which inhibits the phosphorylation and subsequent degradation of IκB, or by inhibiting the proteasome, and thus the proteolytic degradation of IκB.

NF-κB promotes the dysregulated growth and survival of many cancers, including most lymphomas. Efforts to inhibit NF-κB over the years have been fraught with many challenges, not the least of which has been the development of relatively NF-κB non-specific agents. One such example is bortezomib (Velcade), a proteasome inhibitor touted as an NF-κB inhibitor which has been approved for the treatment of myeloma and mantle cell lymphoma. While bortezomib inhibits the degradation of IκB, it also affects more than 90% of the protein turnover in the cell, and thus affects virtually every important cellular process known. Clinically, while effective, the drug is neurotoxic and is associated with an irreversible painful neuropathy. Clearly, more specific NF-κB inhibitors are needed. To date, direct binding of NQBS to the target protein (p65 and p50) has not yet been described within published literature and therefore represents a novel mode of action for a drug that inhibits NF-κB pathway.

3. SUMMARY OF THE INVENTION

The present invention relates to the NQBS class of molecules and related compounds. It is based, at least in part, on the discovery that a representative group of compounds have been observed to inhibit nuclear translocation of NF-κB subunits. Without being bound by any particular theory, this inhibition of nuclear translocation may be mediated by either (i) binding of the NQBS compound to the C-terminus of the RHD, which specifically mediates the nuclear internalization; or (ii) NQBS-mediated stabilization of the NF-κB dimer/IκB complex, disallowing dissociation of the active NF-κB monomers, and thus, inhibiting the generation of the subunits necessary to enter the nucleus. The NQBS class of molecules may be used in therapeutic applications where inhibition of NF-κB translocation is beneficial, including but not limited to the treatment of cancer, autoimmune disorders, and inflammatory states.

4. BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1A-E. Compounds (A) O-1; (B) O-5; (C) O-2; (D) O-3; and (E) O-4. These are examples of NQBS compounds having the C7-locked (A) or C7-open (C-E) configurations.

FIGURE 2. Growth inhibition IC50 values (micromolar; μM) calculated with Calcusyn Software across DLBCL and TNFα unstimulated HUVEC lines for O-1 and O-4 luminescence assays.

FIGURE 3A,B. Flow cytometric analysis showing percentage of apoptotic cells in DLBCL lines treated with (A) O-1 or (B) O-4 (72 hour time point).

FIGURE 4A-B. Laser confocal microscopy showing, via immunofluorescence of p105/p50 NF-κB), the effects of compounds O-1, O-2, O-3, and O-4, as compared to control, in either (A) Ly10 or (B) HBL-1 DLBCL cell lines.

FIGURE 5. Nuclear NF-κB presence in HBL-1 (lanes 1 and 2) or Ly10 (lanes 3-18) DLBCL cell lines (EMSA), either untreated (lanes 1, 3 and 7) or treated with various compounds as indicated.

FIGURE 6. Effect of NQBS compounds on transcription factors other than NF-κB.

FIGURE 7. Results of toxicity studies in SCID beige mice, as assessed by animal weight. Compound O-3 (an open-ring NQBS compound) was administered at doses of 10 mg/kg, 1 mg/kg, or 0.1 mg/kg.

FIGURE 8A-J. Table summarizing all tested NQBS compounds with growth inhibition IC 50 values (luminescence assays) and EMSA and/or IF assay results for NF-κB translocation inhibition.

FIGURE 9. Cytotoxicity of O-4 and O-19 toward solid tumors.

FIGURE 10. Ribbon diagram showing association between O-4 and p65.

FIGURE 11A-C. Thermal shift studies of the interaction between O-4 and p50 or p65.

FIGURE 12A-D. Results of an *in vivo* experiment in which treatment with O-4 resulted in remission of an aggressive lymphoma, as demonstrated in a mouse transgenic for both myc and cherry luciferase reporter genes. (A) day 0 (B) day 4 (C) day 15 (D) NF-κB induced luciferase activity over time; downward arrows indicate treatment with compound O-4 at a dose of 10mg/kg .

FIGURE 13. Results of an *in vivo* experiment in SCID beige mice with human DLBCL xenografts (OCI-Ly1 cell line) treated with O-4, which significantly inhibited growth of tumors when compared to the control mice which were treated with saline and 10% DMSO; downward arrows indicate treatment with compound O-4 at a dose of 10mg/kg.

FIGURE 14. Compound 47, 55, 69, 74 and 75.

5. DETAILED DESCRIPTION OF THE INVENTION

For clarity of description, and not by way of limitation, the detailed description of the invention is divided into the following subsections:

(i) NQBS and related compounds;

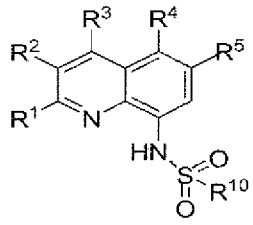
(ii) synthetic schemes for NQBS and related compounds of the invention;

(iii) methods of treatment using NQBS and related compounds; and

(iv) pharmaceutical compositions.

5.1 NQBS AND RELATED COMPOUNDS

In particular non-limiting embodiments, the present invention relates to a compound of Formula I :



and to salts, esters and prodrugs of the compounds of Formula I. Additionally, the present invention describes methods of synthesizing and using compounds of Formula I. In Formula I:

R^1, R^2, R^3, R^4 and R^5 are independently selected for each occurrence from the group consisting of hydrogen, halogen, alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkoxy, aryloxy, alkylthiol, arylthiol, CN, and NO_2; and

R^{10} is selected from the group consisting of substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted alkenyl.

In non-limiting embodiments, R^1, R^2, R^3, R^4 and R^5 are independently selected for each occurrence from the group consisting of hydrogen, branched or unbranched alkyl (e.g., C_1 - C_4 alkyl, for example methyl), alkoxy (e.g., C_1 - C_4 alkoxy, for example methoxy), halogen (e.g. Cl, F, or Br), alkyl halide (e.g. CF_3), aryl, CN, alkoxy, aryloxy, NO_2, alkylthio, and arylthio. In specific non-limiting embodiments, R^4

and R^5 are hydrogen, and R^1, R^2, and R^3 are independently hydrogen, halogen, alkyl (e.g., C_1 - C_4 alkyl), aryl, CN, alkoxy, aryloxy, NO_2, alkylthio, and arylthio.

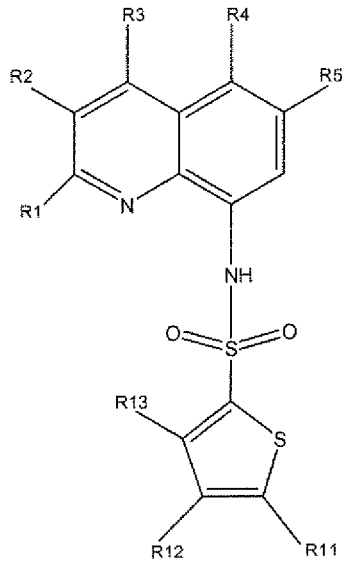
In other particular embodiments, R^{10} is selected from the group consisting of substituted or unsubstituted phenyl, substituted or unsubstituted naphthyl, substituted or unsubstituted thiophenyl, substituted or unsubstituted furanyl, substituted or unsubstituted quinolinyl, substituted or unsubstituted isoquinolinyl, 2-Nitrophenyl, 3-Nitrophenyl, 4-Nitrophenyl, 4-Chlorophenyl, 3-fluorophenyl, 4-fluorophenyl, 4-Methyl-2-nitrophenyl, 2-Methyl-5-nitrophenyl, 2-Nitro-4-(trifluoromethyl)phenyl, 4-Methoxy-2-nitrophenyl, 2-Methyl-5-nitrophenyl, 4-Methyl-2-nitrophenyl, 4-Methylphenyl, 2-Aminophenyl, 2-Amino-4-methyl phenyl, Thiophen-2-yl, 5-Chlorothiophen-2-yl, 5,4-dichlorothiophen-2-yl,5-Bromothiophen-2-yl, 5-chloro-4-bromothiophen-2-yl.

In one subset of non-limiting embodiments, R^{10} of Formula I is a substituted or unsubstituted phenyl, where , if substituted, one or more of the following substituents are present: C_1 - C_4 alkyl, C_1 - C_4 alkoxy, halogen, for example chlorine, fluorine or bromine, halogen-substituted C_1 - C_4 alkyl, amino, or NO_2. In specific non-limiting embodiments of this subset, at least three of R^1- R^5 are H and the remaining R group(s) is C_1 - C_4 alkyl

In one subset of non-limiting embodiments, R^{10} of Formula I is a substituted or unsubstituted thiophenyl, where , if substituted, one or more of the following substituents are present: halogen, for example chlorine or bromine, C_1 - C_4 alkyl, or NO_2. In specific non-limiting embodiments of this subset, at least three of R^1- R^5 are H and the remaining R group(s) is C_1 - C_4 alkyl

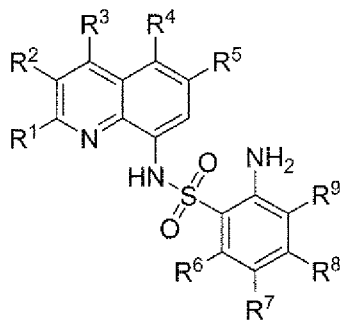
In one subset of non-limiting embodiments, R^{10} of Formula I is a substituted or unsubstituted furan, where , if substituted, one or more of the following substituents are present: halogen, for example chlorine or bromine, C_1 - C_4 alkyl, or NO_2. In specific non-limiting embodiments of this subset, at least three of R^1- R^5 are H and the remaining R group(s) is C_1 - C_4 alkyl

In other non-limiting embodiments, the present invention relates to a compound of Formula Ia:



where R^1, R^2, R^3, R^4 and R^5 are independently selected for each occurrence from the group consisting of hydrogen, branched or unbranched alkyl (e.g., C_1 - C_4 alkyl, for example methyl), alkoxy (e.g., C_1 - C_4 alkoxy, for example methoxy), halogen (e.g. Cl, F, or Br), alkyl halide (e.g. CF_3), aryl, CN, alkoxy, aryloxy, NO_2, alkylthio, and arylthio. In specific non-limiting embodiments, R^4 and R^5 are hydrogen, and R^1, R^2, and R^3 are independently hydrogen, halogen, alkyl (e.g., C_1 - C_4 alkyl), aryl, CN, alkoxy, aryloxy, NO_2, alkylthio, and arylthio; and where R^{11}, R^{12} and R^{13} are independently selected for each occurrence from the group consisting of hydrogen, branched or unbranched alkyl (e.g., C_1 - C_4 alkyl, for example methyl), alkoxy (e.g., C_1 - C_4 alkoxy, for example methoxy), chlorine, bromine, fluorine, NH_2 and NO_2.

In other non-limiting embodiments, the present invention relates to a compound of Formula II:



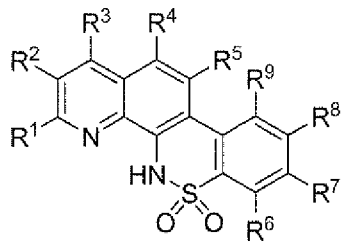
and to salts, esters and prodrugs of the compounds of Formula II. Additionally, the present invention describes methods of synthesizing and using compounds of Formula II. In Formula II:

R^1, R^2, R^3, R^4 and R^5 are independently selected for each occurrence from the group consisting of hydrogen, halogen, alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkoxy, aryloxy, alkylthiol, arylthiol, CN, and NO_2, and in non-limiting embodiments, R^1, R^2, R^3, R^4 and R^5 are independently selected for each occurrence from the group consisting of hydrogen, branched or unbranched alkyl (e.g., C_1 - C_4 alkyl, for example methyl), alkoxy (e.g., C_1 - C_4 alkoxy, for example methoxy), halogen (e.g. Cl, F, or Br), alkyl halide (e.g. CF_3), aryl, CN, alkoxy, aryloxy, NO_2, alkylthio, and arylthio. In specific non-limiting embodiments, R^4 and R^5 are hydrogen, and R^1, R^2, and R^3 are independently hydrogen, halogen, alkyl (e.g., C_1 - C_4 alkyl), aryl, CN, alkoxy, aryloxy, NO_2, alkylthio, and arylthio.

R^6, R^7, R^8 and R^9 are independently selected for each occurrence from the group consisting of hydrogen, halogen, substituted or unsubstituted alkyl, substituted or unsubsituted cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted alkoxy, substituted or unsubstituted aryloxy, substituted or unsubstituted alkylthiol, substituted or unsubstituted arylthiol, CN, NH_2 and NO_2. In a specific non-limiting embodiment, R^4 and R^5 are hydrogen, and R^1, R^2, and R^3 are independently hydrogen, halogen, alkyl, aryl, CN, alkoxy, aryloxy, NO_2, alkylthio, and arylthio.

In a non-limiting embodiment, R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8 and R^9 are independently selected for each occurrence from the group consisting of hydrogen, methyl, Cl, OCH_3, CF_3 and F.

In further non-limiting embodiments, the present invention relates to a compound of Formula III:



and to salts, esters and prodrugs of the compounds of Formula III. Additionally, the present invention describes methods of synthesizing and using compounds of Formula III. In Formula III:

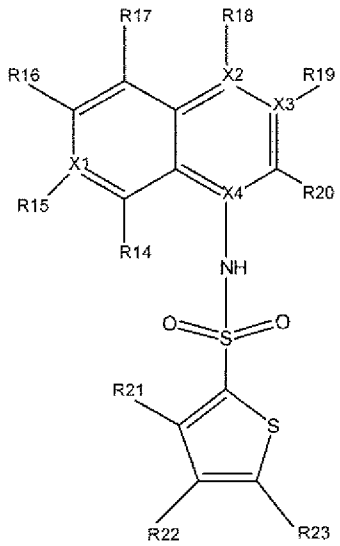
R^1, R^2, R^3, R^4 and R^5 are independently selected for each occurrence from the group consisting of hydrogen, halogen, alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkoxy, aryloxy, alkylthiol, arylthiol, CN, and NO_2, and in non-limiting embodiments, R^1, R^2, R^3, R^4 and R^5 are independently selected for each occurrence from the group consisting of hydrogen, branched or unbranched alkyl (e.g., C_1 - C_4 alkyl, for example methyl), alkoxy (e.g., C_1 - C_4 alkoxy, for example methoxy), halogen (e.g. Cl, F, or Br), alkyl halide (e.g. CF_3), aryl, CN, alkoxy, aryloxy, NO_2, alkylthio, and arylthio. In specific non-limiting embodiments, R^4 and R^5 are hydrogen, and R^1, R^2, and R^3 are independently hydrogen, halogen, alkyl (e.g., C_1 - C_4 alkyl), aryl, CN, alkoxy, aryloxy, NO_2, alkylthio, and arylthio.

R^6, R^7, R^8 and R^9 are independently selected for each occurrence from the group consisting of hydrogen, halogen, substituted or unsubstituted alkyl, substituted or unsubsituted cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted alkoxy, substituted or unsubstituted aryloxy, substituted or unsubstituted alkylthiol, substituted or unsubstituted arylthiol, CN, NH_2 and NO_2. In a specific non-limiting embodiment, R^4 and R^5 are hydrogen, and R^1, R^2, and R^3 are independently hydrogen, halogen, alkyl, aryl, CN, alkoxy, aryloxy, NO_2, alkylthio, and arylthio.

In a non-limiting embodiment, R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8 and R^9 are independently selected for each occurrence from the group consisting of hydrogen, methyl, Cl, OCH_3, CF_3 and F.

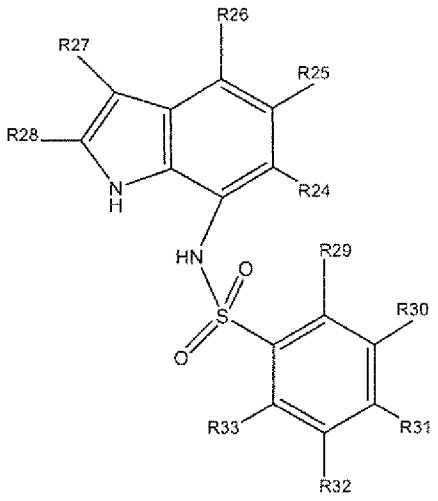
The present invention further contemplates the use of compounds that are structurally related to, but fall outside of, the abovelisted formulas. Examples of such related compounds include compounds of Formulas IV and V, as described below.

In certain non-limiting embodiments, the present invention relates to a compound of Formula IV:



and to salts, esters and prodrugs of the compounds of Formula IV, where X_1, X_2, X_3, and X_4 may independently be C or N, where R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, and R^{20} are independently selected for each occurrence from the group consisting of hydrogen, branched or unbranched alkyl (e.g., C_1 - C_4 alkyl, for example methyl), alkoxy (e.g., C_1 - C_4 alkoxy, for example methoxy), halogen (e.g. Cl, F, or Br), and alkyl halide (e.g. CF_3), and where R^{21}, R^{22}, and R^{23} are independently selected for each occurrence from the group consisting of hydrogen, branched or unbranched alkyl (e.g., C_1 - C_4 alkyl, for example methyl), chlorine, bromine, fluorine, NH_2 and NO_2.

In certain non-limiting embodiments, the present invention relates to a compound of Formula V:



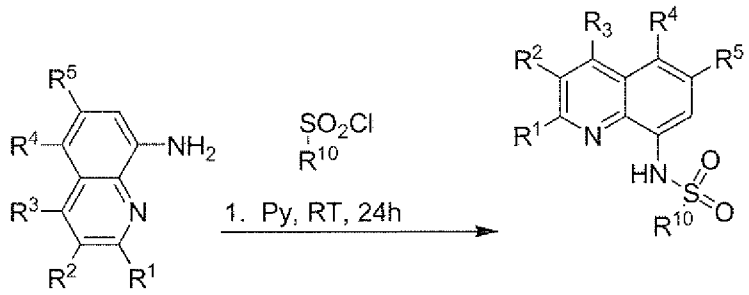
and to salts, esters and prodrugs of the compounds of Formula V, where R^{24}, R^{25}, R^{26}, R^{27}, and R^{28} are independently selected for each occurrence from the group consisting of hydrogen, branched or unbranched alkyl (e.g., C_1 - C_4 alkyl, for example methyl), alkoxy (e.g., C_1 - C_4 alkoxy, for example methoxy), halogen (e.g. Cl, F, or Br), or alkyl halide (e.g. CF_3), R^{24}, R^{25}, R^{26}, R^{27}, and R^{28} are independently selected for each occurrence from the group consisting of hydrogen, branched or unbranched alkyl (e.g., C_1 - C_4 alkyl, for example methyl), alkoxy (e.g., C_1 - C_4 alkoxy, for example methoxy), halogen (e.g. Cl, F, or Br), and alkyl halide (e.g. CF_3),. and where R^{29}, R^{30}, R^{31}, R^{32}, and R^{33} are independently selected for each occurrence from the group consisting of hydrogen, branched or unbranched alkyl (e.g., C_1 - C_4 alkyl, for example methyl), alkoxy (e.g., C_1 - C_4 alkoxy, for example methoxy), halogen (e.g. Cl, F, or Br), alkyl halide (e.g. CF_3), NH_2 and NO_2.

In specific, non-limiting embodiments, compounds that may be used according to the invention include compounds O-1, O-2, O-3, O-4, and O-5 (FIGURE 1A-E) and compounds 1-90 (FIGURE 8A-J; compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, and 90).

In particular non-limiting embodiments, the compound is selected from the group consisting of compounds 19, 47, 55, 69, 74 and 75.

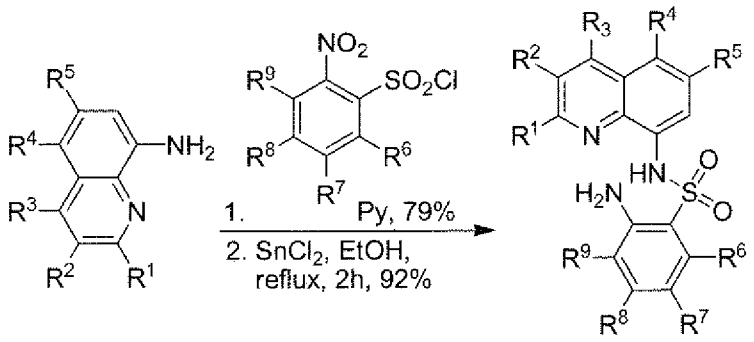
5.2 SYNTHETIC SCHEMES FOR NQBS AND RELATED COMPOUNDS

In one non-limiting embodiment, compounds of Formula I may be synthesized according to the following scheme:



wherein R^1, R^2, R^3, R^4, R^5 and R^{10} are defined as above for Formula I.

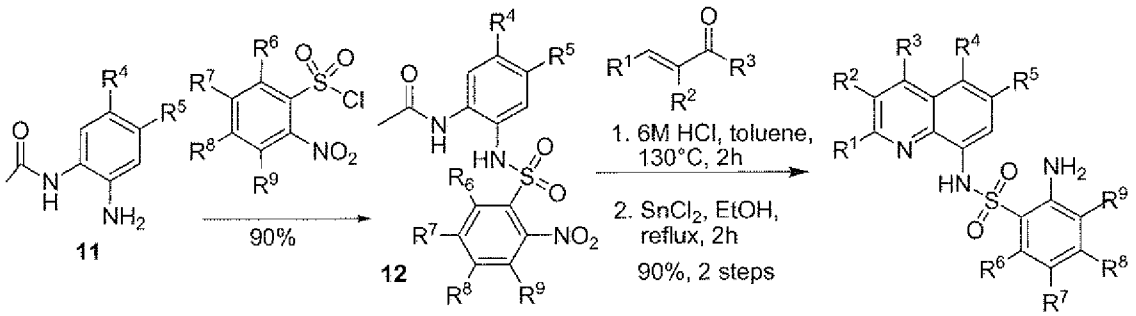
In another non-limiting embodiment, compounds of Formula II may be synthesized according to the following scheme:



wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8 and R^9 are defined as above for Formula II.

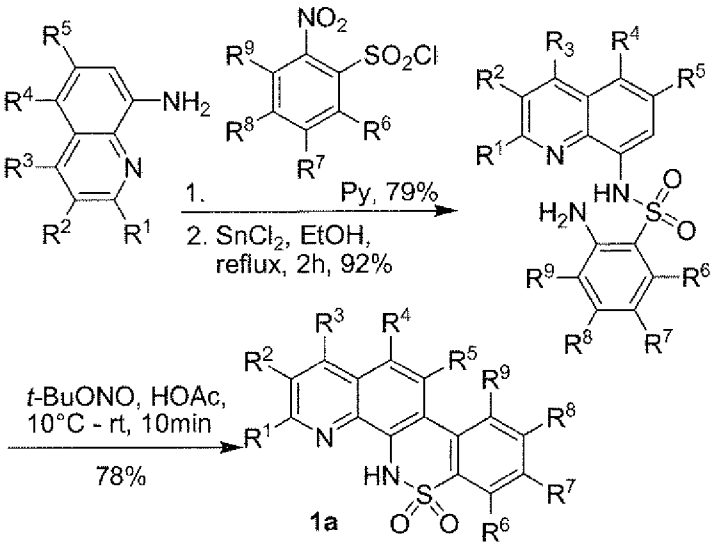
In other non-limiting embodiments, compounds of Formula II may be synthesized by any means known in the art.

In other non-limiting embodiments, the compounds of Formula II may be synthesized according to the following scheme:



wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8 and R^9 are defined as above

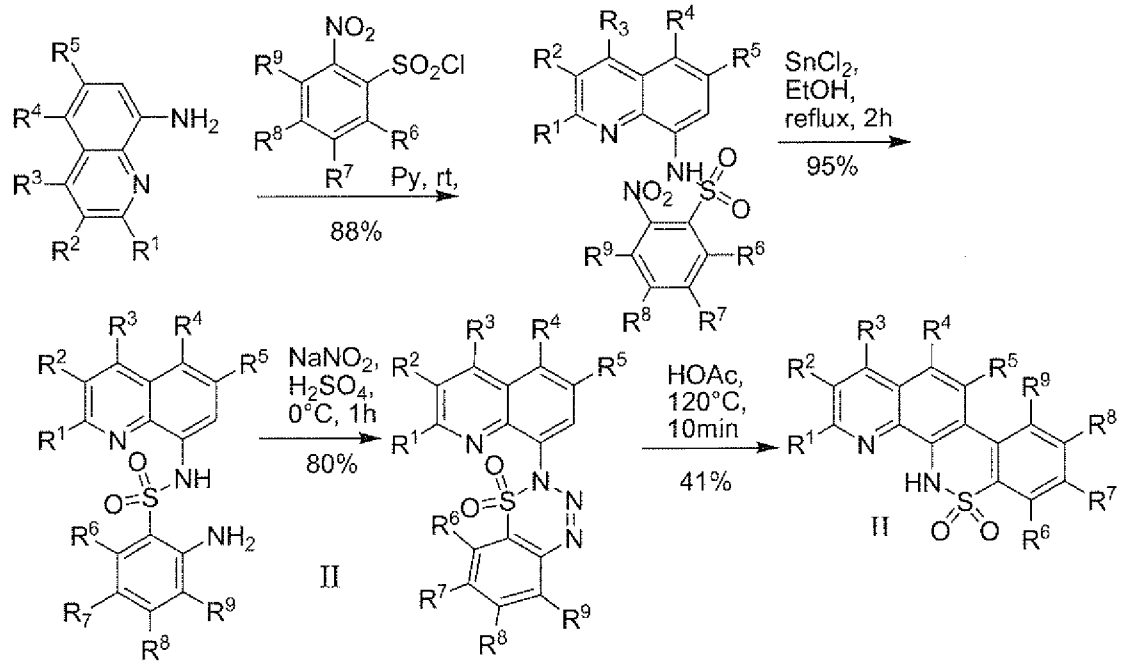
In non-limiting embodiments, compounds of Formula III may be synthesized according to the following scheme:



wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8 and R^9 are defined as above for Formula III.

In other non-limiting embodiments, compounds of Formula III may be synthesized by any means known in the art.

In other non-limiting embodiments, the compounds of Formula III may be synthesized according to the following scheme:



wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8 and R^9 are defined as above for Formula III.

Methods analogous to those set forth above may be used to synthesize compounds of formulas Ia, IV and V.

5.3 METHODS OF TREATMENT USING NQBS AND RELATED COMPOUNDS

In accordance with the invention, there are provided methods of using the compounds of Formulas I-V and compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, and 90. The compounds of the instant disclosure can inhibit NFkB activity to exert beneficial effects. A compound of Formula I, Ia, II, III, IV or V or any one or more of compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90, and in particular one or more of compounds 19, 47, 55, 69, 74 and/or 75,that inhibits NFκB activity may be used, in an effective amount, for the treatment of conditions including, but not limited to, cancer (for example, but not limited to, leukemia, lymphoma, glioblastoma, melanoma, squamous cell carcinoma, breast cancer, lung cancer, gastric cancer, liver cancer, renal cancer, pancreatic cancer, colon cancer, ovarian cancer, uterine cancer, cervical cancer, bladder cancer, prostate cancer, and testicular cancer), and inflammatory conditions including, but not limited to, type I hypersensitivity, atopy, anaphylaxis, asthma, osteoarthritis, rheumatoid arthritis, septic arthritis, gout, juvenile idiopathic arthritis, Still's disease, ankylosing spondylitis, inflammatory bowel disease (Ulcerative colitis and Crohn's disease) or inflammation associated with vertebral disc herniation. In addition, the present invention is directed to the treatment of diseases related to dysfunction of cell proliferation, the immune system and/or inflammation.

5.3.1 Treatment Of Disease Related to Dysfunction of Cell Proliferation, the Immune System and/or Inflammation

In non-limiting embodiments, the present invention provides for methods of treating diseases related to dysfunction of cell proliferation, the immune system and/or inflammation in a subject in need of such treatment by administration of a therapeutic formulation which comprises at least one compound of Formulas I, Ia, II, III, Iv or V, or any one or more of compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42,

43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90 and in particular one or more of compounds 19, 47, 55, 69, 74 and/or 75.

In particular embodiments, the formulation may be administered to a subject in need of such treatment in an amount effective to inhibit NFkB activity. Where the formulation is to be administered to a subject *in vivo*, the formulation may be administered systemically (*e.g.* by intravenous injection, oral administration, inhalation, subcutaneous, intramuscular, etc.), intraventricularly, intrathecally, or by any other means known in the art. The amount of the formulation to be administered may be determined using methods known in the art, for example, by performing dose response studies in one or more model system, followed by approved clinical testing in humans.

In one embodiment, the subject or patient has been diagnosed with, or has been identified as having an increased risk of developing a disease associated with dysfunction of cell proliferation, the immune system and/or inflammation.

In other non-limiting embodiments, the present invention provides for methods of reducing in a subject, the risk of inflammatory damage comprising administering to the subject, an effective amount of a composition according to the invention. An effective amount may be a local concentration or, in a pharmaceutical composition, an amount that, when administered to a subject, results in a therapeutic benefit.

According to the invention, an effective amount is an amount of at least one compound of Formulas I, Ia, II, III, IV or V, or any one or more of compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90, and in particular one or more of compounds 19, 47, 55, 69, 74 and/or 75, which reduces one or more clinical symptom of one or more of the aforementioned diseases. Working examples exemplifying experiments for assessing the efficacy of compounds of the invention are set forth below in section 6. For example, the ability of a compound to inhibit proliferation of a cell manifesting a dysfunction in cell proliferation may be used as an indication of effectiveness in vivo. As another example, the ability of a compound to reduce translocation of NF-κB into the nucleus may be used as an indicator.

In a non-limiting embodiment, the effective amount of at least one compound of Formulas I, Ia, II, III, IV or V, or any one or more of compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90, and in particular one or more of compounds 19, 47, 55, 69, 74 and/or 75, may be determined, for example, via an *in vitro* assay wherein the effective amount of a compound of Formulas I, Ia, II, III, IV or V, or any one or more of compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90, may be correlated with the compound's ability to reduce the nuclear translocation of NFκB. By way of example, and not of limitation, such an assay may comprise a cell-based assay that utilizes an agent, for example, a cytokine such as TNFα, to stimulate nuclear translocation of endogenous NFkB. Stimulation by the agent may result in proteasome degradation of IkBα and subsequent translocation of NFkB from the cytoplasm to the nucleus, while in the absence of such a stimulatory agent, NFkB is sequestered in the cytoplasm due to its binding to IkBα.

In the nuclear-translocation *in vitro* assay, nuclear translocation of NFkB, for example, the endogenous p65 RelA subunit, may be detected and/or measured following stimulation with the agent through the use of, for example, but not limited to, fluorescent antibody detection and an automated imaging platform. Compounds of the invention may be contacted with cells of the *in vitro* assay, wherein a reduction in NFkB nuclear transport compared to a cell not contacted with the compound is indicative of the compound's ability to inhibit NFκB activity. According to the invention, a reduction in nuclear translocation of NFκB may be correlative with the compound's therapeutic efficacy.

In one embodiment, an effective amount of a compound of Formulas I, Ia, II, III, IV or V, or any one or more of compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90, and in particular one or more of compounds 19, 47, 55, 69, 74

and/or 75, may be correlated with the compound's ability to inhibit NFκB induced gene activation , wherein a greater level of inhibition at a lower concentration when compared to a control level of inhibition, for example, as exhibited by a known NFκB nuclear-translocation inhibitor, such as BAY 11-7082, is indicative of greater therapeutic efficacy of the compound.

In one embodiment, an effective amount of a compound of Formulas I, Ia, II, III, IV, or V, or any one or more of compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90, and in particular one or more of compounds 19, 47, 55, 69, 74 and/or 75, may be that amount which inhibits NFκB induced gene activation with an efficacy of at least about 10-20%, at least about 20-50%, at least about 50-80%, or at least about 80-100% or more when compared to the NFκB induced gene activation inhibition achieved by a known inhibitor, such as BAY 11-7082.

In one non-limiting embodiment, an effective amount of a compound of Formulas I, Ia, II, III, IV, or V, or any one or more of compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90, and in particular one or more of compounds 19, 47, 55, 69, 74 and/or 75, may be that amount which inhibits NFκB nuclear-translocation by at least 50% when the compound is administered at a concentration ranging from about 200 μM to about 0.01 μM, preferably from about 100 μM to about 0.01 μM, more preferably from about 50 μM to about 0.01 μM, and more preferably from about 10 μM to about 0.01 μM in the *in vitro* assay, wherein inhibition of NFκB nuclear-translocation at a lower concentration in the *in vitro* assay is correlative with the compound's therapeutic efficacy.

In another non-limiting embodiment, the effective amount of at least one compound of Formulas I, Ia, II, III, IV, or V, or any one or more of compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90, and in particular one or more of compounds 19, 47, 55, 69, 74 and/or 75, may be determined, for example, via an *in vitro*

assay wherein the effective amount of a compound of Formulas I, Ia, II, III, IV, or V, or any one or more of compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90, may be correlated with the compound's ability to reduce expression of an NFκB-dependent reporter construct, for example, a β-lactamase reporter (NFκB-*bla*). By way of example, and not of limitation, such an assay may comprise contacting a cell expressing the NFκB-dependent reporter construct, and monitoring the level of β-lactamase expression, wherein a decrease in expression compared to a cell not contacted with the compound indicates a reduction in NFκB activity. According to the invention, the reduction in expression of the NFκB-dependent reporter may be correlative with the compound's therapeutic efficacy.

In one non-limiting embodiment, an effective amount of a compound of Formulas I, II, III, IV or V, or any one or more of compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90, and in particular one or more of compounds 19, 47, 55, 69, 74 and/or 75, may be that amount which reduces expression of an NFκB-*bla* construct by at least 50% when the compound is administered at a concentration ranging from about 200 μM to about 0.01 μM, preferably from about 100 μM to about 0.01 μM, more preferably from about 50 μM to about 0.01 μM, and more preferably from about 10 μM to about 0.01 μM in the *in vitro* assay, wherein a reduction of NFκB-*bla* expression at a lower concentration in the *in vitro* assay is correlative with the compound's therapeutic efficacy.

In one non-limiting embodiment, an effective amount of a compound of Formulas I, II, III, IV, or V, or any one or more of compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90, and in particular one or more of compounds 19, 47, 55, 69, 74 and/or 75, may be an amount which achieves a local concentration at the therapeutic site of about 100 μM to about 0.01 μM, preferably from about 50 μM to about

0.01 μM, more preferably from about 20 μM to about 0.01 μM, and more preferably from about 10 μM to about 0.01 μM in the *in vitro* assay.

5.3.2 Administration of Treatments

According to the invention, the component or components of a pharmaceutical composition of the invention may be administered to a subject by means including but not limited to intravenous, intra-arterial, intramuscular, intradermal, transdermal, subcutaneous, oral, intraperitoneal, intraventricular, and/or intrathecal administration.

In particular non-limiting embodiments, the therapeutic compound can be delivered in a controlled or sustained release system. For example, a compound or composition may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used (see Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used (see Langer and Wise eds., 1974, Medical Applications of Controlled Release, CRC Press: Boca Raton, Fla; Smolen and Ball eds., 1984, Controlled Drug Bioavailability, Drug Product Design and Performance, Wiley, N.Y.; Ranger and Peppas, 1983, J. Macromol. Sci. Rev. Macromol. Chem., 23:61; Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol., 25:351; Howard et al., 9189, J.Neurosurg. 71:105). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, *i.e.*, the heart or a blood vessel, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in Medical Applications of Controlled Release, supra, Vol. 2, pp. 115-138). Other controlled release systems known in the art may also be used.

5.4 Pharmaceutical Compositions

The compounds and compositions of the invention may be formulated as pharmaceutical compositions by admixture with a pharmaceutically acceptable carrier or excipient.

For example, the pharmaceutical composition may comprise an effective amount of at least one compound of Formulas I, Ia, II, III, IV or V, or any one or more of compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48,

49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90, and in particular one or more of compounds 19, 47, 55, 69, 74 and/or 75, and a physiologically acceptable diluent or carrier. The pharmaceutical composition may further comprise a second drug, for example, but not by way of limitation, an anti-cancer drug, an anti-inflammatory drug, for example, but not limited to, a steroid compound and/or a non-steroidal anti-inflammatory drug.

The phrase "pharmaceutically acceptable" indicates that a substance is physiologically tolerable when administered to a subject. Preferably, but not by way of limitation, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, or, for solid dosage forms, may be standard tabletting excipients. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin, 18th Edition, or other editions.

In a specific embodiment, the therapeutic compound can be delivered in a vesicle, in particular a liposome (see Langer, 1990, Science 249:1527-1533; Treat et al., 1989, in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler eds., Liss: New York, pp. 353-365; Lopez-Berestein, ibid., pp. 317-327; see generally Lopez-Berestein, ibid.).

The present invention is not to be limited in scope by the specific embodiments described herein and the Examples that follow. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying Examples and Figures. Such modifications are intended to fall within the scope of the appended claims.

6. WORKING EXAMPLE

Using a novel cell based assay, a unique scaffold structure of NQBS compounds has been identified having either the C7 locked or C7 open configuration (FIGURE 1) that appears to selectively inhibit NF-κB mediated gene activation. In particular, a series of first, second and third generation compounds have been screened and found to exhibit the following properties.

Both C-7 closed and open configurations of compound were found to exhibit potent and reproducible concentration dependent cytotoxicity and induction of apoptosis in a panel of 12 highly drug resistant lymphoma cell lines (including B- and T-cell lymphomas), with low micromolar potency (FIGURES 2 and 3). The O-1 and O-4 (as well as O-2 and O-3) analogs were found to be highly potent in both GC and ABC DLBCL (O-5 was found to be inactive). While C7 open structures appeared to be more potent, there were minimal differences in the activities of these compounds. Cytotoxicity was not found to be significantly influenced by the duration of exposure. FIGURE 3 shows the results of flow cytometric analysis of DLBCL cell lines treated with compound O-1 or O-4 for 72 hours. Although both compounds were found to induce apoptosis in these cells, the C7 open structure (O-4) was found to induce more apoptosis across a diverse panel of DLBCL cells relative to the O-1, the closed structure.

Treatment of these cell lines with the NQBS analogs was observed to sequester the p50 subunit to the cytoplasm (FIGURE 4A-B), which has been confirmed using a 'gold standard' electrophoretic mobility shift assay ("EMSA"; FIGURE 5). The NQBS compounds tested were found not to affect other non-NF-κB dependent transcription factors involved in normal cell cycle regulation (eg: MCM3 and Ku80) (FIGURE 6). Further, open ring structure NQBS compounds were found not to exhibit significant toxicity in SCID beige mice (no weight loss and no toxic deaths) at doses as high as 10 mg/kg, well in excess of the in vitro IC50 (FIGURE 7).

The foregoing analysis was used to generate structure activity relationships (SAR) that have informed new rounds of synthesis, producing additional NQBS structures (FIGURES 8A-J). The results of *in vitro* cytotoxicitry screens of these compounds demonstrating their activity is shown in FIGURE 9.

A binding site has been predicted for NQBS within the RHD of both p65 and p50 (FIGURE 10), supporting the hypothesis regarding the potential novel

mechanism of action (MOA) for this class of compounds. Direct binding data using thermal shift assays was also obtained, further corroborating the MOA (FIGURE 11A-C).

Finally, using a transgenic mouse created to represent an in vivo model for aggressive lymphoma, it has been shown that one of the NQBS analogs (O-4) produces a complete remission of the lymphoma following daily dosing (FIGURE 12A-D).

Furthermore, activity of O-4 was shown in SCID beige mice with human DLBCL xenografts, where O-4 significantly inhibited growth of tumors compared to the control (Figure 13).

Various publications are cited herein, the contents of which are hereby incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A method for inhibiting the activity of a Nuclear factor-κ B (NFκB) in a cell which comprises contacting the cell with a compound selected from the group consisting of compounds 47, 55, 69, 74 and/or 75 in an amount effective to inhibit Nuclear factor-κ B (NFκB) activity.

2. The method of claim 1, wherein the inhibition of Nuclear factor-κ B (NFκB) activity reduces nuclear translocation of the Nuclear factor-κ B (NFκB).

3. The method of claim 1, wherein the cell is a mammalian cell.

4. The method of claim 1, wherein the cell is contacted *in vitro*.

5. A method for treating a disease related to dysfunction of cell proliferation, the immune system, and/or inflammation in an individual, which method comprises administering to the individual an effective amount of a compound selected from the group consisting of compounds 47, 55, 69, 74 and/or 75.

6. The method of claim 5, where the disease is cancer.

7. The method of claim 5, where the cancer is lymphoma.

8. A composition comprising compound 47, 55, 74 and/or 75 for use in a method for inhibiting the activity of a Nuclear factor-κ B (NFκB) in a cell.

9. The composition of claim 8 wherein the inhibition of Nuclear factor-κ B (NFκB) activity reduces nuclear translocation of the Nuclear factor-κ B (NFκB).

10. A composition comprising compound 47, 55, 74 and/or 75 for use in treating a disease related to dysfunction of cell proliferation, the immune system, and/or inflammation.

11. The composition of claim 10, where the disease is cancer.

12. The composition of claim 11, where the disease is lymphoma.

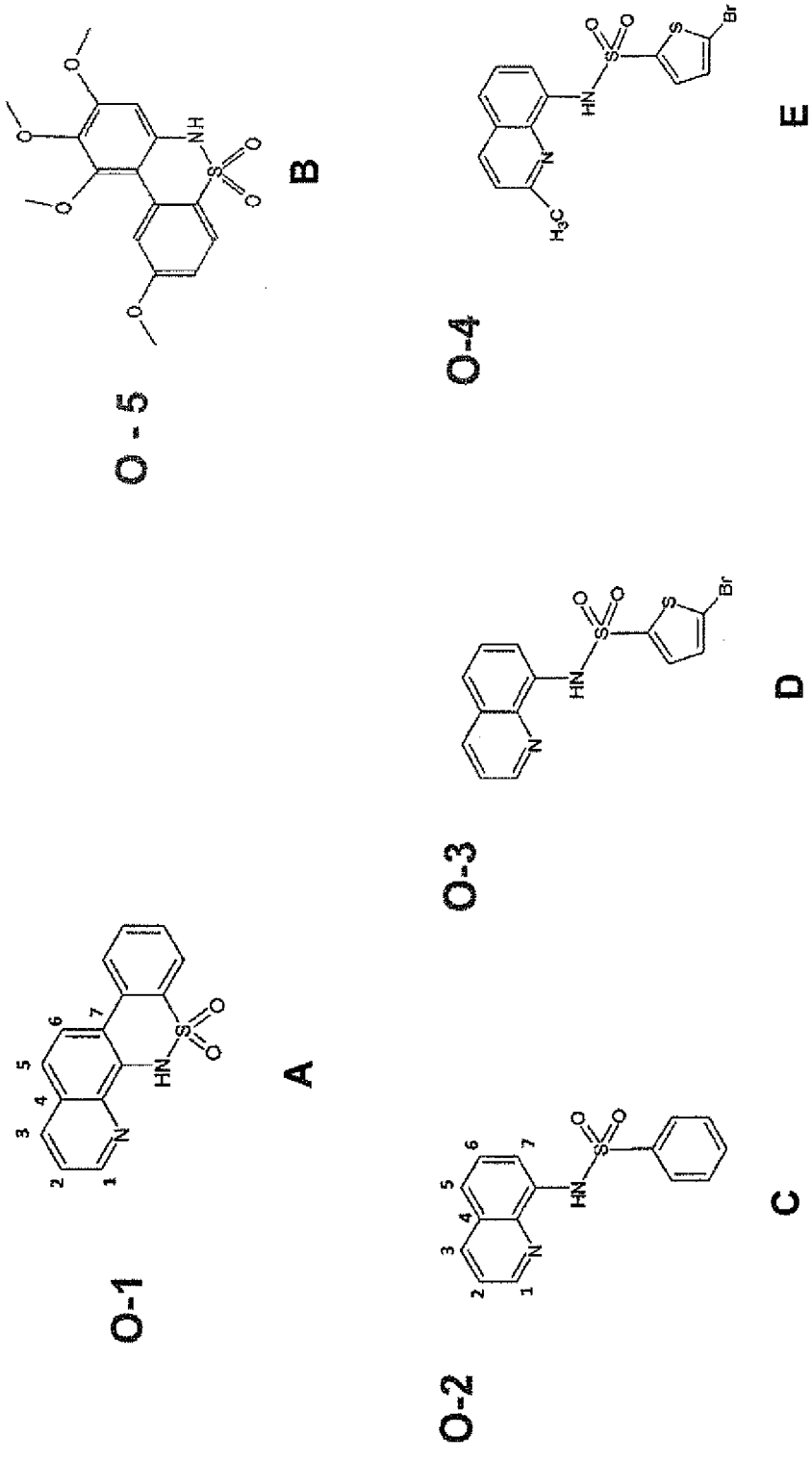


Figure 1

Growth Inhibition IC50 Values (μM) Calculated with Calcusyn Software Across DLBCL and HUVEC Lines for O-1 and O-4 - Luminescence Assays

Cell line	**O-1** (μM) (Closed Ring Structure)			**O-4** (μM) (Open Ring Structure)		
	24h	48h	72h	24h	48h	72h
Ly1 (GCB)	5.31	2.96	3.7	2.1	1.4	1.4
Ly7 (GCB)	3.29	2.02	1.67	4.01	3.44	3.3
Ly10 (ABC)	4.56	5.64	1.5	1.6	1.6	1.5
HBL-1 (ABC)	>10	6.2	5.7	3.09	2	3.42
RIVA (ABC)	8.29	4.29	2.88	6.95	4.1	2.72
SUDHL6 (GCB)	7.42	4.49	3.97	4.29	4.01	4.79
HUVEC	>10	>10	6.74	>10	>10	>10

Figure 2

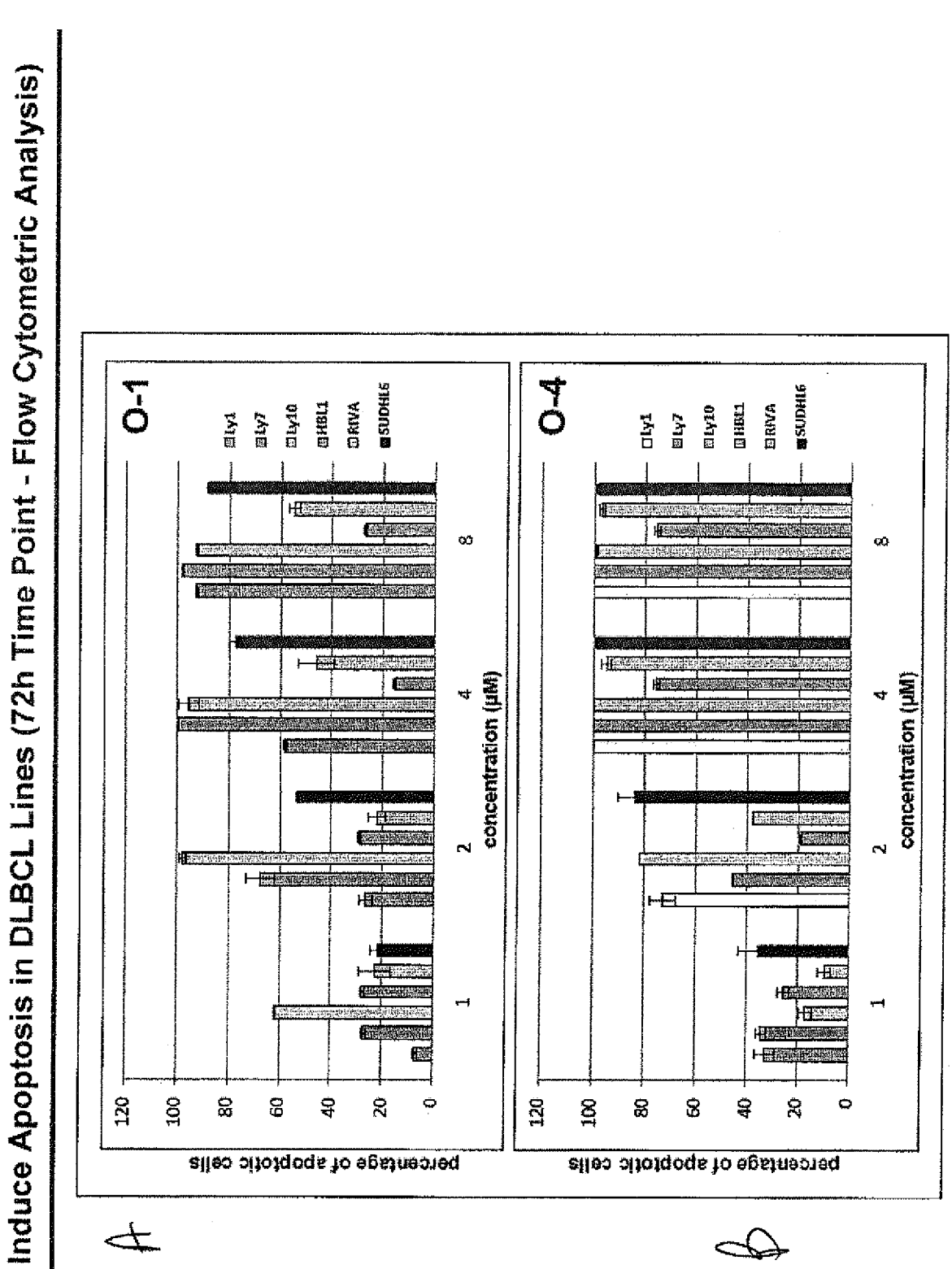
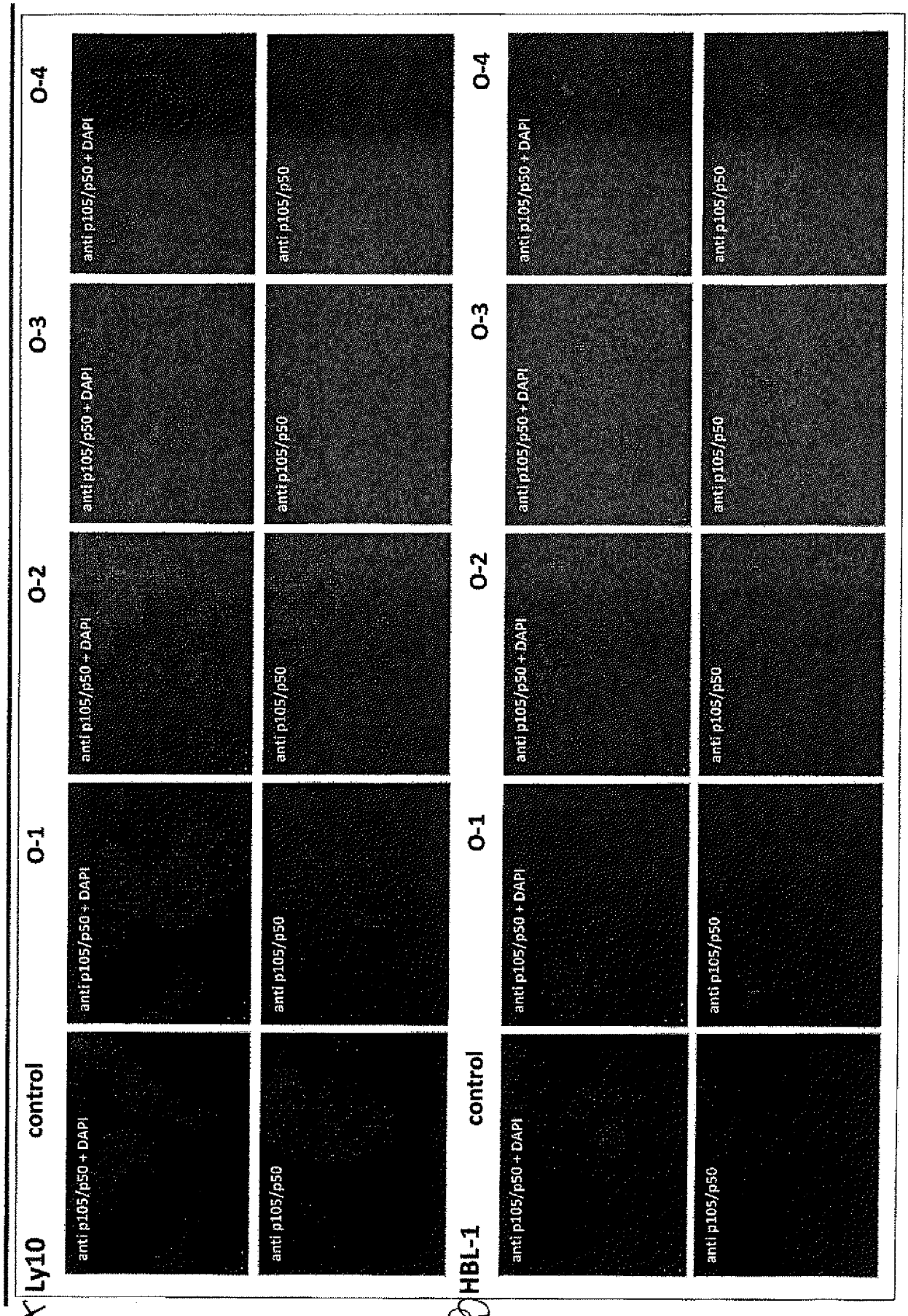
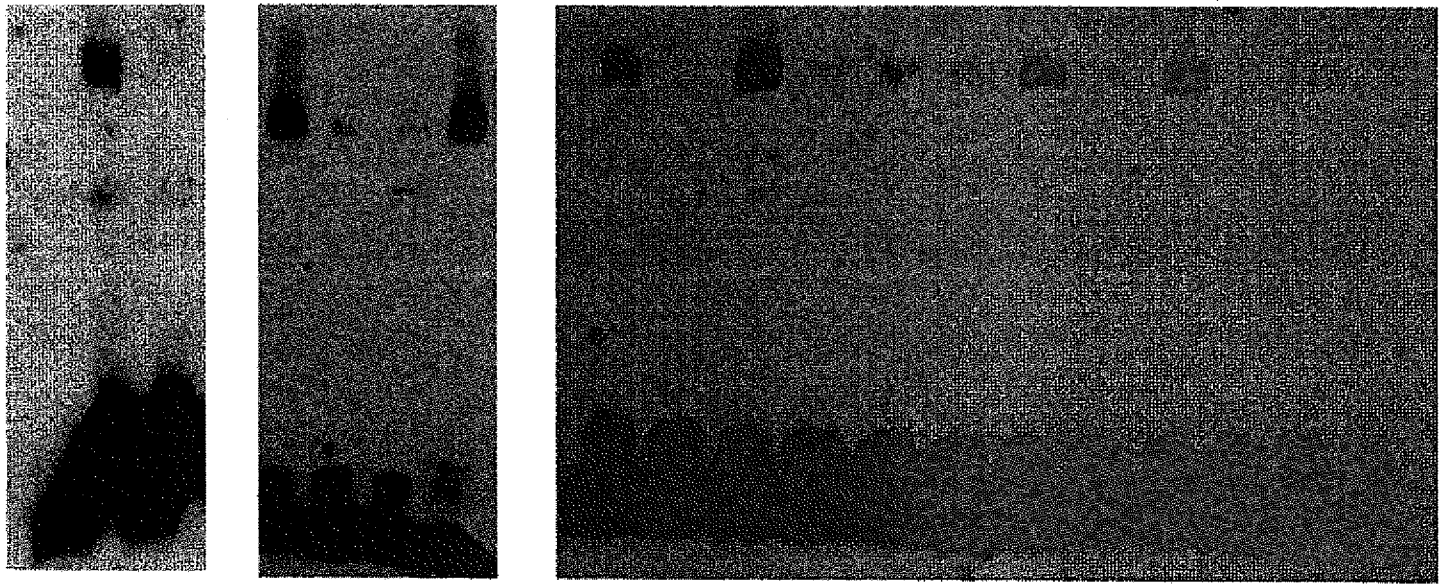

O-1 and O4 Induce Apoptosis in DLBCL Lines (72h Time Point - Flow Cytometric Analysis)
O-1
O-4
Ly1
Ly7
Ly10
HBL1
RIVA
SUDHL6
percentage of apoptotic cells
concentration (μM)
0
20
40
60
80
100
120
1
2
4
8
A
B

Figure 3

FIGURE 4A-B


Immunofluoresence Microscopy (p105/p50 NF-κB) in ABC DLBCL Lines (Ly10, HBL-1)
Demonstrates the NBQS Analogs Inhibit Nuclear Translocation
A
Ly10
control
O-1
O-2
O-3
O-4
anti p105/p50 + DAPI
anti p105/p50
B
HBL-1
control
O-1
O-2
O-3
O-4
anti p105/p50 + DAPI
anti p105/p50

Electrophoretic mobility shift assay (EMSA) for NF-κB in DLBCL Lines treated with NQBS



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Lanes:
1 – HBL-1 untreated control
2 – HBL-1 O-4 4μM for 3h
3 - Ly10 untreated control
4 - Ly10 comp. 55 4μM for 3h
5 - Ly10 comp. 55 2μM for 3h
6 – Ly10 O-5 4μM for 3h

Lanes:
7 - Ly10 untreated control
8 - Ly10 bortezomib 6 nM
9 - Ly10 comp 2 4μM for 3h
10 – Ly10 comp 6 4μM for 3h
11 - Ly10 comp 8 4μM for 3h
12 - Ly10 comp 12 4μM for 3h
13 - Ly10 comp 13 4μM for 3h

Lanes:
14 - Ly10 comp 14 4μM for 3h
15 - Ly10 comp 15 4μM for 3h
16 - Ly10 comp 16 4μM for 3h
17 – Ly10 comp 67 4μM for 3h
18 - Ly10 comp 68 4μM for 3h

Figure 5

NQBS Analogs Do Not Affect Other Non-NF-kB (p105/p50) Transcription Factors (Ku80)

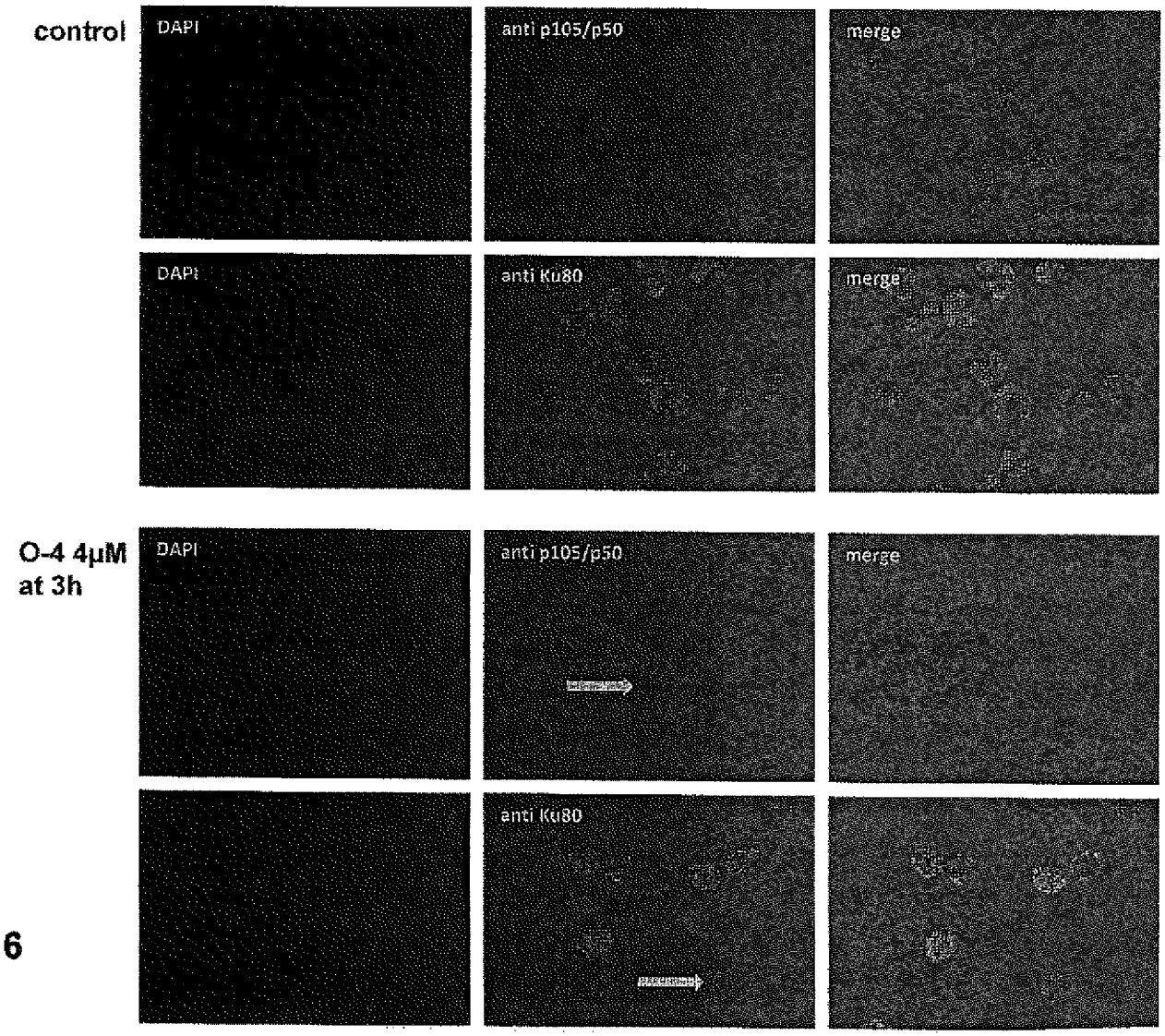


Figure 6

Open Ring Structure Analogs (O-3) Do Not Exhibit Significant Toxicity In SCID Beige Mouse Models

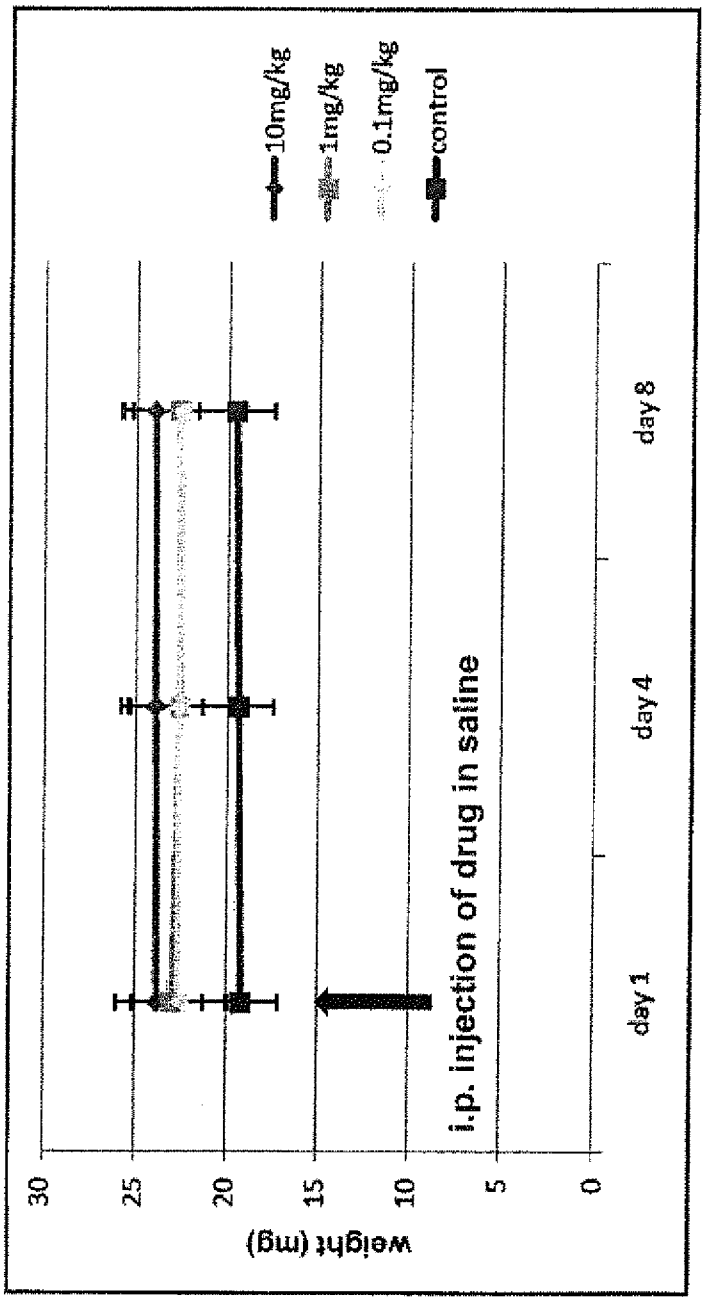


Figure 7

FIGURE 8A

NQBS analogs - summary

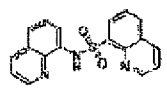
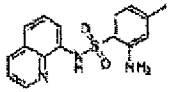
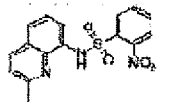
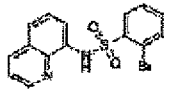
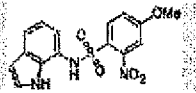
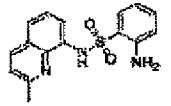
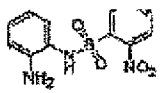
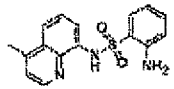
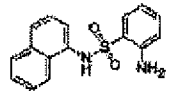
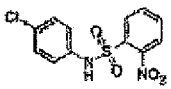
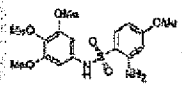
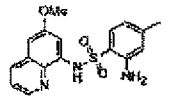
Compound No.	Chemical structure	IC50 (μM) Ly1/Ly10	Likely orally active (Lipinski)	ICM binding	EMSA	IF
1		10 - 20 / 10 - 20	yes			
2		2.4 / 2.3	yes	Site 2	-	
3		> 100 / > 100	yes			
4		10 - 20 / 10 - 20	yes			
5		10 - 20 / 10 - 20	yes	Site 3		
6		1.7 / 1.6	yes		+	
7		> 100 / > 100	yes	Site 1		
8		1.9 / 2.8	yes	Site 2	+	
9		10 - 20 / 10 - 20	yes	Site 1		
10		20 - 50 / 20-50	yes	Sites 2 and 3		
11		5.1 / 1.5	yes		+	
12		1.3 / 1.2	yes	Site 3	+	

FIGURE 8B

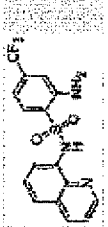
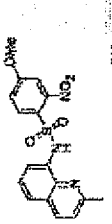
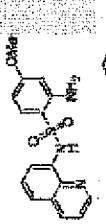
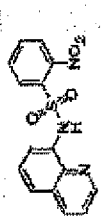
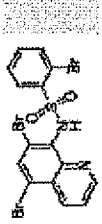
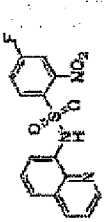
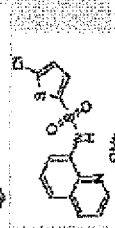
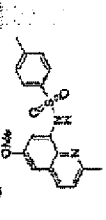
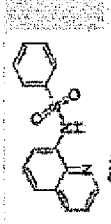
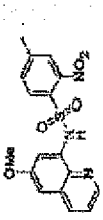
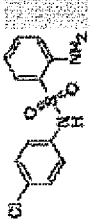
Compound No.	Chemical structure	IC50 (μM) Ly1/Ly10	Likely orally active (Lipinski)	ICM binding	EMSA	IF
13		1.5 / 1.4	yes		-	
14		2.4 / 2.1	yes	Site 2	+	
15		7.2 / 7.7	yes	Site 1	-	
16		1.9 / 1.5	yes	Site 3	+	
17		20 / 10 - 20	no			
18		1.7 / 1.6	yes	Site 2		
19		0.8 / 0.7	yes	Site 3	+	
20		20 - 50 / 20 - 50	yes	Site 2		
21		10 - 20 / 10 - 20	yes	No		
22		2.6 / 2.3	yes	Site 1		
23		20 / 10 - 20	yes	Site 2		

FIGURE 8C

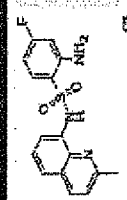
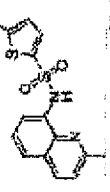
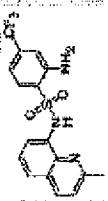
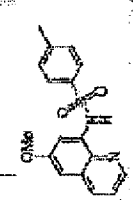
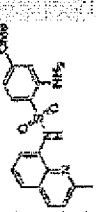
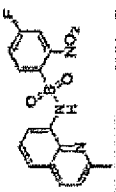
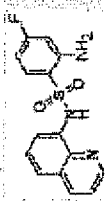
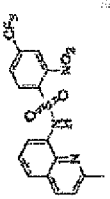
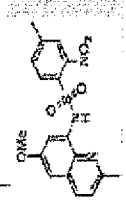
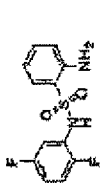
Compound No.	Chemical structure	IC50 (μM) Ly1/Ly10	Likely orally active (Lipinski)	ICM binding	EMSA	IF
24		1.3 / 1.2	yes	Site 3		
25		1.3 / 0.8	yes		+	
26		1.4 / 1.3	yes	Site 1		
27		1.6 / 1.6	yes		+/-	
28		1.4 / 0.8	yes	Site 3	-	
29		1.9 / 1.5	yes	Site 1	+	
30		1.4 / 1.5	yes	Site 1	-	
31		3.2 / 3.0	yes	Site 2	+/-	
32		10 - 20 / 10 - 20	yes	Site 2		
33		20 / 10 - 20	yes	Site 2		

FIGURE 8D

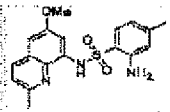
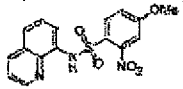
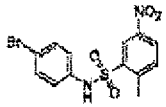
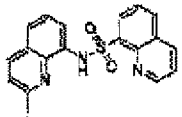
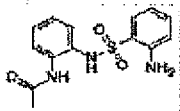
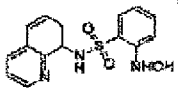
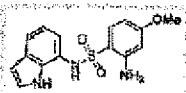
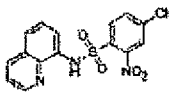
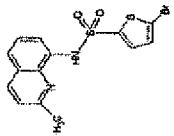
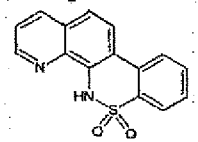
Compound No.	Chemical structure	IC50 (μM) Ly1/Ly10	Likely orally active (Lipinski)	ICM binding	EMSA	IF
34		1.2 / 1.0	yes	Site 1	+/-	
35		3.2 / 2.8	yes	Site 2	-	
36		10 - 20 / 10 - 20	yes	No		
37		20 - 50 / 20 - 50	yes	Site 1		
38		> 100 / > 100	yes	Site 3		
39		3.5 / 1.3	yes	Site 3	+	
40		5.0 / 1.2	yes	Site 1	+	
41		4.1 / 5.8	yes		-	
O-4 (42)		1.4 / 1.5	yes	Sites 1 and 3	+	+
O-1 (43)		3.7 / 1.5	yes	Site 1	+	+

FIGURE 8E

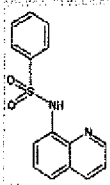
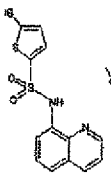
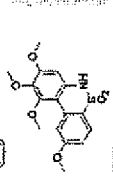
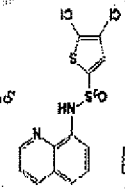
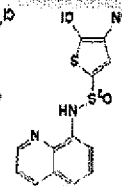
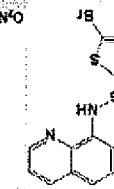
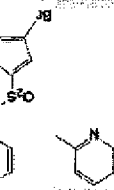
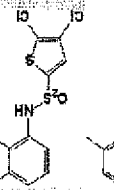
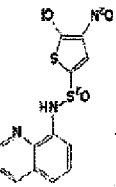
Compound No.	Chemical structure	IC50 (μM) Ly1/Ly10	Likely orally active (Lipinski)	ICM binding	EMSA	IF
O-2 (44)		1.7 / 1.7	yes	Site 1		+
O-3 (45)		1.4 / 1.4	yes	Site 1		+
O-5 (46)		> 100 / > 100	yes	No	-	-
47		0.7 / 0.7	yes			
48		10 - 20 / 10 - 20	yes			
49		1.4 / 1.5	yes			
50		10 - 20 / 10 - 20	yes			
51		10 - 20 / 10 - 20	yes			
52		10 - 20 / 10 - 20	yes			

FIGURE 8F

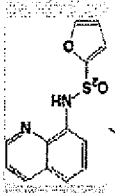
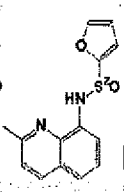
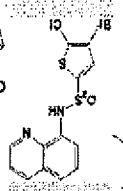
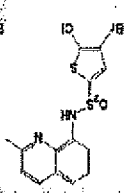
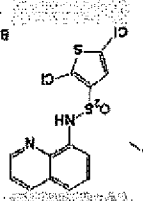
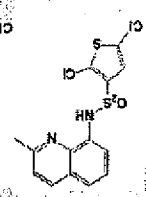
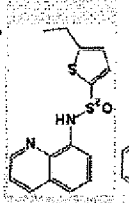
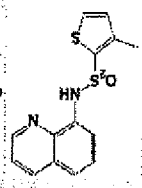
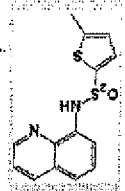
Compound No.	Chemical structure	IC50 (μM) Ly1/Ly10	Likely orally active (Lipinski)	ICM binding	EMSA	IF
53		20 - 50 / 20 - 50	yes			
54		6.3 / 5.4	yes			
55		0.5 / 0.5	yes		+	
56		10 - 20 / 10 - 20	yes			
57		1.7 / 3.1	yes		-	
58		1.4 / 2.6	yes			
59		1.4 / 2.6	yes		+	
60		1.3 / 2.6	yes		+	
61		1.1 / 2.4	yes		+	

FIGURE 8G

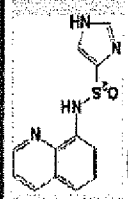
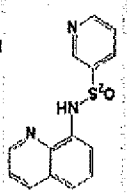
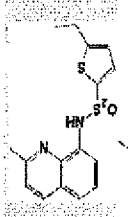
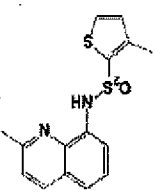
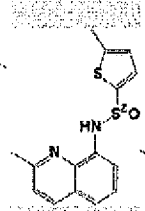
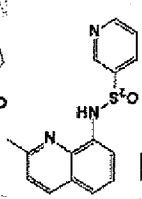
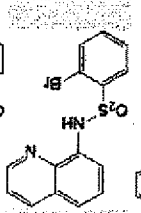
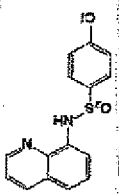
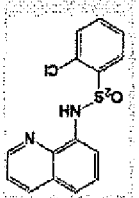
Compound No.	Chemical structure	IC50 (μM) Ly1/Ly10	Likely orally active (Lipinski)	ICM binding	EMSA	IF
62		20 - 50 / 20 - 50	yes			
63		10 - 20 / 10 - 20	yes			
64		1.6 / 3.2	yes		-	
65		10 - 20 / 10 - 20	yes			
66		0.6 / 1.7	yes		+	
67		1.3 / 1.8	yes		+	
68		1.5 / 1.2	yes		+	
69		0.7 / 0.7	yes		+	
70		1.1 / 1.2	yes		+	

FIGURE 8H

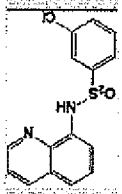
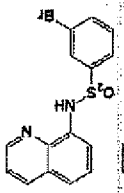
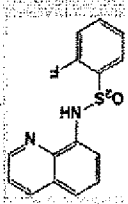
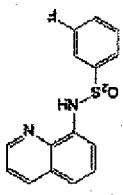
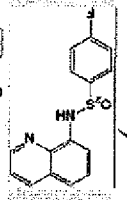
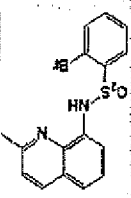
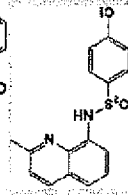
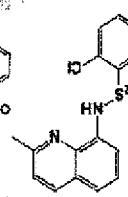
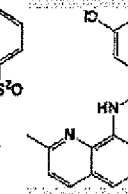
Compound No.	Chemical structure	IC50 (μM) Ly1/Ly10	Likely orally active (Lipinski)	ICM binding	EMSA	IF
71		1.2 / 1.2	yes		+	
72		1.9 / 1.5	yes		-	
73		0.9 / 1.0	yes		-	
74		0.7 / 0.7	yes		+/-	
75		0.7 / 0.7	yes		+	
76		3.7 / 3.9	yes		-	
77		1.5 / 1.2	yes		-	
78		3.8 / 3.0	yes		-	
79		1.7 / 1.6	yes			

FIGURE 6I

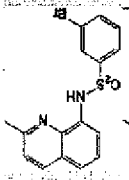
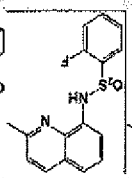
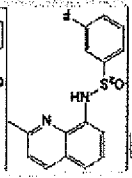
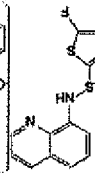
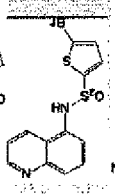
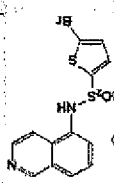
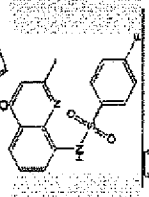
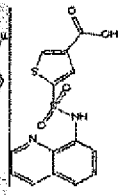
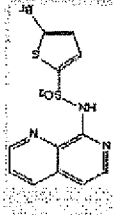
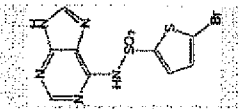
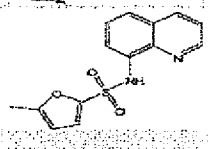
Compound No.	Chemical structure	IC50 (μM) Ly1/Ly10	Likely orally active (Lipinski)	ICM binding	EMSA	IF
80		1.2 / 1.0	yes			
81		1.8 / 1.9	yes			
82		1.5 / 1.0	yes			
83		1.5 / 1.1	yes			
84		> 100 / 20-50	yes			
85		50-100 / 20-50	yes			
86		2.1 / 2.3	yes			
87		> 100 / > 100	yes			
88		50 / 50-100	yes			

FIGURE 85

Compound No.	Chemical structure	IC50 (μM) Ly1/Ly10	Likely orally active (Lipinski)	ICM binding	EMSA	IF
89		> 100 / > 100	yes			
90		50-100 / 50-100	yes			
91						
92						
93						
94						
95						
96						
97						

O-4 and C19 Also Demonstrate Significant Cytotoxicity in Solid Tumors

Cell line	Origin	NF-κB constitutive activation	IC50 (μM) O-4 at 72h	IC50 (μM) C19 at 72h
DLD-1 myc +; myb + ; ras +; fos +; sis +; p53 +	Colorectal adenocarcinoma	yes	3.2	3.0
SW480 myc +; myb + ; ras +; fos +; sis +; p53 +	Colorectal adenocarcinoma	intermediate	4.1	4.0
LNCaP androgen sensitive	Prostate adenocarcinoma	no	3.9	1.6
PC-3 androgen insensitive	Prostate adenocarcinoma	yes	4.1	4.0
SKBR 3 HER2/c-erb-2 +	Breast adencarcinoma	no	9.9	>10
MDA231 Triple negative	Breast adencarcinoma	yes	>10	>10
FM29	Melanoma	unclear	7.4	6.3
M44	Melanoma	unclear	2.4	0.9
SK19	Melanoma	unclear	8.1	2.3

Figure 9

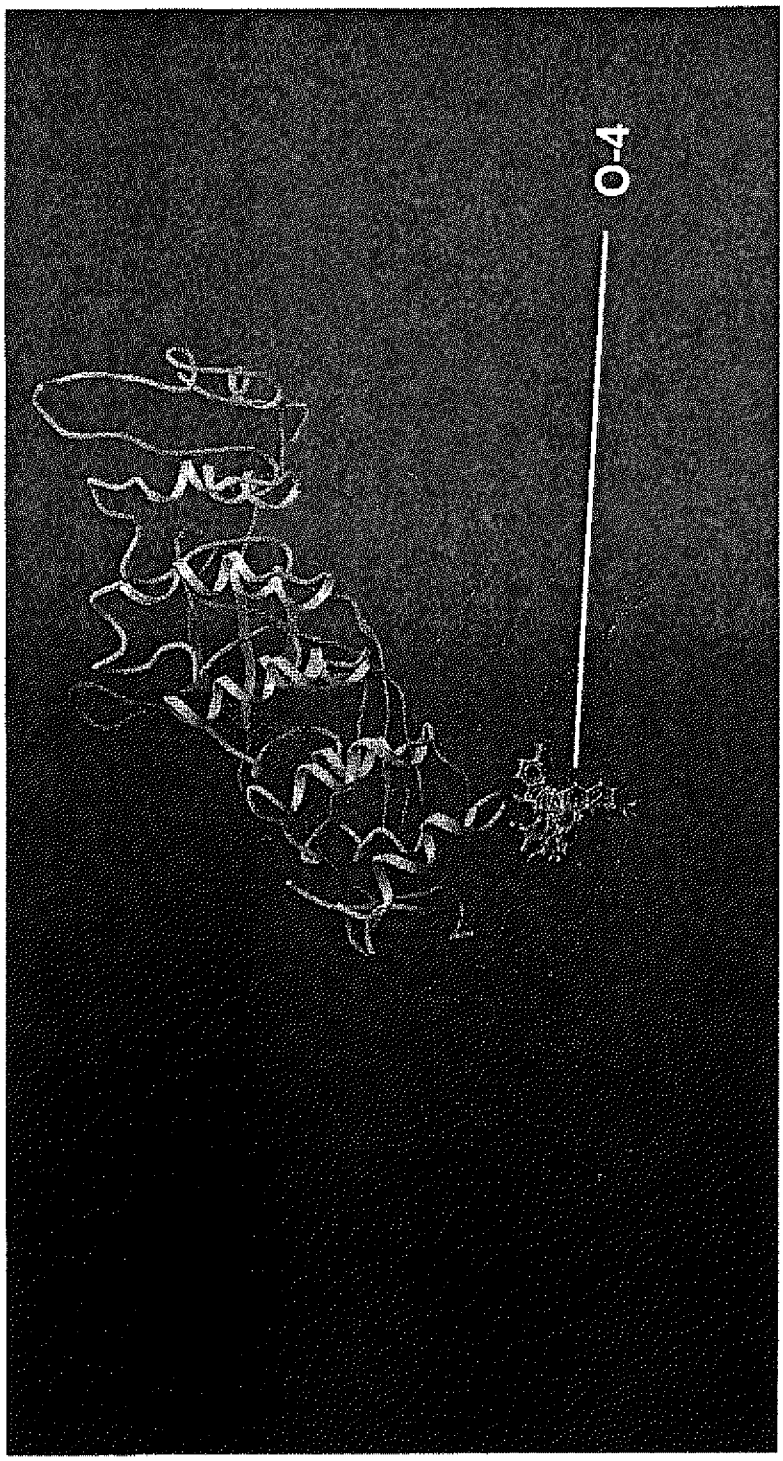

O-4
IκBα
p65
p50
Amino acid residues that interact with O-4:
53, 52, 51, 49, 30, 28, 244, 242, 241, 240,
239, 238, 237, 236, 221, 222, 223, 224,
225, 226, 273, 275, 276

Figure 10

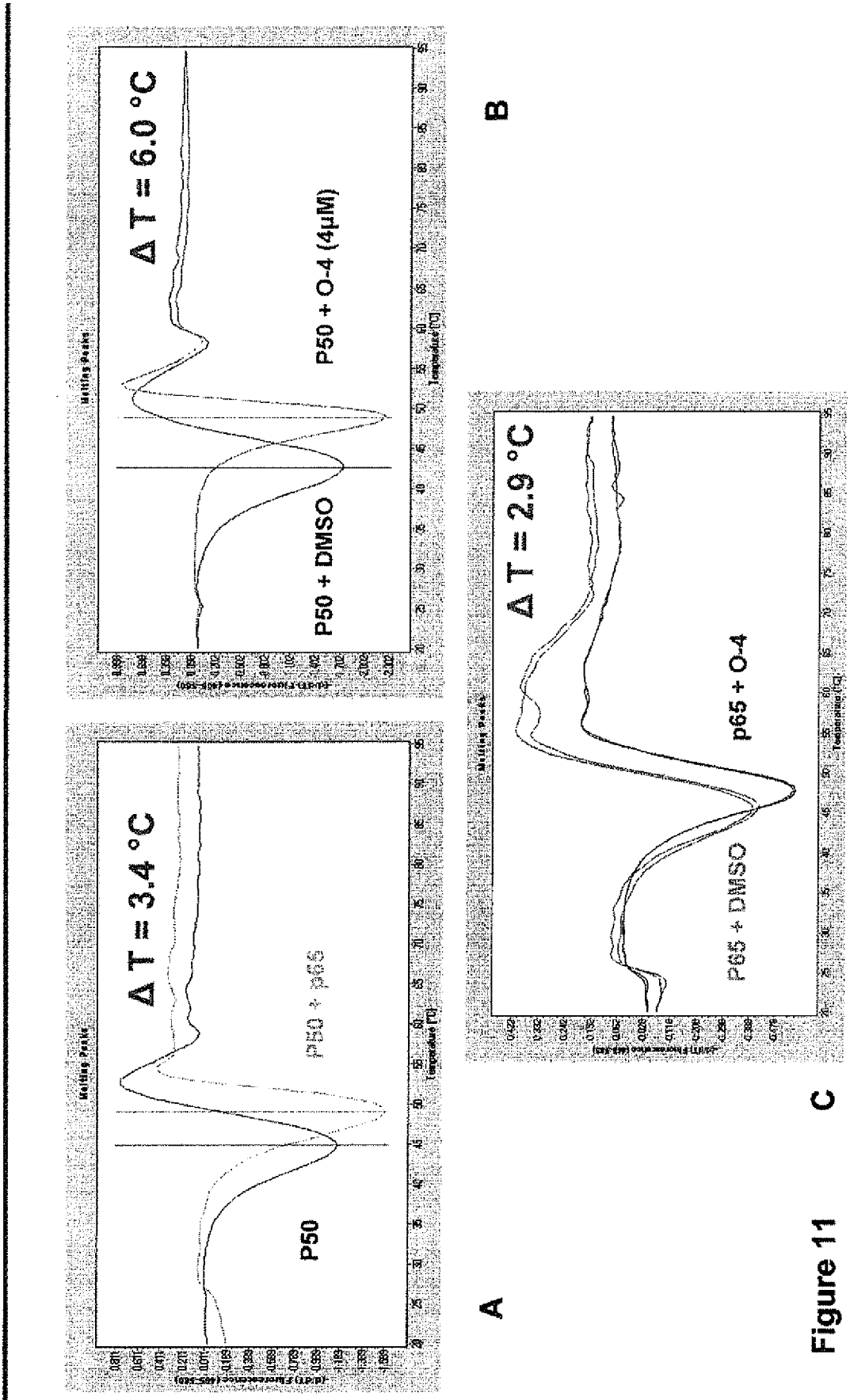

Thermal shift assay – p50+O-4 and p50+p65 – overnight incubation (4 °C)
ΔT = 3.4 °C
P50
P50 + p65
ΔT = 6.0 °C
P50 + DMSO
P50 + O-4 (4μM)
ΔT = 2.9 °C
P65 + DMSO
p65 + O-4
A
B
C

Figure 11

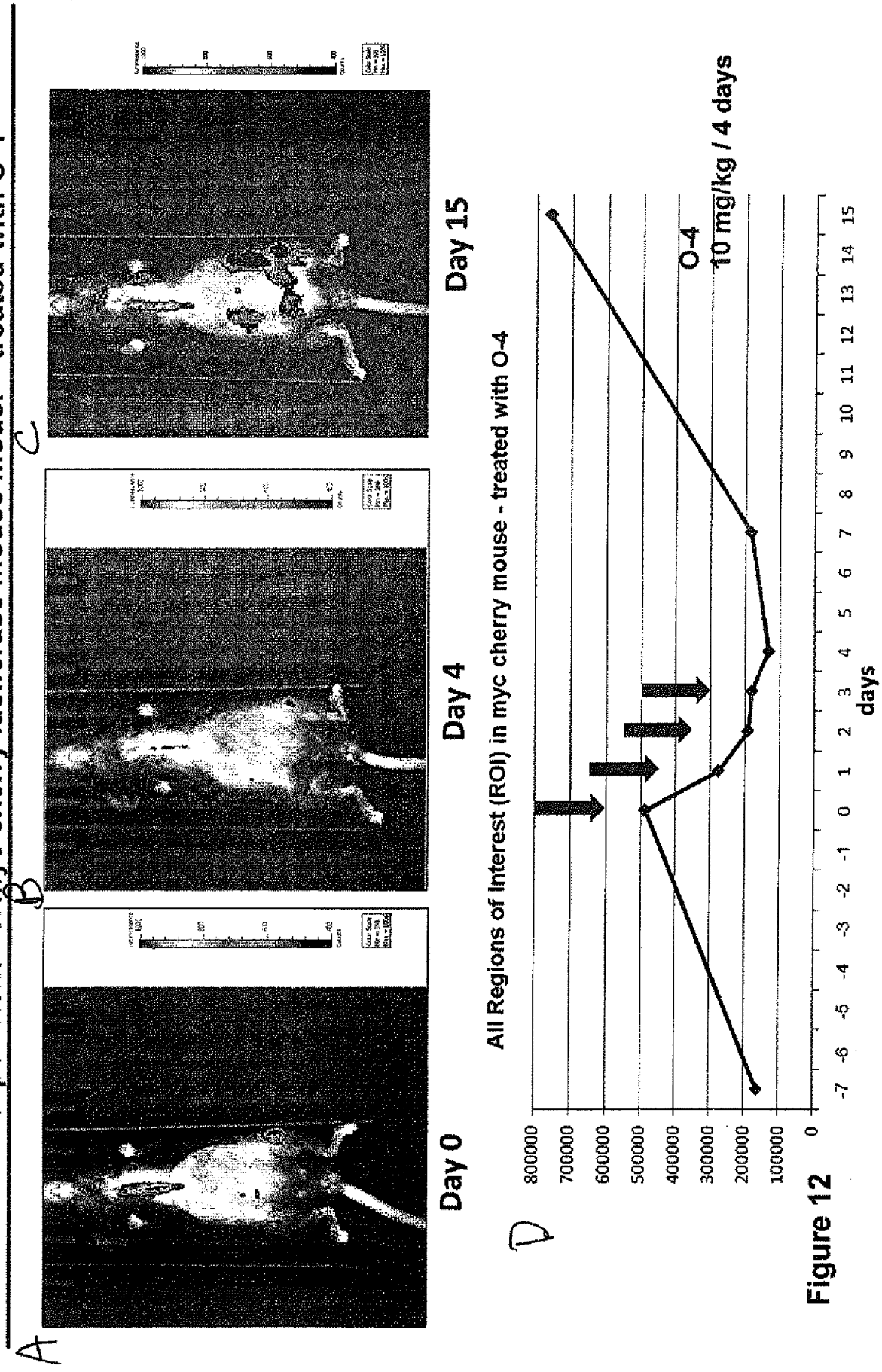
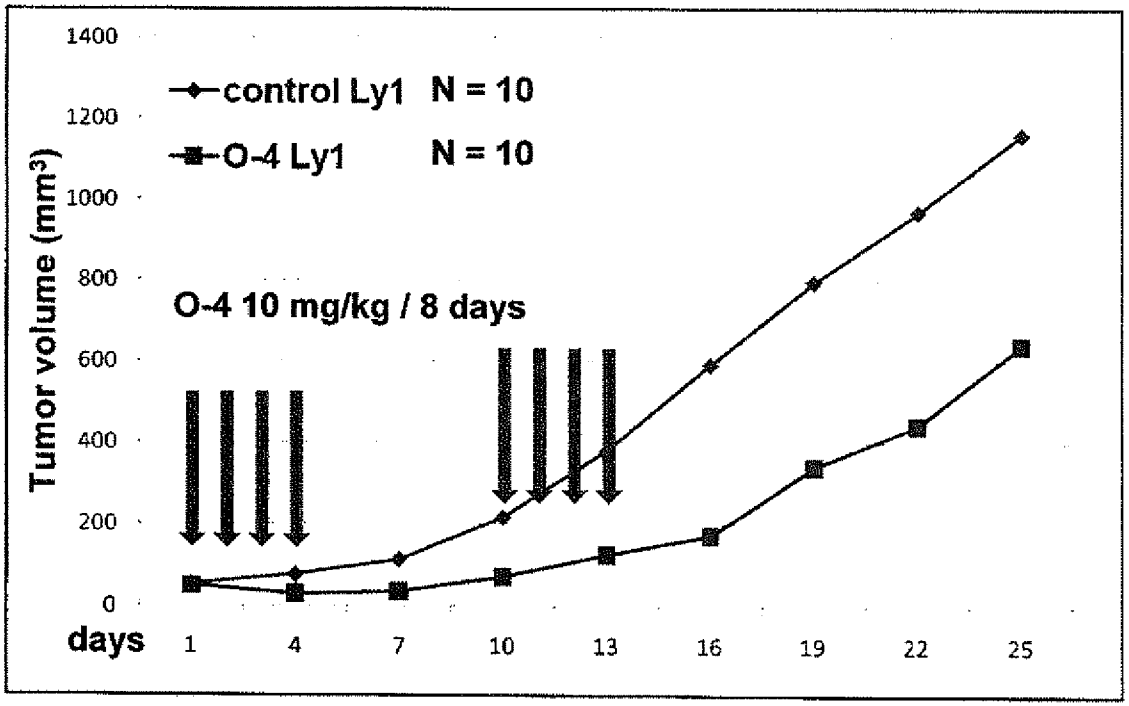

In vivo experiment – λ myc cherry luciferase mouse model – treated with O-4
A
B
C
D
Day 0
Day 4
Day 15
All Regions of Interest (ROI) in myc cherry mouse - treated with O-4
O-4
10 mg/kg / 4 days
days
800000
700000
600000
500000
400000
300000
200000
100000
0
-7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Figure 12

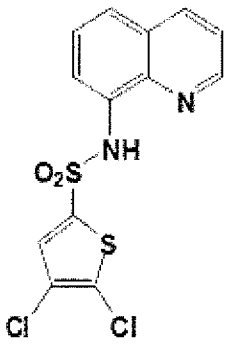
FIGURE 13

In vivo experiment – human DLBCL (Ly1 cell line) xenograft in SCID beige mice – treated with O-4

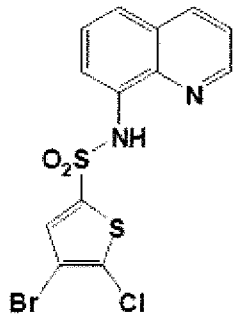


	T test	Wilcoxon rank sum test
Tumor volumes	p = 0.0007	p < 0.001
Area under the curve	p = 0.001	p = 0.01

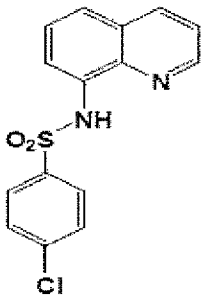
47



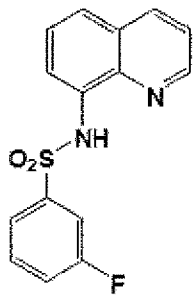
55

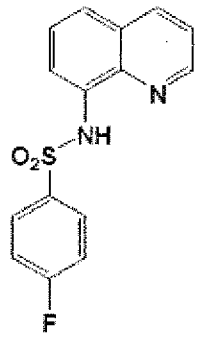


69



74





75

FIGURE 14