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The present invention is directed to combinations of compounds useful in the treatment and prevention of cancer and inflammatory conditions or diseases. In particular embodiments, the combinations comprise one or more compounds that are NF-KB inhibitors or p38 MAPK inhibitors. The invention further provides pharmaceutical compositions and methods of treatment using the combinations. In one embodiment, the NF-KB inhibitor is a curcumin analog.
COMBINATION THERAPIES FOR TREATMENT OF CANCER AND INFLAMMATORY DISEASES

FIELD OF THE INVENTION

The invention relates to combinations of compounds useful for the treatment of cancer and inflammatory diseases. In particular, the combination provides a synergistic effect allowing for improved treatments.

BACKGROUND OF THE INVENTION

Curcumin (diferuloylmethane), the aromatic yellow pigment in curry, turmeric and mustard, has been shown to have anti-angiogenic, anti-tumor, and anti-tumor promoting properties. In addition, curcumin exhibits numerous other therapeutic effects, including anti-oxidative, anti-thrombotic, anti-inflammatory, anti-cholesterol and anti-diabetic properties. Curcumin has particularly demonstrated great potential as a chemopreventative and therapeutic agent due to its ability to negatively modulate cancer-related biomarkers and inhibit the proliferation of tumor cells but retain pharmacological safety. This anti-cancer activity has been in part attributed to the inhibition of nuclear factor-kappa B (NF-κB), a transcription factor that controls the expression of genes involved in cellular survival, the anti-apoptotic machinery, and the inflammatory response.

Despite the broad activity of curcumin, its in vivo activity can be limited due to its rapid excretion. Recently, various curcumin-derived analogs and derivatives have been developed having similar activity to curcumin; however, these synthetic compounds have been shown in various models to have greater potency both in vitro and in vivo. See, for example, U.S. Patent No. 6,664,272 and U.S. Patent No. 7,371,766, both of which are incorporated herein by reference in their entirety.

Like curcumin, curcumin analogs have also been demonstrated to be anti-NF-kB agents, although the mechanism may differ. Many intercellular pathways cross talk in order to transduce highly regulated cellular signals, and there are multiple hypotheses around the interplay between the NF-kB pathway and other cell signaling events. Published reports include the establishment of links between NF-kB and the

The MAPK pathways are three-tiered kinase pathways, which: 1) are activated upon stimulation with growth factors; 2) signal to transcription factors and other protein kinases; and 3) ultimately elicit some biological response. The three major MAPK family members include p38 MAPK along with the extracellular-regulated kinase (ERK) and the c-jun N-terminal kinase (JNK). Activation of ERK has been widely accepted to lead to cell growth and differentiation while JNK and p38 MAPK are considered stress-induced kinases since they not only can respond to mitogens but also a variety of cellular stresses including inflammatory cytokines. See Raingeaud J, et al. "Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine", J. Biol. Chem. 270 (1995): 7420-6; and Johnson GL and Lapadat R. "Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases", Science 298 (2002): 191 1-2, which are incorporated herein by reference. Recently, p38 MAPK has also been implicated in the transcriptional up-regulation of NF-kB, further suggesting a connection between inflammation, cancer, and NF-kB. See Madrid LV, et al. "Akt stimulates the transactivation potential of the RelA/p65 Subunit of NF-kappa B through utilization of the Ikappa B kinase and activation of the mitogen-activated protein kinase p38", J. Biol. Chem. 276 (2001): 18934-40, which is incorporated herein by reference.

There are reports that p38 MAPK is selectively activated in homogenates of non-small cell lung tumors compared with normal tissue and thus, may be involved in malignant cell growth or transformation at least in this particular cancer. See Greenberg AK, et al. "Selective p38 activation in human non-small cell lung cancer", Am. J. Respir. Cell. Mol. Biol. 26 (2002): 558-64, which is incorporated herein by reference. Research on the development and use of p38 MAPK inhibitors is ongoing,
particularly in relation to rheumatoid arthritis, skin disorders, and other inflammatory diseases. For example, p38 MAPK inhibitors have been found to inhibit the production of pro-inflammatory cytokines and therefore inhibit the propagation of the inflammatory response.

While there are numerous reported treatments for various types of cancer and inflammatory diseases, many have toxic side effects. Thus, there is a need for improved treatments for cancer and inflammatory diseases with high efficacy and reduced clinical toxicity.

SUMMARY OF THE INVENTION

The present invention is directed to combinations of NF-κB (nuclear factor-kappa B) inhibiting compounds and p38 MAPK (mitogen activated protein kinase) inhibiting compounds, compositions providing the combinations, and methods of using the combinations. Such combinations are useful in the treatment of multiple diseases and conditions, including but not limited cancers and inflammatory conditions. For example, the combinations can be useful for decreasing the toxic effect of cancer therapies. The combinations can also be useful for decreasing the toxic effect of inflammatory disease therapies. The combinations can further be useful for increasing the effectiveness of cancer treatments and/or inflammatory condition treatments. For example, specific NF-κB inhibitors (e.g., curcumin analogs) exhibit specific activity in the treatment of cancers as well as inflammatory conditions. The combination of an NF-κB inhibitor with a p38 MAPK inhibitor according to the invention can be effective to increase the activity of the NF-κB inhibitor. Such increase particularly exceeds any additive effect of the combination and actually exhibits synergism (i.e., an increase in activity that exceeds any effect that may be expected from combining the compounds).

In one aspect, the present invention provides combinations of NF-κB inhibiting compounds and p38 MAPK inhibiting compounds. The combinations may be formed of a single NF-κB inhibitor and a single p38 inhibitor, a single NF-κB inhibitor and a plurality of p38 inhibitors, a plurality of NF-κB inhibitors and a single p38 inhibitor, or a plurality of NF-κB inhibitors and a plurality of p38 inhibitors. The active agents forming the combination may also be provided as one or more pharmaceutical compositions (e.g., in combination with pharmaceutically acceptable carrier). The type of pharmaceutical composition can vary depending upon the
desired use. For example, the invention encompasses a single composition formed with the NF-KB inhibitor(s) and the p38 inhibitor(s). The invention also encompasses multiple compositions (e.g., a composition formed with the NF-κB inhibitor(s) and a separate composition formed with the p38 inhibitor(s)). Thus, it is clear that the combination of the invention can be administered in a variety of embodiments. For example, the NF-κB inhibitor and the p38 inhibitor could be administered simultaneously, either in the same composition or in separate compositions administered at the same time or in very close proximity of time (e.g., within only a few minutes). The active agents could also be administered sequentially (e.g., administration of one composition comprising either the NF-KB inhibitor or the p38 inhibitor followed thereafter by a composition comprising the remaining active agent. Of course, a skilled person would recognize the variety of methods whereby the active agents could be administered depending upon the final desired effect of the combination. For example, the methods of treatment described herein may exhibit optimized effectiveness arising from separate administration of the NF-KB inhibitor and the p38 inhibitor spaced over a specific time frame, and the present invention would encompass any such methods of administration.

The NF-KB inhibiting compound may be chosen from a wide variety of compounds, as described herein. In specific embodiments, the NF-κB inhibiting compound is a compound that performs one or more of the following functions: inhibits activity of NF-κB directly, inhibits activation of the NF-KB pathway, increases the sensitivity of NF-κB to conventional chemotherapy, or inhibits phosphorylation or degradation of naturally occurring NF-κB inhibitors.

In preferred embodiments, the NF-κB inhibiting compound is a curcumin analog. For example, the curcumin analog can be a compound according to the structure of Formula (1)

\[
\text{(1)}
\]

wherein:
Ar is an optionally substituted ring structure comprising 5-20 ring atoms and is selected from the group consisting of aryl, substituted aryl, heteroaryl having 1-3 heteroatoms selected from O, S, and N, substituted heteroaryl, heterocycle having 1-3 heteroatoms selected from O, S, and N, and substituted heterocycle;

any substituent on Ar is selected from the group consisting of halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkyamino, dialkyamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, azide, alkylcarbonyl, acyl, trialkylammonium, NH-aa, and O-aa, where aa is an amino acid;

A is selected from the group consisting of:
n is 1-8;
X is selected from the group consisting of -CH₂-, -CH₂-CH₂-, -CH₂-CH₂-CH₂-;

Q is NH or NR₃;
Vi₋₄ are each independently OH, OR₂, or halogen;
R₁, R₂, and R₃ are independently H, alkyl, substituted alkyl, alkoxy, aryl,
substituted aryl, heteroaryl, substituted heteroaryl, heterocycle, substituted
heterocycle, acyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl or
dialkylaminocarbonyl;

the dashed lines indicate the presence of optional double bonds; and

L is the point of bonding of A to the compound structure;
as well as pharmaceutically acceptable esters, amides, salts, solvates,
enantiomers, and prodrugs thereof.

Of course, particular curcumin analogs could be chosen from the group
encompassed by Formula (1). For example, in certain embodiments, a curcumin
analog useful as an NF-κB inhibiting compound according to the invention may be a
compound according to the structure of Formula (3)

wherein:
X is optional and is selected from the group consisting of -CH₂⁻, -CH₂-CH₂⁻, -CH₂-CH₂-CH₂⁻, -CH₂-CH₂-CH₂⁻, -CH₂-CH₂-CH₂⁻, -CH₂-CH₂-CH₂⁻, O, -O-NR₃⁻, S, SO, SO₂, -S-S-, NR₃, and -NR₃⁻NR₃⁻:

R₃ is H, alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, acyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl or dialkylaminocarbonyl;

each Yᵢ and Y₂ᵢ is optional and is independently selected from the group consisting of halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, azide, alkyl carbonyl, acyl, trialkylammonium, NH-aa, and O-aa, where aa is an amino acid;

due to the dashed lines indicate the presence of optional double bonds;

as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

Particularly preferred curcumin analogs are those according to the structure of Formula (3), wherein X is NR₃, R₃ is H, alkyl, substituted alkyl, or alkoxy, Yᵢ is absent, and Y₂ᵢ is halo, hydroxyl, alkoxy, or CF₃. An especially useful curcumin analog in the inventive combination is the compound provided in Formula (6).

![Formula 6](image)

The p38 MAPK inhibiting compounds useful according to the invention may also encompass a large variety of compounds. Many p38 inhibiting compounds have been identified and may have great diversity of structure. The synergistic effect arising from the combination of the invention, however, is believed to extend across the diversity of structure and arise from the activity of the compounds, which is not necessarily limited by structure. For example, p38 inhibitors useful in the inventive combinations may be chosen from the following groups of compounds having multiple representatives known to exhibit p38 inhibiting activity: a) 1-aryl-2-pyridinyl/pyrimidinyl heterocycles; b) 2-methoxypyrimidinylimidazoles; c) pyrazoles;
d) oxazoles; e) thiazoles; f) compounds formed of a central heterocycle with vicinal
groups selected from benzoimidazoles, benzothiazoles, and quinolines; g) compounds
formed of a 6-membered heterocyclic cores selected from the group of pyridazines,
pyrazines, pyridines; h) compounds formed of a fused bicyclic heterocyclic core that
is an imidazopyrimidine; i) compounds formed of ketone-containing compounds
selected from benzophenones and pyrazoles; j) cyclic ureas; k) pipazine-based
indole compounds; l) urea-based compounds; m) methylbenzamides; n) N-p-
tolylamides; o) bis-aryl amides; and p) bis-aryl benzamides.

In some embodiments, a p38 MAPK inhibiting compound useful according to
the invention may be defined by a variety of generic structures. For example, useful
p38 inhibitors may include:

structures according to Formula (23)

\[
\begin{array}{c}
\text{Ar} \\
\text{Het} \\
\text{Z} \\
\text{Ar}
\end{array}
\]

(23)

wherein:

Het indicates a 5-membered heterocycle with 1-3 ring carbons replaced with
an atom selected from N, O, and S or a 6-membered heterocycle with 1-4 ring carbons
replaced with an atom selected from N, O, or S;

each Ar is optionally substituted and is independently a ring structure
comprising 5-20 ring atoms selected from the group consisting of aryl, substituted
aryl, heteroaryl having 1-3 heteroatoms selected from O, S, and N, substituted
heteroaryl, heterocycle having 1-3 heteroatoms selected from O, S, and N, and
substituted heterocycle;

a Z substituent may be present on any ring atom not bonded to an Ar group;

Z and any substituent on Ar are independently selected from the group
consisting of H, halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl,
carbonyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl,
substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino,
dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide,
imide, azide, alkylcarbonyl, acyl, sulfonyl, alkylsulfonyl, sulfinyl, alkylsulfanyl,
sulfenyl, alkylsulfenyl, and trialkylammonium, or two Z groups may be combined to form a five or six-membered fused ring with Het, optionally including one or more heteroatoms in the fused ring, and the fused ring optionally including one or more substituents as described above; as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof;

structures according to Formula (25)

\[
\begin{align*}
\text{Z} & \quad \text{Het} \\
& \quad \text{Z} \\
\end{align*}
\]

wherein:

Het indicates a 5-membered heterocycle with 1-3 ring carbons replaced with an atom selected from N, O, or S;

A is a heteroatom selected from N, O, or S;

each Z is independently selected from the group of H, halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkysulfonyl, sulfinyl, alkylsulfinyl, sulfenyl, alkylsulfenyl, and trialkylammonium, or two Z groups may be combined to form a five or six-membered fused ring with its respective base ring, optionally including one or more heteroatoms in the fused ring, and the fused ring optionally including one or more substituents as described above in relation to Z; as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof;

structures according to Formula (56)
wherein:
each Ar is optionally substituted and is independently a ring structure
comprising 5-20 ring atoms and is selected from the group consisting of aryl,
substituted aryl, heteroaryl having 1-3 heteroatoms selected from O, S, and N,
substituted heteroaryl, heterocycle having 1-3 heteroatoms selected from O, S, and N,
and substituted heterocycle;

Ri and R₂ are optionally substituted and are independently selected from the
group consisting of halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy,
hydroxyl, carbonyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, aroyl,
substituted heteroaryl, heterocycle, substituted heterocycle, amino,
alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro,
cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkylsulfonyl, sulfanyl,
akylsulfanyl, sulfinyl, alkylsulfinyl, and trialkylammonium;

X is -CH₂⁻, -CH₂-CH₂⁻, -CH₂-CH₂-CH₂⁻, O, -O-NR₄⁻, S, SO, SO₂⁻, -S-S⁻, NR₄⁻,
and -NR₄-NR₄⁻;

R₄ is H, alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, heteroaryl,
substituted heteroaryl, heterocycle, substituted heterocycle, acyl, alkoxyacarbonyl,
aminocarbonyl, alkylaminocarbonyl or dialkylaminocarbonyl; and

Z and any substituent on Ar, R₁, or R₂ are independently selected from the
group consisting of H, halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy,
hydroxyl, carbonyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, aroyl,
substituted heteroaryl, heterocycle, substituted heterocycle, amino,
akylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro,
cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkylsulfonyl, sulfanyl,
akylsulfanyl, sulfinyl, alkylsulfinyl, and trialkylammonium; as well as
pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs
thereof;

structures according to Formula (85)


wherein:

\[ \text{Nc is a nitrogen atom that may be replaced by a carbon atom;} \]

\[ \text{each } Z \text{ is independently selected from the group consisting of halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF}_3\text{, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkylsulfonyl, sulfinyl, alkylsulfinyl, sulfonyl, alkylsulfenyl, and trialkylammonium, or two } Z \text{ groups may be combined to form a five or six-membered fused ring with the respective base ring, optionally including one or more heteroatoms in the fused ring, and the fused ring optionally including one or more substituents as described above; as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof; or structures according to Formula (98)} \]

\[ \begin{align*}
\text{O} & \quad \text{Ar} \\
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{N} \\
\text{Ar} & \quad \text{Ar}
\end{align*} \]

\[ \text{(98)} \]

wherein:

\[ \text{each } \text{Ar is optionally substituted and is independently a ring structure comprising 5-20 ring atoms and is selected from the group consisting of aryl, substituted aryl, heteroaryl having 1-3 heteroatoms selected from O, S, and N, substituted heteroaryl, heterocycle having 1-3 heteroatoms selected from O, S, and N, and substituted heterocycle; and any substituents on } \text{Ar are independently selected from the group consisting of halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF}_3\text{, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, } \]

\[ \begin{align*}
\text{O} & \quad \text{Ar} \\
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{N} \\
\text{Ar} & \quad \text{Ar}
\end{align*} \]
heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamidie, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkylsulfonyl, sulfinyl, alkylsulfinyl, sulfenyl, alkylsulfenyl, and trialkylammonium; as well as 
5 pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

In other embodiments, p38 MAPK inhibiting compounds for use in the invention may be chosen from specific structures. Examples of such compounds are described herein by Formulas (26) - (55), (58) - (84), (86) - (97), and (99) - (153).

Any of these particular p38 inhibiting compounds could be combined with an NF-κB inhibitor, as described herein, particularly for use in the various methods of the invention.

In one preferred embodiment, a combination according to the invention comprises an NF-κB inhibiting compound that is a curcumin analog according to the structure of Formula (6) and a p38 MAPK inhibiting compound according to any of the structures of Formulas (26) - (55), (58) - (84), (86) - (97), and (99) - (153).

In another aspect, the present invention provides a variety of methods of treatment. In one embodiment, the invention provides a method of treating cancer. In other words, the invention provides a combination of an NF-κB inhibiting compound and a p38 MAPK inhibiting compound for use in a method of treating cancer. The method may comprise the use of any combination of NF-κB inhibitors and p38 inhibitors described herein.

In further embodiments, the invention provides a method of treating an inflammatory disease or condition. In other words, the invention provides a combination of an NF-κB inhibiting compound and a p38 MAPK inhibiting compound for use in a method of treating an inflammatory condition. Again, the method may comprise the use of any combination of NF-κB inhibitors and p38 inhibitors described herein.

In still further embodiments, the invention provides methods for increasing the therapeutic activities of NF-κB inhibitors. For example, various curcumin analogs exhibit activity for treating cancer and inflammatory conditions. The present invention provides methods for increasing such activity. In specific embodiments, the invention provides a method for enhancing the anti-cancer activity of an NF-κB inhibitor, and particularly curcumin analogs. Specifically, the method may comprise
administering a p38 MAPK inhibiting compound in combination with the NF-κB inhibitor, thus enhancing the activity of the NF-κB inhibitor. In other words, the invention can be directed to a p38 MAPK inhibiting compound for use in a method of enhancing the anti-cancer activity of a curcumin analog, the method comprising 

administering the p38 MAPK inhibiting compound in combination with the curcumin analog. As noted herein, the administration of the combination can vary (e.g., can be simultaneous in the same or different compositions or may be sequential).

Further, the enhanced activity of the NF-κB inhibitor can vary. It is believed that the synergistic effect exhibited by the inventive combinations is conserved across a wide array of NF-κB inhibitor activities, a wide array of NF-κB inhibitor compounds, and a wide array of p38 inhibitor compounds. For example, in specific embodiments, the inventive methods are useful for increasing the activity of the NF-κB inhibitor for inhibiting growth of cancer cells. In a further embodiment, the inventive methods are useful for increasing the activity of the NF-κB inhibitor for inducing apoptosis of cancer cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Having thus described the invention in general terms, reference will now be made to the accompanying drawings, wherein:

FIG. 1 is a graph illustrating cell viability over time of A549 lung cancer cells treated with curcumin or the curcumin analog EF24;

FIG. 2 is images of immunoblot assays of A549 lung cancer cells treated with curcumin or EF24 to evaluate activation of ERK, p38 MAPK, or JNK using phospho-specific antibodies for the Thr/Tyr activation motifs;

FIG. 3A is a graph illustrating cell viability of A549 lung cancer cells treated with EF24 alone, SB203580 (SB80) alone, or a combination of EF24 and SB80 at varying concentrations;

FIG. 3B is a graph illustrating cell viability of A549 lung cancer cells treated with EF24 alone, SB202190 (SB90) alone, or a combination of EF24 and SB90 at varying concentrations;

FIG. 3C is a graph comparing the ability of SB80 and SB90 at varying concentrations to affect viability of A549 lung cancer cells;
FIG. 3D is images of a western blot of A549 lung cancer cells treated with EF24 and varying concentrations of SB80 to evaluate phosphorylation and protein expression of MAPKAPK-2 using specific antibodies;

FIGS. 4A and 4B are graphs illustrating growth inhibition of A549 lung cancer cells with treatment of EF24 alone and in combination with SB80 as measured by 48h SRB assay and graphed as a function of dose-response;

FIGS. 4C and 4D are graphs illustrating growth inhibition of A549 lung cancer cells with treatment of curcumin alone and in combination with SB80 as measured by 48h SRB assay and graphed as a function of dose-response;

FIG. 5A is images from immunoblot assays illustrating A549 lung cancer cells transfected with siRNA targeting p38 MAPK;

FIG. 5B provides two graphs illustrating levels of reduction of p38 and ERK expressed after transfection with the siRNA;

FIG. 5C is a graph illustrating cell viability of cells transfected and then treated with EF24, SB80, or the combination of EF24 and SB80;

FIG. 6A is images illustration colony formation of A549 lung cancer cells 10 days after no treatment, treatment with SB80 alone, treatment with EF24 along, or treatment with the combination of EF24 and SB80;

FIG. 6B is a graph illustrating the number of colonies formed based on the specified treatment;

FIG. 7A is an illustration of cell cycle distribution for A549 lung cancer cells treated with EF24 and SB80 and evaluated by flow cytometry to define the cycle cell distribution in comparison to the control (0.5% DMSO);

FIG. 7B is a graph illustrating the % of sub-G1 cells after no treatment, treatment with EF24, treatment with SB80, treatment with EF24 and SB80, treatment with curcumin, or treatment with curcumin and SB80;

FIG. 7C is a graph illustrating the % of total cells undergoing apoptosis as a function of the specific treatment as evaluated using Western blot analysis of A549 lysates and using a specific antibody to evaluate the amount of full length and cleaved PARP;

FIG. 7D is an image from a Western blot analysis of A549 lung cancer cells treated with EF24, curcumin, SB80, or combinations thereof and evaluated for evidence of apoptosis; and
FIG. 7E is a graph illustrating viability of A549 lung cancer cells pretreated with the caspase inhibitor zVAD-fmk for 1 hr before treatment with EF24, SB80, or combinations thereof.

DETAILED DESCRIPTION OF THE INVENTION

The invention now will be described more fully hereinafter through reference to various embodiments. These embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art. Indeed, the invention may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. As used in the specification, and in the appended claims, the singular forms "a", "an", "the", include plural referents unless the context clearly dictates otherwise.

The present invention is directed to combinations of compounds, pharmaceutical compositions providing the combinations, and methods of using the combinations and compositions for treating cancer and inflammatory diseases. In certain embodiments, the combinations comprise NF-κB inhibiting compounds and/or p38 MAPK inhibiting compounds.

1. Definitions

The term "compound" as used herein means a chemical entity, whether in the solid, liquid or gaseous phase, and whether in a crude mixture or purified and isolated.

The term "NF-κB inhibiting compound” as used herein means any compound that inhibits activity of NF-κB directly, inhibits activation of the NF-κB pathway, increases the sensitivity of NF-κB to conventional chemotherapy, or inhibits phosphorylation or degradation of naturally occurring NF-κB inhibitors (e.g., naturally occurring inhibitory IκB proteins, such as IκBα, IκBβ, IκBε, pIκBα, and pIκBε).

The term "p38 MAPK inhibiting compound" as used herein means any compound that inhibits activity of p38 MAPK either directly or indirectly.

The term "alkyl" as used herein means saturated straight, branched, or cyclic hydrocarbon groups. In particular embodiments, alkyl refers to groups comprising 1
to 10 carbon atoms ("C_{1-10} alkyl"). In further embodiments, alkyl refers to groups comprising 1 to 8 carbon atoms ("C_{1-8} alkyl"), 1 to 6 carbon atoms ("C_{1-6} alkyl"), or 1 to 4 carbon atoms ("C_{1-4} alkyl"). In specific embodiments, alkyl refers to methyl, trifluoromethyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, isobutyl, t-butyl, pentyl, cyclopentyl, isopentyl, neopentyl, hexyl, isohexyl, cyclohexyl, cyclohexymethyl, 3-methylpentyl, 2,2-dimethylbutyl, and 2,3-dimethylbutyl. Substituted alkyl refers to alkyl substituted with one or more non-interfering substituents, such as but not limited to halo (e.g., Cl, F, Br, and I); halogenated alkyl (e.g., CF_3, 2-Br-ethyl, CH_2F, CH_2Cl, CH_2CF_3, or CF_2CF_3); hydroxyl; amino; carboxylate; carboxamido; alkyamino; aroylamino; alkoxy; aryl; aryloxy; nitro; cycloalkyl; acetylene; alkanoyloxy; ketone; azido; cyano; thio; sulfonic acid; sulfate; phosphonic acid; phosphate; and phosphonate.

The term "alkenyl" as used herein means alkyl moieties wherein at least one saturated C-C bond is replaced by a double bond. In particular embodiments, alkenyl refers to groups comprising 1 to 10 carbon atoms ("C_{1-10} alkenyl"). In further embodiments, alkenyl refers to groups comprising 1 to 8 carbon atoms ("C_{1-8} alkenyl"), 1 to 6 carbon atoms ("C_{1-6} alkenyl"), or 1 to 4 carbon atoms ("C_{1-4} alkenyl"). In specific embodiments, alkenyl can be vinyl, allyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, or 5-hexenyl.

The term "alkynyl" as used herein means alkyl moieties wherein at least one saturated C-C bond is replaced by a triple bond. In particular embodiments, alkynyl refers to groups comprising 1 to 10 carbon atoms ("C_{1-10} alkynyl"). In further embodiments, alkynyl refers to groups comprising 1 to 8 carbon atoms ("C_{1-8} alkynyl"), 1 to 6 carbon atoms ("C_{1-6} alkynyl"), or 1 to 4 carbon atoms ("C_{1-4} alkynyl"). In specific embodiments, alkynyl can be ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl, 3-butynyl, 1-pentylnyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, or 5-hexynyl.

The term "alkoxy" as used herein means straight or branched chain alkyl groups linked by an oxygen atom (i.e., -O-alkyl or -alkyl-O-alkyl), wherein alkyl is as described above. In particular embodiments, alkoxy refers to oxygen-linked groups comprising 1 to 10 carbon atoms ("C_{1-10} alkoxy"). In further embodiments, alkoxy refers to oxygen-linked groups comprising 1 to 8 carbon atoms ("C_{1-8} alkoxy"), 1 to 6 carbon atoms ("C_{1-6} alkoxy"), or 1 to 4 carbon atoms ("C_{1-4} alkoxy").
The term "halo" or "halogen" as used herein means fluorine, chlorine, bromine, or iodine.

The term "heterocycle" or "heterocyclic" as used herein means one or more rings of 5, 6 or 7 atoms with or without unsaturation or aromatic character and having at least one ring atom which is not carbon. Preferred heteroatoms include sulfur, oxygen, and nitrogen. Multiple rings may be fused, as in quinoline or benzofuran. "Substituted heterocycle" is heterocycle having one or more side chains formed from non-interfering substituents.

The term "aryl" as used herein means a stable monocyclic, bicyclic, or tricyclic carbon ring of up to 8 members in each ring, wherein at least one ring is aromatic as defined by the Huckel 4n+2 rule. Multiple aryl rings may be fused, and aryl rings may be fused or unfused with one or more cyclic hydrocarbon, heteroaryl, or heterocyclic rings. Exemplary aryl groups according to the invention include phenyl, naphthyl, tetrahydronaphthyl, and biphenyl. The aryl group can be substituted with one or more non-interfering substituents, such as, for example, hydroxyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate.

The term "heteroaryl" as used herein means an aryl group containing from one or more (particularly one to four) non-carbon atom(s) (particularly N, O, or S) or a combination thereof, which heteroaryl group is optionally substituted at one or more carbon or nitrogen atom(s) with alkyl, -CF₃, phenyl, benzyl, or thienyl, or a carbon atom in the heteroaryl group together with an oxygen atom form a carbonyl group, or which heteroaryl group is optionally fused with a phenyl ring. Heteroaryl rings may also be fused with one or more cyclic hydrocarbon, heterocyclic, aryl, or heteroaryl rings. Heteroaryl includes, but is not limited to, 5-membered heteroaryls having one hetero atom (e.g., thiophenes, pyrroles, furans); 5 membered heteroaryls having two heteroatoms in 1,2 or 1,3 positions (e.g., oxazoles, pyrazoles, imidazoles, thiazoles, purines); 5-membered heteroaryls having three heteroatoms (e.g., triazoles, thiadiazoles); 5-membered heteroaryls having 3 heteroatoms; 6-membered heteroaryls with one heteroatom (e.g., pyridine, quinoline, isoquinoline, phenanthrine, 5,6-cycloheptenopyridine); 6-membered heteroaryls with two heteroatoms (e.g., pyridazines, cinnolines, phthalazines, pyrazines, pyrimidines, quinazolines); 6-
membered heretoaryls with three heteroatoms (e.g., 1,3,5-triazine); and 6-membered heteroaryls with four heteroatoms. Substituted heteroaryl is heteroaryl having one or more non-interfering groups as substituents.

The terms "aralkyl" and "arylalkyl" as used herein mean an aryl group as defined above linked to the molecule through an alkyl group as defined above.

The terms "alkaryl" and "alkylaryl" as used herein means an alkyl group as defined above linked to the molecule through an aryl group as defined above.

The term "acyl" as used herein means a carboxylic acid ester in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic alky or lower alkyl; alkoxyalkyl including methoxymethyl; aralkyl including benzy; aryloxyalkyl such as phenoxyethyl; aryl including phenyl optionally substituted with one or more non-interfering substituents, such as halogen, C₁-C₆ alkyl or C₁-Çe alkoxy; sulfonate esters such as alkyl or aralkyl sulphonyl including methanesulfonyl; mono-, di-, or triphosphate ester; trityl or monomethoxytrityl; substituted benzyl; trialkylsilyl such as dimethyl-t-butyldisilyl or diphenylmethylsilyl. Aryl groups in the esters optimally comprise a phenyl group.

The term "amino" as used herein means a moiety represented by the structure NR₂, and includes primary amines, and secondary and tertiary amines substituted by alkyl (i.e., alkylamino). Thus, R₂ may represent two hydrogen atoms, two alkyl moieties, or one hydrogen atom and one alkyl moiety.

The terms "alkylamino" and "arylamino" as used herein mean an amino group that has one or two alkyl or aryl substituents, respectively.

The term "non-interfering substituents" as used herein means any groups that yield stable compounds. Suitable non-interfering substituents or radicals include, but are not limited to, halo, C₁-C₆ alkyl, C₂-C₁₀ alkenyl, C₂-C₁₀ alkynyl, C₁-C₁₀ alkoxy, C₇-C₁₂ aralkyl, C₇-C₁₂ alkaryl, C₃-C₁₀ cycloalkyl, C₃-C₁₀ cycloalkenyl, phenyl, substituted phenyl, toluoyl, xylene, biphenyl, C₂-C₁₂ alkoxyalkyl, C₇-C₁₂ alkoxyaryl, C₇-C₁₂ aryloxyalkyl, C₆-C₁₂ oxyaryl, C₁-Çe alkylsulfinyl, C₁-C₁₀ alkylsulfanyl, -(CH₂)₃O-(C₁-C₁₀ alkyl) wherein m is from 1 to 8, aryl, substituted aryl, substituted alkoxy, fluoralkyl, heterocyclic radical, substituted heterocyclic radical, nitroalkyl, -NO₂, -CN, -NRC(O)-(C₁-C₁₀ alkyl), -C(O)-(C₁-C₁₀ alkyl), C₂-C₁₀ thioalkyl, -C(O)- (C₁-C₁₀ alkyl), -OH, -SO₂=O, -COOH, -NR₂, carbonyl, -C(O)-(C₁-C₁₀ alkyl)-CF₃, -C(O)-CF₃, -C(O)NR₂, -(C₁-C₁₀ alkyl)-S-(C₆-C₁₂ ary), -C(O)-(C₆-C₁₂ ary), -(CH₂)m-
0-(CH₂)m-O-(C₁-C₆ alkyl) wherein each m is from 1 to 8, -C(O)NR₂, -C(S)NR₂, -SO₂NR₂, -NRC(O)NR₂, -NRC(S)NR₂, salts thereof, and the like. Each R as used herein is H, alkyl or substituted alkyl, aryl or substituted aryl, aralkyl, or alkaryl.

The term "analogue" as used herein means a compound in which one or more individual atoms or functional groups have been replaced, either with a different atom or a different functional, generally giving rise to a compound with similar properties.

The term "derivative" as used herein means a compound that is formed from a similar, beginning compound by attaching another molecule or atom to the beginning compound. Further, derivatives, according to the invention, encompass one or more compounds formed from a precursor compound through addition of one or more atoms or molecules or through combining two or more precursor compounds.

The term "prodrug" as used herein means any compound which, when administered to a mammal, is converted in whole or in part to a compound of the invention.

The term "active metabolite" as used herein means a physiologically active compound which results from the metabolism of a compound of the invention, or a prodrug thereof, when such compound or prodrug is administered to a mammal.

The term "intermittent administration" as used herein means administration of a therapeutically effective dose of a composition according to the invention, followed by a time period of discontinuance, which is then followed by another administration of a therapeutically effective dose, and so forth.

"Pharmaceutically acceptable excipient" or "pharmaceutically acceptable carrier" refers to an excipient that can be included in the compositions of the invention and that causes no significant adverse toxicological effects to the patient.

"Pharmacologically effective amount," "physiologically effective amount," "therapeutically effective amount", and "therapeutically effective dose" are used interchangeably herein to mean the amount of a conjugate of the invention present in a pharmaceutical preparation that is needed to provide a desired level of active agent and/or conjugate in the bloodstream or in the target tissue. The precise amount will depend upon numerous factors, e.g., the particular active agent, the components and physical characteristics of the pharmaceutical preparation, intended patient population, patient considerations, and the like, and can readily be determined by one skilled in the art, based upon the information provided herein and available in the relevant literature.
The term "antiproliferative agent" as used herein means a compound that decreases the hyperproliferation of cells.

The term "abnormal cell proliferation" as used herein means a disease or condition characterized by the inappropriate growth or multiplication of one or more cell types relative to the growth of that cell type or types in an individual not suffering from that disease or condition.

The term "cancer" as used herein means a disease or condition characterized by uncontrolled, abnormal growth of cells, which can spread locally or through the bloodstream and lymphatic system to other parts of the body. The term includes both tumor-forming or non-tumor forming cancers, and includes various types of cancers, such as primary tumors and tumor metastasis.

The term "tumor" as used herein means an abnormal mass of cells within a multicellular organism that results from excessive cell division that is uncontrolled and progressive, also called a neoplasm. A tumor may either be benign or malignant.

The term "inflammatory disease" or "inflammatory condition" as used herein means any disease or condition causing a localized protective response resulting from injury or destruction of tissues and evidenced by one or more of pain, heat, redness, swelling, and loss of function. The inflammation may arise from a variety of events, including but not limited to dilatation of arterioles, capillaries, and venules, with increased permeability and blood flow; exudation of fluids, including plasma proteins; and leukocyte migration into the site of inflammation.

II. Compounds

It has been discovered according to the present invention that various compounds can be combined to provide a synergistic effect useful in the treatment of cancer, as well as inflammatory conditions. It has particularly been recognized that combinations of compounds from the class of NF-κB inhibitors and compounds from the class of p38 MAPK inhibitors can provide improved treatment that extends well beyond any additive effect of the individual compounds. Rather, the combination of compounds acts synergistically to treat the diseases and conditions described herein.

A. NF-κB Inhibiting Compounds

The transcription factor NF-κB can be involved in cellular alterations such as self-sufficiency in growth signals; insensitivity to growth inhibition; evasion of
apoptosis; immortalization; sustained angiogenesis; and tissue invasion and metastasis. It has also been shown to be constitutively activated in some types of cancer cell. Activated NF-κB has been associated with several aspects of tumorigenesis, including promoting cancer cell proliferation, preventing apoptosis, and increasing a tumor's angiogenic and metastatic potential. Furthermore, constitutive activation of NF-κB in cancer cells may be critical in their development of drug resistance to certain cytotoxicity agents. Inhibitors of NF-κB activation can thus be useful anticancer agents, particularly antitumor agents. Still further, NF-κB inhibitors can also be useful for reversing chemoresistance in cancer cells.

The activity of NF-κB is tightly regulated by its interaction with inhibitory IKB proteins. In most resting cells, NF-κB is sequestered in the cytoplasm in an inactive form associated with inhibitory molecules such as IκBα, IκBβ, IκBε, p105, and p100. This interaction blocks the ability of NF-κB to bind to DNA and results in the NF-κB complex being primarily localized to the cytoplasm due to a strong nuclear export signal in IκBα. Following exposure to stimulus, such as inflammatory cytokines, UV light, reactive oxygen species, or bacterial and viral toxins, the NF-κB signaling cascade is activated, leading to the complete degradation of IKB. This allows the translocation of unmasked NF-κB to the nucleus where it binds to the enhancer or promoter regions of target genes and regulates their transcription. In the nucleus, acetylation of NF-κB determines its active or inactive state, and acetylated NF-κB is active and resistant to the inhibitory effects of IKB. However, when histone deacetylase 3 (HDAC3) deacetylates NF-κB, IKB readily binds to NF-κB and causes its translocation into the cytoplasm. Here HDAC3 serves as an intranuclear molecular switch that turns off the biological processes triggered by NF-κB. One of the target genes activated by NF-κB is that encoding IκBα. Newly synthesized IκBα can enter the nucleus, remove NF-κB from DNA, and export the complex back to the cytoplasm to restore its original latent state.

Any compound exhibiting activity for inhibiting NF-κB (i.e., an NF-κB inhibiting compound) could be used in the combination of the present invention. In particular embodiments, the NF-κB inhibiting compound can be any compound that inhibits activity of NF-κB directly. In other embodiments, the NF-κB inhibiting compound can be any compound that inhibits activation of the NF-κB pathway. In
further embodiments, the NF-κB inhibiting compound can be any compound that increases the sensitivity of NF-κB to conventional chemotherapy. In yet further embodiments, the NF-κB inhibiting compound can be any compound that inhibits phosphorylation or degradation of naturally occurring NF-κB inhibitors, such as described herein.

In certain embodiments, the combination of the invention can comprise one or more NF-κB inhibiting compounds selected from the group consisting of lipoic acid, tocopherol, allicin, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, N-acetyldopamine dimmers (e.g., from P. cicadae), allopurinol, anetholdithiolthione, apocynin, apple juice extracts, artemisia p7F (5,6,3′,5′-tetramethoxy-7,4′-hydroxyflavone), astaxanthin, autumn olive extracts, avenanthramides (e.g., from oats), benidipine, bis-eugenol, bruguiera gymnorrhiza compounds, butylated hydroxyanisole (BHA), cepharanthine, caffeic acid phenethyl ester (3,4-dihydroxycinnamic acid, or CAPE), carnosol, beta-carotene, carvedilol, catechol derivatives, centaurea L (asteraceae) extracts, chalcone, chlorogenic acid, Cholestirn, chroman-2-carboxylic acid N-substituted phenylamides, cocoa polyphenols, coffee extract (3-methyl-1,2-cyclopentanedi one), Crataegus pinnatifida polyphenols, curcumin, dimethoxycrucurmin, curcumin analogs, dehydroepiandrosterone (DHEA), DHEA-sulfate, dibenzylbutyrolactone lignans, diethylthiocarbamate (DDC), diferoxamine, dihydroisoeugenol, isoegenol, dihydrolipoic acid, dilazep and fenofibric acid, dimethylthiocarbamates (DMDTC), dimethylsulfoxide (DMSO), disulfiram, ebeselen, edaravone, EPC-KI (phosphodiester compound of vitamin E and vitamin C), epigallocatechin-3-gallate (EGCG or green tea polyphenols), ergothioneine, ethyl pyruvate (glutathione depletion), ethylene glycol tetraacetic acid (EGTA), fisetin, flavonoids (e.g., Crataegus, hoerhaavia diffusa root, xanthohumol, eupatorium arnottianum, genistein, kaempferol, quercetin, daidzein, flavone, isorhamnetin, naringenin, pelargonidin, and sophora flavescens), folic acid, gamma-gluatamylcysteine synthetase (gamma-GCS), ganoderma lucidum polysaccharides, garcinol (e.g., from extract of garcinia indica fruit rind), ginko biloba extract, glutathione, guaiacol (2-methoxyphenol), hematein, HMC05 herbal extract, hydroquinone, 23-hydroxyursolic acid, IRFI 042, iron tetrakis, isovitexin, isoliquiritinigenin, kallistatin, kangen-karyu extract, L-cysteine, lacidipine, lazaroids, ligonberries, lupeol, magnolol, maltol, manganese superoxide dismutase (Mn-SOD),
extract of the stem bark of *mangifera indica* L., melatonin, 21 (alpha, beta)-
methylmelianodiol, mulberry anthocyanins, N-acetyl-L-cysteine (NAC),
nordihydroguaiaritic acid (NGDA), nacyselyn (NAL), ochnaflavone, onion extract
(2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyranone), orthophenanthroline, phenolic
antioxidants (e.g., hydroquinone and tert-butyl hydroquinone), alkylphenols from
piper obliquum, alpha-phenyl-n-tert-butyl-nitrone (PBN), phenylarsine oxide (PAO),
phyllanthus urinaria, *peper longum* Linn. Extract, pitavastatin, prodelphinidin B2 3,3’
di-O-gallate, pyrrolinedithiocarbamate (PDTC), quercetin, red orange extract,
rotenone, roxithromycin, rutin, S-allyl-cysteine (SAC), salogaviolide (Centaurea
ainetensis), sauchinone, silybin, spironolactone, strawberry extracts, taxifolin, tempol,
tepoxaline, thioavarol derivatives, thymoquinone, tocotrienol, tomato peel
polysaccharide, UDN glycoprotein (*ulmus davidiana* nakai), vaccinium stamineum
(deerberry) extract, vanillin (2-hydroxy-3-methoxybenzaldehyde), vitamin B6, alpha-
torphyryl succinate, alpha-torphyryl acetate, PMC (2,2,5,7,8-pentamethyl-6-
hydroxychromane), Yakuchinone A and B, astragalus membranaceus polysaccharide,
azithromycin, canthariden, *cornus officinalis* extract, dan-shao-hua-zian, gamboic
acid, glycine, *Korean mistletoe* lectin, *lyceum* seed oil, meiduoluomi, neomycin,
omapatrilat, enalapril, oconase, paeniflorin, rapamycin, sargassum hemiphyllum
methanol extract, shrnfu, tripterygium polyglycosides, and combinations thereof. Yet
further examples of compounds that may be useful according to the invention as NF-
κB inhibiting compound in relation to phosphorylation or degradation of IKB,
activation of NF-κB, IKB upregulation, nuclear translocation, nuclear expression,
DNA binding, transactivation, or other mechanism of action that may result in
inhibition of NF-κB may be found in the disclosure provided on-line at
http://people.bu.edu/gilmore/nf-kb/inhibitors/index.html, the disclosure of which is
incorporated herein by reference in its entirety.

In particular embodiments, a NF-κB inhibiting compound useful according to
the invention may particularly comprise a curcumin analog. Multiple examples of
curcumin analogs useful according to the invention are provided in U.S. Patent No.
6,664,272 and U.S. Patent No. 7,371,766, both of which are incorporated herein by
reference in their entirety.
In specific embodiments, a curcumin analog useful as a NF-κB inhibiting compound according to the invention is any compound encompassed by the structure of Formula (1) provided below

\[
\begin{align*}
\text{Ar} & \text{Ar} & \text{Ar} \\
\end{align*}
\]  

(1)

wherein:

- each Ar is optionally substituted and is independently a ring structure, typically comprising 5-20 ring atoms, selected from the group consisting of aryl (e.g., \(C_6-C_{10}\) aryl), substituted aryl, heteroaryl (e.g., 5-10-membered rings with 1-3 heteroatoms selected from O, S, and N), substituted heteroaryl, heterocycle (e.g., 5-10-membered rings with 1-3 heteroatoms selected from O, S, and N), and substituted heterocycle;
- any substituent on Ar is selected from the group consisting of halo (i.e., chloro, bromo, iodo, or fluoro), alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, \(CF_3\), alkenyl, alkynyl, aryl, substituted aryl, alkaryl, aryalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, azide, alkylcarbonyl, acyl, trialkylammonium, NH-aa, and O-aa, where aa is an amino acid (e.g., Gly);
- A is selected from the group consisting of:

\[
\begin{align*}
L & \text{L} & \text{L} \\
\text{L} & \text{L} & \text{L} \\
\text{L} & \text{L} & (\text{CH}_2)_n \\
\end{align*}
\]
n is 1-8;
X is selected from the group consisting of -CH₂-, -CH₂-CH₂-, -CH₂-CH₂-CH₂-,
O, -0-NR₃-, S, SO, SO₂, -S-S-, NR₃, and -NR₃-NR₃-;
Q is NH or NR₃;
Vi₄ are each independently OH, OR₂, or halogen;
10 R₁, R₂, and R₃ are independently H, alkyl, substituted alkyl, alkoxy, aryl,
substituted aryl, heteroaryl, substituted heteroaryl, heterocycle, substituted
heterocycle, acyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl or
dialkylaminocarbonyl;
the dashed lines indicate the presence of optional double bonds; and
L is the point of bonding of A to the compound structure;
as well as pharmaceutically acceptable esters, amides, salts, solvates,
enantiomers, and prodrugs thereof.
In other embodiments, a curcumin analog useful as a NF-κB inhibiting compound according to the invention is any compound encompassed by the structure of Formula (2) provided below

![Chemical structure](image)

wherein:

- each Ar is optionally substituted and is independently a ring structure, typically comprising 5-20 ring atoms, selected from the group consisting of aryl (e.g., C₆₋C₁₀ aryl), substituted aryl, heteroaryl (e.g., 5-10-membered rings with 1-3 heteroatoms selected from O, S, and N), substituted heteroaryl, heterocycle (e.g., 5-10-membered rings with 1-3 heteroatoms selected from O, S, and N), and substituted heterocycle;

- any substituent on Ar is selected from the group consisting of halo (i.e., chloro, bromo, iodo, or fluoro), alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, azide, alkylcarbonyl, acyl, trialkylammonium, NH-aa, and O-aa, where aa is an amino acid (e.g., Gly);

- X, which is optional (i.e., the ring structure can be a five-membered carbocycle), is selected from the group consisting of -CH₂⁻, -CH₂-CH₂⁻, -CH₂-CH₂⁻, CH₂⁻, O, -O-NR₃⁻, S, SO, SO₂⁻, S-S⁻, NR₃⁻, and -NR₃⁻ NR₃⁻;

- R₃ is H, alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, acyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl or dialkylaminocarbonyl;

- Yᵢ represents one or more optional substituents of any carbon atom of the designated ring structure, and may be present as one or two substituents on the same carbon atom or as multiple substituents on different carbon atoms, each of the one or more Yᵢ groups being independently selected from the group consisting of halo (i.e.,
chloro, bromo, iodo, or fluoro), alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, azide, alkylcarbonyl, acyl, trialkylammonium, NH-aa, and O-aa, where aa is an amino acid (e.g., Gly), or Y forms a fused ring structure with the central ring comprising X (e.g., a fused ring of about 10 to about 12 total ring atoms selected from C, N, O, and S, which are optionally substituted and can be selected from X above), the ring structure being carbocyclic, heterocyclic, aryl, or heteroaryl, and wherein preferred ring structures for fusing to the central ring including any of the Ar ring structures described above;
the dashed lines indicate the presence of optional double bonds;
as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

In further embodiments, a curcumin analog useful as a NF-κB inhibiting compound according to the invention is any compound encompassed by the structure of Formula (3) provided below

![Chemical structure](image)

wherein:
X, which is optional (i.e., the ring structure can be a five-membered carbocycle), is selected from the group consisting of -CH₂-, -CH₂-CH₂-, -CH₂-CH₂-, CH₂-, O, -O-NR₃-, S, SO, SO₂-, S-S-, NR₃, and -NR₃-NR₃-;
R₃ is H, alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, acyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl or dialkylaminocarbonyl;
each Y₁ and Y₂ individually represents one or more optional substituents of any carbon atom of the designated ring structures, and may be present as one or two substituents on the same carbon atom or as multiple substituents on different carbon
atoms, each of the one or more Yi and Y2 groups being independently selected from
the group consisting of halo (i.e., chloro, bromo, iodo, or fluoro), alkyl, substituted
alkyl, alkoxy, substituted alkoxy, hydroxyl, CF3, alkenyl, alkynyl, aryl, substituted
aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted
heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester,
carboxamide, nitro, cyano, azide, alkylcarbonyl, acyl, trialkylammonium, NH-aa, and
O-aa, where aa is an amino acid (e.g., Gly), or Yi forms a fused ring structure with the
central ring comprising X (e.g., a fused ring of about 10 to about 12 total ring atoms
selected from C, N, O, and S, which are optionally substituted and can be selected
from X above), the ring structure being carbocyclic, heterocyclic, aryl, or heteroaryl,
and wherein preferred ring structures for fusing to the central ring including any of the
Ar ring structures described above;

the dashed lines indicate the presence of optional double bonds;

as well as pharmaceutically acceptable esters, amides, salts, solvates,
enantiomers, and prodrugs thereof.

For each of the above Formulas (1) - (3), Ar is preferably selected from the
following ring structures, which may be optionally substituted with one or more Y2
substituents: phenyl, naphthyl, indyl, azulyl, pentaryl, heptaryl, biphenyl
indacenyl, acenaphthyl, phenalyl, isodiazolidinyl, indolyl, isoindolyl, morpholinyl,
piperazinyl, piperidinyl, pyrazolidinyl, pyrrolidinyl, benzofuranyl, carbazolyl,
benzopyranyl, furanyl, imidazoyl, indazoyl, indolizinyl, isobenzofuryl, isoindolyl,
isoquinolinyl, isothiazoyl, isoaxazolyl, naphthyridinyl, oxazolyl, pteridinyl, purinyl,
pyranyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridinyl, pyrimidinyl, pyridyl,
pyrrolidinyl, quinazolinyl, quinolinyl, quinolizinyl, quinoxalinyl, thiazolyl,
and thiophenyl. Particularly preferred Ar groups include substituted or unsubstituted
phenyl, furanyl, pyridinyl, pyrimidinyl, quinolinyl, and naphthyridinyl. In one
embodiment, each Ar group is substituted or unsubstituted phenyl, substituted or
unsubstituted naphthyl, substituted or unsubstituted naphthyridinyl, substituted or
unsubstituted quinolinyl, or a substituted or unsubstituted six-membered heteroaryl
ring comprising 1-3 nitrogen atoms.

The number of Yi and/or Y2 groups present on any give ring structure,
whether the central ketone ring or the outer Ar rings, can vary, but is typically 0-8
(i.e., 0, 1, 2, 3, 4, 5, 6, 7, or 8 Y groups), more typically 0-4. As noted above, two Y
groups can be substituted on the same carbon atom in the case of non-aromatic rings. In one preferred embodiment, the outer Ar rings are ortho-substituted with \( Y_2 \) substituents, particularly halo, alkoxy (e.g., methoxy), hydroxyl, or \( \text{CF}_3 \).

In further preferred embodiments, \( X \) is \( \text{NH} \) or \( \text{NR}_3 \). Preferably, \( R_3 \) is \( \text{H} \) or lower alkyl (e.g., \( \text{C}_1 \text{-Ce} \)).

In preferred embodiments, curcumin analogs useful as a NF-\( \kappa \text{B} \) inhibiting compound according to the invention are provided by the structure of Formula (4)

\[ \text{Formula (4)} \]

\[ \text{Y}_1 \text{ represents one or more optional substituents of any carbon atom of the designated ring structure, and may be present as one or two substituents on the same carbon atom or as multiple substituents on different carbon atoms, each of the one or more } Y \text{ groups being independently selected from the group consisting of halo (i.e., chloro, bromo, iodo, or fluoro), alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, C}_3 \text{, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, alkyaryl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkyamino, dialkyamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, azide, alkylcarbonyl, acyl, trialkylammonium, NH-aa, and O-aa, where aa is an amino acid (e.g., Gly); } \]

\[ Y_2 \text{ represents one or more optional substituents of any carbon atom of the designated ring structure, and may be present as one or two substituents on the same carbon atom or as multiple substituents on different carbon atoms, each of the one or more } Y_2 \text{ groups being independently selected from the group consisting of halo (i.e., chloro, bromo, iodo, or fluoro), alkoxy (e.g., methoxy), hydroxyl, and C}_3 \text{; } \]
the dashed lines indicate the presence of optional double bonds;
as well as pharmaceutically acceptable esters, amides, salts, solvates,
enantiomers, and prodrugs thereof.

In further preferred embodiments, curcumin analogs useful as a NF-κB
inhibiting compound according to the invention are provided by the structure of
Formula (5)

![Formula (5)]

wherein

\[ \text{Y}_2 \] represents one or more optional substituents of any carbon atom of the
designated ring structure, and may be present as one or two substituents on the same
carbon atom or as multiple substituents on different carbon atoms, each of the one or
more \[ \text{Y}_2 \] groups being independently selected from the group consisting of halo (i.e.,
chloro, bromo, iodo, or fluoro), alkoxy (e.g., methoxy), hydroxyl, and \( \text{CF}_3 \);

the dashed lines indicate the presence of optional double bonds;
as well as pharmaceutically acceptable esters, amides, salts, solvates,
enantiomers, and prodrugs thereof.

In a particularly preferred embodiment, a curcumin analog useful as a NF-κB
inhibiting compound according to the invention is provided by the structure of
Formula (6), which may be referred to herein as the compound EF24.

![Formula (6)]
The compound EF24 may particularly be in the form of a salt, such as the acetate salt. In such embodiments, the compound may have the structure provided in Formula (6b)

![Formula (6b)](image)

wherein $A^-$ is an appropriate salt forming anion, such as OAc.

Still further examples of curcumin analogs useful as NF-κB inhibiting compounds according to the invention are provided below in Tables I-IV.

### Table I

<table>
<thead>
<tr>
<th>Compound</th>
<th>$Y$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF2</td>
<td>2-OH</td>
</tr>
<tr>
<td>EF3</td>
<td>3-OH</td>
</tr>
<tr>
<td>EF1</td>
<td>4-OH</td>
</tr>
<tr>
<td>EF8</td>
<td>2-F</td>
</tr>
<tr>
<td>EF9</td>
<td>2,4-F</td>
</tr>
<tr>
<td>EF10</td>
<td>3,4-F</td>
</tr>
<tr>
<td>EF(23)</td>
<td>2,6-F</td>
</tr>
<tr>
<td>MD6</td>
<td>3,4-(OMe)</td>
</tr>
<tr>
<td>EF16</td>
<td>2-OMe</td>
</tr>
<tr>
<td>EF17</td>
<td>3-OMe</td>
</tr>
<tr>
<td>EF18</td>
<td>4-OMe</td>
</tr>
</tbody>
</table>
### Table II

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>(EF4)</td>
<td>C</td>
<td>2-OH</td>
</tr>
<tr>
<td>(EF31)</td>
<td>C-l</td>
<td>2-OH</td>
</tr>
<tr>
<td>(EF25)</td>
<td>O</td>
<td>2-OH</td>
</tr>
<tr>
<td>(EF29)</td>
<td>O</td>
<td>2-F</td>
</tr>
<tr>
<td>(EF30)</td>
<td>O</td>
<td>2,4-F</td>
</tr>
<tr>
<td>(EF36)</td>
<td>O</td>
<td>3,4(OMe)</td>
</tr>
<tr>
<td>(EF28)</td>
<td>O</td>
<td>2-OMe</td>
</tr>
<tr>
<td>(EF27)</td>
<td>O</td>
<td>4-OMe</td>
</tr>
<tr>
<td>(EF34)</td>
<td>NMe</td>
<td>2-OH</td>
</tr>
<tr>
<td>(EF33)</td>
<td>NMe</td>
<td>2-F</td>
</tr>
<tr>
<td>(EF47)</td>
<td>NMe</td>
<td>2,4-F</td>
</tr>
<tr>
<td>(EF35)</td>
<td>NMe</td>
<td>3,4-(OMe)</td>
</tr>
<tr>
<td>(EF24)</td>
<td>NH$_2$OAc</td>
<td>2-F</td>
</tr>
<tr>
<td>(EF26)</td>
<td>NH$_2$Cl</td>
<td>H</td>
</tr>
</tbody>
</table>

### Table III

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>(EF15)</td>
<td>0</td>
<td>H</td>
</tr>
<tr>
<td>(EF13)</td>
<td>1</td>
<td>H, (E, E)</td>
</tr>
<tr>
<td>(EF14)</td>
<td>1</td>
<td>H, (E, Z)</td>
</tr>
<tr>
<td>(EF11)</td>
<td>2</td>
<td>2-OH</td>
</tr>
<tr>
<td>(EF12)</td>
<td>2</td>
<td>H</td>
</tr>
</tbody>
</table>

### Table IV

<table>
<thead>
<tr>
<th>Compound</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>(EF32)</td>
<td>2-OH</td>
</tr>
<tr>
<td>(EF48)</td>
<td>2-F</td>
</tr>
<tr>
<td>(EF19)</td>
<td>2,4-F$_2$</td>
</tr>
<tr>
<td>(EF20)</td>
<td>3,4-F$_2$</td>
</tr>
<tr>
<td>(MD279L)</td>
<td>3,4-(OMe)</td>
</tr>
</tbody>
</table>
Several further exemplary curcumin analogs useful as NF-κB inhibiting compounds according to the invention are set forth in the formulas provided below:

(7)

(8)

(9)

(10)

(11)
The curcumin analogs useful as a NF-κB inhibiting compounds according to the present invention may be prepared according to methods known in the art, particularly in light of the disclosure and examples set forth herein. The starting materials used to synthesize the curcumin analog compounds are commercially available or capable of preparation using methods known in the art. For example, some curcumin analogs according to the present invention may be prepared by reaction of an aromatic aldehyde, such as hydroxybenzaldehyde or fluoro-substituted benzaldehyde, with a ketone, such as acetone, cyclohexanone, cyclopentanone, tetrahydro-4-H-pyran-4-one, N-methyl-4-piperidone, piperidin-4-one, and the like, under basic aldol condensation conditions. Similarly, other curcumin analogs according to the present invention may be prepared by reaction of an alkoxy-substituted benzaldehyde or anisaldehyde with a ketone. As would be understood, the actual ketone or aldehyde utilized will depend on the type and position of the substituents of the desired final compound. Salts of the curcumin analogs may be prepared, in general, by reaction of a curcumin analog according to the invention with the desired acid or base in solution. After the reaction is complete, the salts can be crystallized from solution by the addition of an appropriate amount of solvent in which the salt is insoluble.

B. p38 MAPK Inhibiting Compounds

The mitogen-activated protein kinase (MAPK) pathways are three-tiered kinase pathways, which are activated upon stimulation with growth factors, signal to transcription factors and other protein kinases, and ultimately elicit some biological response. The three major MAPK family members include p38 MAPK along with the extracellular-regulated kinase (ERK) and the c-jun N-terminal kinase (JNK). Activation of ERK has been widely accepted to lead to cell growth and differentiation while JNK and p38 MAPK are considered stress-induced kinases since they not only can respond to mitogens but also a variety of cellular stresses including inflammatory cytokines. Recently, p38 MAPK has also been implicated in the transcriptional up-regulation of NF-κB, further suggesting a connection between inflammation, cancer, and NF-κB.

Like other MAPKs, p38 kinases are activated by MAP kinase kinases (MAPKKs/MKKs) with MKK3 and MKK6 being the two main MAPKKs known to activate p38. Further, p38 MAPK has been identified as the upstream kinase of MAP
kinase-activated protein kinase-2 (MAPKAPK-2/MK2), activating transcription factor-2 (ATF-2), mitogen- and stress-activated kinase (MSK), and even p53. It is believed that p38 MAPK plays a role in human cancers, and there are reports that p38 MAPK is selectively activated in homogenates of non-small cell lung tumors compared with normal tissue and thus, may be involved in malignant cell growth or transformation.

Development of MAPK inhibitors is on-going, and p38 MAPK inhibiting compounds are believed to be useful in the treatment of at least rheumatoid arthritis, skin disorders, and other inflammatory diseases. Moreover, p38 MAPK inhibitors can inhibit the production of pro-inflammatory cytokines and therefore inhibit the propagation of the inflammatory response.

Any compound useful to inhibit activity of p38 MAPK (i.e., a p38 inhibiting compound or p38 inhibitor) may be used in a combination according to the present invention. For example, many known p38 inhibiting compounds are characterized by inclusion of an azole or imidazole ring, typically bonded to one or more further aromatic ring structures. The combinations of the present invention particularly encompass any azole or imidazole containing compound having activity to inhibit p38 MAPK. This general structure may also give rise to further p38 inhibitors, such as compounds based on a 6-membered central ring structure.

In various embodiments, a p38 inhibiting compound useful according to the present invention may be any compound having the structure provided in Formula (23) below

\[
\begin{align*}
\text{Het} & \quad \text{Z} \\
\text{Ar} & \quad \text{Ar}
\end{align*}
\]

(23)

wherein:

Het indicates a 5-membered heterocycle with 1-3 ring carbons replaced with an atom selected from N, O, or S or a 6-membered heterocycle with 1-4 ring carbons replaced with an atom selected from N, O, or S;
each Ar is optionally substituted and is independently a ring structure, typically comprising 5-20 ring atoms, selected from the group consisting of aryl (e.g., C₆₋C₁₀ aryl), substituted aryl, heteroaryl (e.g., 5-10-membered rings with 1-3 heteroatoms selected from O, S, and N), substituted heteroaryl, heterocycle (e.g., 5-10-membered rings with 1-3 heteroatoms selected from O, S, and N), and substituted heterocycle;

a Z substituent may be present on any ring atom not bonded to an Ar group;

Z and any substituent on Ar are independently selected from the group consisting of H, halo (i.e., chloro, bromo, iodo, or fluoro), alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarboxyl, acyl, sulfonyl, alkylsulfonyl, sulfinyl, alkysulfinyl, sulflenyl, alkylsulfenyl, and trialkylammonium, or two Z groups may be combined to form a five or six-membered fused ring with Het, optionally including one or more heteroatoms in the fused ring, and the fused ring optionally including one or more substituents as described above;

as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

In certain embodiments, a p38 inhibiting compound useful according to the invention is any compound having the structure provided in Formula (24) below

\[
\begin{array}{c}
\text{Ar} \\
\text{Het} \\
\text{Z}
\end{array}
\]

(24)

wherein:

Het indicates a 5-membered heterocycle with 1-3 ring carbons replaced with an atom selected from N, O, or S;

each Ar is optionally substituted and is independently a ring structure, typically comprising 5-20 ring atoms, selected from the group consisting of aryl (e.g., C₆₋C₁₀ aryl), substituted aryl, heteroaryl (e.g., 5-10-membered rings with 1-3 heteroatoms selected from O, S, and N), and substituted heterocycle;
heteroatoms selected from O, S, and N), substituted heteroaryl, heterocycle (e.g., 5-10-membered rings with 1-3 heteroatoms selected from O, S, and N), and substituted heterocycle;

each Z and any substituent on Ar are independently selected from the group consisting of H, halo (i.e., chloro, bromo, iodo, or fluoro), alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkylsulfonyl, sulfanyl, alkylsulfanyl, sulfenyl, alkylsulfenyl, and trialkylammonium, or two Z groups may be combined to form a five or six-membered fused ring with Het, optionally including one or more heteroatoms in the fused ring, and the fused ring optionally including one or more substituents as described above;

as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

In other embodiments, a p38 inhibiting compound useful according to the invention is any compound having the structure provided in Formula (25) below

![Formula (25)](image)

wherein:

Het indicates a 5-membered heterocycle with 1-3 ring carbons replaced with an atom selected from N, O, and S;

A is a heteroatom selected from N, O, and S;

each Z is independently selected from the group consisting of H, halo (i.e., chloro, bromo, iodo, or fluoro), alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino,
alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkylsulfonyl, sulfinyl, alkylsulfinyl, sulfonyl, alkylsulfenyl, and trialkylammonium, or two Z groups may be combined to form a five or six-membered fused ring with its respective base ring, optionally including one or more heteroatoms in the fused ring, and the fused ring optionally including one or more substituents as described above in relation to Z; as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

In specific embodiments, a p38 inhibiting compound useful according to the invention can be any compound that is a 1-aryl-2-pyridinyl/pyrimidinyl heterocycle. For example, the p38 inhibiting compound could be a compound such as shown in Formula (26) - also known as SB203580- and Formula (27) - also known as SB202190.

Further, the invention encompasses 2-methoxypyrimidinylimidazole compounds, such as the compound exemplified in Formula (28).
In further embodiments, the central imidazole ring may be replaced with non-imidazole 5-membered rings (e.g., pyrazoles, oxazoles, and thiazoles). Examples of such compounds useful according to the invention are illustrated in Formulas (29) - (38).
While it is useful for p38 inhibitor compounds according to the invention to include a pyridine or pyrimidine group vicinal to the central heterocycle, such groups may be replaced (e.g., with benzoimidazoles, benzothiazoles, and quinolines). Such compounds are exemplified below in Formulas (40) - (44).

![Chemical Structures](image)

Although it is useful for the p38 inhibiting compounds of the invention to include a core 5-membered heterocycle, other types of p38 inhibiting compounds may also be useful in the inventive combinations. For instance, in accordance with Formula (23) described above, the compounds may be based on 6-membered heterocyclic cores, such as pyridazines, pyrazines, pyridines, and fused bicyclic heterocycle, such as imidazopyrimidines. Such compounds are exemplified below in Formulas (45) - (55).
The p38 inhibiting compounds useful in the combinations of the present invention may also encompass further types of compounds. For example, p38 inhibitors based on ketone-containing compounds (e.g., benzophenone scaffolds or pyrazoles) may also be used. In certain embodiments, benzophenone-based compounds useful according to the invention can include any compound encompassed by the structure of Formula (56)

\[
\begin{align*}
\text{Ar} & \quad \text{Ar} \\
R_1 & \quad X & \quad Z \\
\end{align*}
\]

(56)

wherein:

- each Ar is optionally substituted and is independently a ring structure, typically comprising 5-20 ring atoms, selected from the group consisting of aryl (e.g., C_6^\text{C}_{10} \text{aryl}), substituted aryl, heteroaryl (e.g., 5-10-membered rings with 1-3 heteroatoms selected from O, S, and N), substituted heteroaryl, heterocycle (e.g., 5-10-membered rings with 1-3 heteroatoms selected from O, S, and N), and substituted heterocycle;

- Ri and R₂ are optionally substituted and are independently selected from the group consisting of halo (i.e., chloro, bromo, iodo, or fluoro), alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamid, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkylsulfonyl, sulfinyl, alkylsulfinyl, sulfenyl, alkylsulfenyl, and trialkylammonium, or two Ri or R₂ groups may be combined to form a five or six-membered fused ring with its respective base ring, optionally including one or more heteroatoms in the fused ring, and the fused ring optionally including one or more substituents as described above in relation to Ri or R₂;

- X is \(-\text{CH}_2^\text{-}, -\text{CH}_2^\text{-CH}_2^\text{-}, -\text{CH}_2^\text{-CH}_2^\text{-CH}_2^\text{-}, \text{O}, -0\text{-NR}_4^\text{-}, \text{S}, \text{SO}, \text{SO}_2, -\text{S-S-}, \text{NR}_4^\text{-}, \text{or } -\text{NR}_4^\text{-NR}_4^\text{-}:

- 46 -
R₄ is H, alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, acyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl or dialkylaminocarbonyl; and

Z and any substituent on Ar, R₁, or R₂ are independently selected from the group consisting of H, halo (i.e., chloro, bromo, iodo, or fluoro), alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyle, alkylsulfonyl, sulfinyl, alkylsulfinyl, sulfenyl, alkylsulfenyl, and trialkylammonium;

as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

In other embodiments, benzophenone-based compounds useful according to the invention can include any compound encompassed by the structure of Formula (57) below

![Formula (57)](image)

wherein:

R₁, R₂, and R₃ are independently selected from the group consisting of halo (i.e., chloro, bromo, iodo, or fluoro), alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyle, alkylsulfonyl, sulfinyl, alkylsulfinyl, sulfenyl, alkylsulfenyl, and trialkylammonium, or two R₃ groups may be combined to form a five or six-membered fused ring with its respective base ring, optionally including one or more heteroatoms in the fused ring, and the fused ring optionally including one or more substituents as described above in relation to R₁ or R₂;
as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

Further, specific examples of benzophenone-based compounds useful according to the invention are shown below in Formulas (58) - (63). Exemplary pyrazole ketones and amino pyrroles ketones are shown in Formulas (64) - (66).
Inhibitors of p38 useful according to the present invention may also include compounds based on cyclic ureas. Exemplary embodiments of such compounds are provided below in Formulas (67) - (84).
In yet further embodiments, p38 inhibiting compounds useful according to the invention include piperazine-based indole molecules. In specific embodiments, piperazine-based indole compounds useful according to the invention can include any compound encompassed by the structure of Formula (85)

![Formula 85](image)

wherein:

Nc is a nitrogen atom that may be replaced by a carbon atom;

each Z is independently selected from the group consisting of H, halo (i.e., chloro, bromo, iodo, or fluoro), alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, aryalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonl, alkylsulfonl, sulfanyl, alkylsulfanyl, sulphenyl, alkylsulphenyl, and trialkylammonium, or two Z groups may be combined to form a five or six-membered fused ring with the respective base ring, optionally including one or more heteroatoms in the fused ring, and the fused ring optionally including one or more substituents as described above;

as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

Exemplary compounds according to this embodiment of the invention are provided below in Formulas (86) - (97).
In specific relation to the compounds of Formulas (89) - (97),

\[ x = \begin{array}{c}
\text{structure}
\end{array} \quad \text{and} \quad \begin{array}{c}
\text{structure}
\end{array} \]

Urea-based compounds exhibiting p38 inhibiting activity may also be used in the combinations of the present invention. In specific embodiments, urea-based compounds useful according to the invention can include any compound encompassed by the structure of Formula (98)

\[ \text{structure} \]

wherein:

- each Ar is optionally substituted and is independently a ring structure, typically comprising 5-20 ring atoms, selected from the group consisting of aryl (e.g., C_6-C_{10} aryl), substituted aryl, heteroaryl (e.g., 5-10-membered rings with 1-3 heteroatoms selected from O, S, and N), substituted heteroaryl, heterocycle (e.g., 5-10-membered rings with 1-3 heteroatoms selected from O, S, and N), and substituted heterocycle; and

- any substituent on Ar are independently selected from the group consisting of halo (i.e., chloro, bromo, iodo, or fluoro), alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF_3, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkylsulfonyl, sulfinyl, alkylsulfinyl, sulfonyl, alkylsulfenyl, and trialkylammonium;

- as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

Exemplary urea-based p38 inhibitor compounds are provided below in Formulas (99) - (104).
The invention also encompasses the use of p38 inhibiting compounds based on methylbenzamides, N-p-tolylamides, bis-aryl amides, and bis-aryl benzamides.

Exemplary embodiments of such compounds are provided below in Formulas (105) - (128).
Of course, the above illustrated embodiments of p38 inhibiting compounds useful according to the invention should not be viewed as limiting the scope of the invention. Rather further compounds having p38 inhibiting activity could also be used in the invention. For example, additional compounds known to have p38 inhibiting activity that may also be used in the invention include the following compounds provided in Formulas (129) - (153).
Still further compounds having p38 inhibiting activity may be used in the combinations of the invention. For example, compounds known by the following names could also be used: ARRY-791; SB681323; ISIS101757; VX-702; SCIO323; PS540446; SB856553; KC706; and SB281832.
Any of the above-provided specific p38 inhibiting compounds, as well as further compounds exhibiting p38 inhibiting activity, may be disclosed in additional documents. In particular, any p38 inhibiting compound disclosed in any of the following documents may be used in the combinations of the present invention. All of the following documents are incorporated herein by reference in their entirety:


International Patent Application Numbers WO02/32862; WO02/060869;
WO00/10563; WO00/31063; WO00/31072; WO00/39116; WO00/63204;
WO1/30778; WO02/072571; WO03/035638; WO00/64894; WOO 1/10865;
WO1/0748 11; WO02/072579; WO2004/0 14900; WO2004/026302; WOO0/25791;
WO00/40243; WO01/34605; WO02/16359; WO01/57018; WO2004/076450;
WO03/024973; WO03/024971; WO01/90074; WO02/083622; WO02/076447;
WO02/092087; WO03/008413; WO03/053967; WO03/076405; WOO3/091229;
WO01/21591; WO03/020715; WO98/27098; WOO0/17204; WOO0/17175;
WO01/70695; WO01/37837; WO01/38312; WO01/38313; WO01/38314;
WO01/64679; WO02/058695; WOO3/103950; WO2004/024699; WOO2/059083;
WO03/088972; WO2004/073628; WO03/033502; WO2004/0 14920;
WO2004/031188; WOO0/12074; WOO0/59904; WOO0/71535; WOO2/42292;
WO02/46158; WO03/043988; WO2004/022712; WO2004/021988;
WO2004/0328742; WO03/084539; WOO0/41698; WOO2/085859; WOO3/087087;
WO2004/060306; WO2004/014870; WOO0/20402; WOO0/07980; WOO0/07991;
III. Biologically Active Variants

Biologically active variants of the compounds set forth above are particularly also encompassed by the invention. Such variants should retain the general biological activity of the original compounds; however, the presence of additional activities would not necessarily limit the use thereof in the present invention. Such activity may be evaluated using standard testing methods and bioassays recognizable by the skilled artisan in the field as generally being useful for identifying such activity.

According to one embodiment of the invention, suitable biologically active variants comprise one or more analogues or derivatives of the compounds described above. Indeed, a single compound, such as those described above, may give rise to an entire family of analogues or derivatives having similar activity and, therefore, usefulness according to the present invention. Likewise, a single compound, such as those described above, may represent a single family member of a greater class of compounds useful according to the present invention. Accordingly, the present invention fully encompasses not only the compounds described above, but analogues and derivatives of such compounds, particularly those identifiable by methods commonly known in the art and recognizable to the skilled artisan.

The compounds disclosed herein may contain chiral centers, which may be either of the \((R)\) or \((S)\) configuration, or may comprise a mixture thereof. Accordingly, the present invention also includes stereoisomers of the compounds described herein, where applicable, either individually or admixed in any proportions. Stereoisomers may include, but are not limited to, enantiomers, diastereomers, racemic mixtures, and combinations thereof. Such stereoisomers can be prepared and separated using conventional techniques, either by reacting enantiomeric starting materials, or by separating isomers of compounds of the present invention. Isomers may include geometric isomers. Examples of geometric isomers include, but are not limited to, cis isomers or trans isomers across a double bond. Other isomers are
contemplated among the compounds of the present invention. The isomers may be
used either in pure form or in admixture with other isomers of the compounds
described herein.

Various methods are known in the art for preparing optically active forms and
determining activity. Such methods include standard tests described herein other
similar tests which are well known in the art. Examples of methods that can be used
to obtain optical isomers of the compounds according to the present invention include
the following:

i) physical separation of crystals whereby macroscopic crystals of the
individual enantiomers are manually separated. This technique may particularly be
used when crystals of the separate enantiomers exist (i.e., the material is a
conglomerate), and the crystals are visually distinct;

ii) simultaneous crystallization whereby the individual enantiomers are
separately crystallized from a solution of the racemate, possible only if the latter is a
conglomerate in the solid state;

iii) enzymatic resolutions whereby partial or complete separation of a
racemate by virtue of differing rates of reaction for the enantiomers with an enzyme;

iv) enzymatic asymmetric synthesis, a synthetic technique whereby at least
one step of the synthesis uses an enzymatic reaction to obtain an enantiomerically
pure or enriched synthetic precursor of the desired enantiomer;

v) chemical asymmetric synthesis whereby the desired enantiomer is
synthesized from an achiral precursor under conditions that produce asymmetry (i.e.,
chirality) in the product, which may be achieved using chiral catalysts or chiral
auxiliaries;

vi) diastereomer separations whereby a racemic compound is reacted with an
enantiomerically pure reagent (the chiral auxiliary) that converts the individual
enantiomers to diastereomers. The resulting diastereomers are then separated by
chromatography or crystallization by virtue of their now more distinct structural
differences and the chiral auxiliary later removed to obtain the desired enantiomer;

vii) first- and second-order asymmetric transformations whereby
diastereomers from the racemate equilibrate to yield a preponderance in solution of
the diastereomer from the desired enantiomer or where preferential crystallization of
the diastereomer from the desired enantiomer perturbs the equilibrium such that
eventually in principle all the material is converted to the crystalline diastereomer from the desired enantiomer. The desired enantiomer is then released from the diastereomers;

viii) kinetic resolutions comprising partial or complete resolution of a racemate (or of a further resolution of a partially resolved compound) by virtue of unequal reaction rates of the enantiomers with a chiral, non-racemic reagent or catalyst under kinetic conditions;

ix) enantiospecific synthesis from non-racemic precursors whereby the desired enantiomer is obtained from non-chiral starting materials and where the stereochemical integrity is not or is only minimally compromised over the course of the synthesis;

x) chiral liquid chromatography whereby the enantiomers of a racemate are separated in a liquid mobile phase by virtue of their differing interactions with a stationary phase. The stationary phase can be made of chiral material or the mobile phase can contain an additional chiral material to provoke the differing interactions;

xi) chiral gas chromatography whereby the racemate is volatilized and enantiomers are separated by virtue of their differing interactions in the gaseous mobile phase with a column containing a fixed non-racemic chiral adsorbent phase;

xii) extraction with chiral solvents whereby the enantiomers are separated by virtue of preferential dissolution of one enantiomer into a particular chiral solvent; and

xiii) transport across chiral membranes whereby a racemate is placed in contact with a thin membrane barrier. The barrier typically separates two miscible fluids, one containing the racemate, and a driving force such as concentration or pressure differential causes preferential transport across the membrane barrier. Separation occurs as a result of the non-racemic chiral nature of the membrane which allows only one enantiomer of the racemate to pass through.

The NF-κB inhibiting compounds and/or p38 inhibiting compounds of the invention may be provided in an enantiomerically enriched form, such as a mixture of enantiomers in which one enantiomer is present in excess (given as a mole fraction or a weight fraction). Enantiomeric excess is understood to exist where a chemical substance comprises two enantiomers of the same compound and one enantiomer is present in a greater amount than the other enantiomer. Unlike racemic mixtures, these mixtures will show a net optical rotation. With knowledge of the specific rotation of
the mixture and the specific rotation of the pure enantiomer, the enantiomeric excess (abbreviated "ee") can be determined by known methods. Direct determination of the quantities of each enantiomer present in the mixture is possible with NMR spectroscopy and chiral column chromatography. The compounds of the invention can have a specific degree of enantiomeric purity for a single enantiomer (e.g., at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.5%).

The compounds described herein can also be in the form of an ester, amide, salt, solvate, prodrug, or metabolite provided they maintain pharmacological activity according to the present invention. Esters, amides, salts, solvates, prodrugs, and other derivatives of the compounds of the present invention may be prepared according to methods generally known in the art, such as, for example, those methods described by J. March, Advanced Organic Chemistry: Reactions, Mechanisms and Structure, 4th Ed. (New York: Wiley-Interscience, 1992), which is incorporated herein by reference.

Examples of pharmaceutically acceptable salts of the compounds useful according to the invention include acid addition salts. Salts of non-pharmaceutically acceptable acids, however, may be useful, for example, in the preparation and purification of the compounds. Suitable acid addition salts according to the present invention include organic and inorganic acids. Preferred salts include those formed from hydrochloric, hydrobromic, sulfuric, phosphoric, citric, tartaric, lactic, pyruvic, acetic, succinic, fumaric, maleic, oxaloacetic, methanesulfonic, ethanesulfonic, p-toluenesulfonic, benzenesulfonic, and isethionic acids. Other useful acid addition salts include propionic acid, glycolic acid, oxalic acid, malic acid, malonic acid, benzoic acid, cinnamic acid, mandelic acid, salicylic acid, and the like. Particular example of pharmaceutically acceptable salts include, but are not limited to, sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, phosphates, monohydrogen phosphates, dihydrogen phosphates, metaphosphates, pyrophosphates, chlorides, bromides, iodides, acetates, propionates, decanoates, caprylates, acrylates, formates, isobutyrate, caproates, heptanoates, propiolates, oxalates, malonates, succinates, suberates, sebacates, fumarates, maleates, butyne-1,4-dioates, hexyne-1,6-dioates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, hydroxybenzoates, methoxybenzoates, phthalates, sulfonates, xylenesulfonates, phenylacetates,
phenylpropionates, phenylbutyrates, citrates, lactates, γ-hydroxybutyrates, glycolates, tartrates, methanesulfonates, propanesulfonates, naphthalene-1-sulfonates, naphthalene-2-sulfonates, and mandelates.

An acid addition salt may be reconverted to the free base by treatment with a suitable base. Preparation of basic salts of acid moieties which may be present on a compound useful according to the present invention may be prepared in a similar manner using a pharmaceutically acceptable base, such as sodium hydroxide, potassium hydroxide, ammonium hydroxide, calcium hydroxide, triethylamine, or the like.

Esters of the compounds according to the present invention may be prepared through functionalization of hydroxyl and/or carboxyl groups that may be present within the molecular structure of the compound. Amides and prodrugs may also be prepared using techniques known to those skilled in the art. For example, amides may be prepared from esters, using suitable amine reactants, or they may be prepared from anhydride or an acid chloride by reaction with ammonia or a lower alkyl amine.

Moreover, esters and amides of compounds of the invention can be made by reaction with a carboxylating agent (e.g., ethyl formate, acetic anhydride, methoxyacetyl chloride, benzoyl chloride, methyl isocyanate, ethyl chloroformate, methanesulfonyl chloride) and a suitable base (e.g., 4-dimethylaminopyridine, pyridine, triethylamine, potassium carbonate) in a suitable organic solvent (e.g., tetrahydrofuran, acetone, methanol, pyridine, N,N-dimethylformamide) at a temperature of 0 °C to 60 °C.

Prodrugs are typically prepared by covalent attachment of a moiety, which results in a compound that is therapeutically inactive until modified by an individual's metabolic system. Examples of pharmaceutically acceptable solvates include, but are not limited to, compounds according to the invention in combination with water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid, or ethanolamine.

In the case of solid compositions, it is understood that the compounds used in the compositions of the invention may exist in different forms. For example, the compounds may exist in stable and metastable crystalline forms and isotropic and amorphous forms, all of which are intended to be within the scope of the present invention.

If a compound useful according to the invention is a base, the desired salt may be prepared by any suitable method known to the art, including treatment of the free base with an inorganic acid, such as hydrochloric acid, hydrobromic acid, sulfuric...
acid, nitric acid, phosphoric acid and the like, or with an organic acid, such as acetic acid, maleic acid, succinic acid, mandelic acid, fumaric acid, malonic acid, pyruvic acid, oxalic acid, glycolic acid, salicylic acid, pyranosidyl acids such as glucuronic acid and galacturonic acid, alpha-hydroxy acids such as citric acid and tartaric acid, amino acids such as aspartic acid and glutamic acid, aromatic acids such as benzoic acid and cinnamic acid, sulfonic acids such a p-toluensulfonic acid or ethanesulfonic acid, or the like.

If a compound of the invention is an acid, the desired salt may be prepared by any suitable method known to the art, including treatment of the free acid with an inorganic or organic base, such as an amine (primary, secondary or tertiary), an alkali metal or alkaline earth metal hydroxide or the like. Illustrative examples of suitable salts include organic salts derived from amino acids such as glycine and arginine, ammonia, primary, secondary and tertiary amines, and cyclic amines such as piperidine, morpholine and piperazine, and inorganic salts derived from sodium, calcium, potassium, magnesium, manganese, iron, copper, zinc, aluminum and lithium.

The present invention further includes prodrugs and active metabolites of the compounds of the invention. Any of the compounds described herein can be administered as a prodrug to increase the activity, bioavailability, or stability of the compound or to otherwise alter the properties of the compound. Typical examples of prodrugs include compounds that have biologically labile protecting groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolyzed, dehydrolyzed, alkylated, dealkylated, acylated, deacetylated, phosphorylated, and/or dephosphorylated to produce the active compound.

In specific embodiments, the curcumin analogs described herein particularly could be provided as prodrugs. For example, U.S. Patent Application Publication No. 2007/0270464, the disclosure of which is incorporated herein by reference, describes a number of curcumin analog prodrugs that could be used according to the present invention.

In particular, prodrugs useful according to the invention are provided below in Formulas (154), (155), (156), or (157):
wherein:

- $Z$ is $S$ or $NR'$, where $R'$ is $H$ or the residue of an amine-containing molecule;
- $R$ is the residue of a thiol-containing molecule when $Z$ is $S$ or the residue of an amine-containing molecule when $Z$ is $NR'$;
- each $R_1$ and $R_2$, which can be the same or different, is selected from the group consisting of hydrogen, alkyl (e.g., C1-C8 alkyl), substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocycle, and substituted heterocycle;
- $R_3$ is selected from the group consisting of CF$_3$, alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocycle, and substituted heterocycle, or $R_2$ and $R_3$ together complete a 5 to 8-membered carbocycle ring or heterocycle ring comprising one heteroatom selected from the group consisting of O, S, and $NR_4$, wherein $R_4$ is $H$, alkyl, substituted alkyl, acyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, or dialkylaminocarbonyl;
X, which is optional (i.e., the ring structure can be a five-membered carbocycle), is selected from the group consisting of -CH₂-, -CH₂-CH₂-, -CH₂-CH₂-CH₂-, O, -0-NR₄-, S, SO, SO₂-, -S-S-, NR₄, and -NR₄-NR₄-:

X' is selected from the group consisting of -CH₂-, O, -0-NR₄-, S, SO, SO₂-, -S-S-, NR₄, and -NR₄-NR₄-:

Y represents one or more optional substituents of any carbon atom of the designated ring structures, and may be present as one or two substituents on the same carbon atom or as multiple substituents on different carbon atoms, each of the one or more Y groups being independently selected from the group consisting of halo (i.e., chloro, bromo, iodo, or fluoro), alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, substituted heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamid, nitro, cyano, azide, alkylcarbonyl, acyl, trialkylammonium, NH-aa, and O-aa, where aa is an amino acid (e.g., Gly), or Y forms a fused ring structure with the central ring comprising X (e.g., a fused ring of about 10 to about 12 total ring atoms selected from C, N, O, and S, which are optionally substituted and can be selected from X above), the ring structure being carbocyclic, heterocyclic, aryl, or heteroaryl, and wherein preferred ring structures for fusing to the central ring including any of the Ar ring structures set forth below;

Ar is a ring structure, typically comprising 5-20 ring atoms, selected from the group consisting of aryl (e.g., C₆-C₁₀ aryl), substituted aryl, heteroaryl (e.g., 5-10-membered rings with 1-3 heteroatoms selected from O, S, and N), substituted heteroaryl, heterocycle (e.g., 5-10-membered rings with 1-3 heteroatoms selected from O, S, and N), and substituted heterocycle, the substituting groups preferably selected from the Y groups set forth above;

each dotted line indicates an optional bond;

as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

Certain exemplary sulfur-linked curcumin analog conjugates according to each one of Formulas (I)-(IV) are set forth in Tables V-IX below.
### Table V

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* The phenyl or naphthyl ring is optionally ortho-, meta-, or para- substituted with 1-3 substituents selected from halo (e.g., F), alkoxy (e.g., methoxy), hydroxyl, CF₃, NO₂, NH₂, NH-aa, or O-aa, wherein aa is an amino acid.

** The nitrogen atom of the pyridinyl ring can be at the 2, 3, or 4 position.
### Table VI

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* The phenyl or napthyl ring is optionally ortho-, meta-, or para- substituted with 1-3 substituents selected from halo (e.g., F), alkoxy (e.g., methoxy), hydroxyl, CF₃, NO₂, NH₂, NH-aa, or O-aa, wherein aa is an amino acid.

** The nitrogen atom of the pyridinyl ring can be at the 2, 3, or 4 position.
Table VII

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* The phenyl or naphthyl ring is optionally ortho-, meta-, or para- substituted with 1-3 substituents selected from halo (e.g., F), alkoxy (e.g., methoxy), hydroxyl, CF₃, NO₂, NH₂, NH-aa, or O-aa, wherein aa is an amino acid.

** The nitrogen atom of the pyridinyl ring can be at the 2, 3, or 4 position.
Table VIII

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** The nitrogen atom of the pyridinyl ring can be at the 2, 3, or 4 position.
The use of prodrugs applies universally to the various compounds described herein and is not simply limited to prodrugs of curcumin analogs. Of course, a skilled person viewing the above disclosure would be able to envision the preparation of prodrugs of any of the curcumin analogs described herein.

A number of prodrug ligands are known. In general, alkylation, acylation, or other lipophilic modification of one or more heteroatoms of the compound, such as a free amine or carboxylic acid residue, reduces polarity and allows passage into cells. Examples of substituent groups that can replace one or more hydrogen atoms on the free amine and/or carboxylic acid moiety include, but are not limited to, the

### Table IX

<table>
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<tr>
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** The nitrogen atom of the pyridinyl ring can be at the 2, 3, or 4 position.
following: aryl; steroids; carbohydrates (including sugars); 1,2-diacylglycerol; alcohols; acyl (including lower acyl); alkyl (including lower alkyl); sulfonate ester (including alkyl or arylalkyl sulfonyl, such as methanesulfonyle and benzyl, wherein the phenyl group is optionally substituted with one or more substituents as provided in the definition of an aryl given herein); optionally substituted arylsulfonyl; lipids (including phospholipids); phosphatidylcholine; phosphocholine; amino acid residues or derivatives; amino acid acyl residues or derivatives; peptides; cholesterol; or other pharmaceutically acceptable leaving groups which, when administered in vivo, provide the free amine and/or carboxylic acid moiety. Any of these can be used in combination with the disclosed compounds to achieve a desired effect.

IV. Pharmaceutical Compositions

While it is possible for the individual compound used in the composition of the present invention to be administered in the raw chemical form, it is preferred for the compounds to be delivered as a pharmaceutical composition. Accordingly, there are provided by the present invention pharmaceutical compositions comprising combinations of compounds as described herein. As such, the compositions of the present invention comprise the pharmaceutically active compounds, as described above, or pharmaceutically acceptable esters, amides, salts, solvates, analogs, derivatives, or prodrugs thereof. Further, the inventive compositions can be prepared and delivered in a variety of combinations. For example, the composition can comprise a single composition containing all of the active ingredients. Alternately, the composition can comprise multiple compositions comprising separate active ingredients but intended to be administered simultaneously, in succession, or in otherwise close proximity of time.

The compounds of the invention can be prepared and delivered together with one or more pharmaceutically acceptable carriers therefore, and optionally, other therapeutic ingredients. Carriers should be acceptable in that they are compatible with any other ingredients of the composition and not harmful to the recipient thereof. A carrier may also reduce any undesirable side effects of the agent. Such carriers are known in the art. See, Wang et al. (1980) J. Parent. Drug Assn. 34(6):452-462, herein incorporated by reference in its entirety.
Compositions of the present invention may include short-term, rapid-onset, rapid-offset, controlled release, sustained release, delayed release, and pulsatile release compositions, providing the compositions achieve administration of a compound as described herein. See Remington's Pharmaceutical Sciences (18th ed.; Mack Publishing Company, Eaton, Pennsylvania, 1990), herein incorporated by reference in its entirety.

Pharmaceutical compositions according to the present invention are suitable for various modes of delivery, including oral, parenteral (including intravenous, intramuscular, subcutaneous, intradermal, intra-articular, intra-synovial, intrathecal, intra-arterial, intracardiac, subcutaneous, intraorbital, intracapsular, intraspinal, intrastemal, and transdermal), topical (including dermal, buccal, and sublingual), vaginal, urethral, and rectal administration. Administration can also be via nasal spray, surgical implant, internal surgical paint, infusion pump, or via catheter, stent, balloon or other delivery device. The most useful and/or beneficial mode of administration can vary, especially depending upon the condition of the recipient and the disorder being treated.

The pharmaceutical compositions may be conveniently made available in a unit dosage form, whereby such compositions may be prepared by any of the methods generally known in the pharmaceutical arts. Generally speaking, such methods of preparation comprise combining (by various methods) the active compounds of the invention with a suitable carrier or other adjuvant, which may consist of one or more ingredients. The combination of the active ingredients with the one or more adjuvants is then physically treated to present the composition in a suitable form for delivery (e.g., shaping into a tablet or forming an aqueous suspension).

Pharmaceutical compositions according to the present invention suitable for oral dosage may take various forms, such as tablets, capsules, caplets, and wafers (including rapidly dissolving or effervescing), each containing a predetermined amount of the active agent. The compositions may also be in the form of a powder or granules, a solution or suspension in an aqueous or non-aqueous liquid, and as a liquid emulsion (oil-in-water and water-in-oil). The active agents may also be delivered as a bolus, electuary, or paste. It is generally understood that methods of preparations of the above dosage forms are generally known in the art, and any such method would be suitable for the preparation of the respective dosage forms for use in delivery of the compositions according to the present invention.
In one embodiment, compound may be administered orally in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an edible carrier. Oral compositions may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets or may be incorporated directly with the food of the patient's diet. The percentage of the composition and preparations may be varied; however, the amount of substance in such therapeutically useful compositions is preferably such that an effective dosage level will be obtained.

Hard capsules containing the compound may be made using a physiologically degradable composition, such as gelatin. Such hard capsules comprise the compound, and may further comprise additional ingredients including, for example, an inert solid diluent such as calcium carbonate, calcium phosphate, or kaolin. Soft gelatin capsules containing the compound may be made using a physiologically degradable composition, such as gelatin. Such soft capsules comprise the compound, which may be mixed with water or an oil medium such as peanut oil, liquid paraffin, or olive oil.

Sublingual tablets are designed to dissolve very rapidly. Examples of such compositions include ergotamine tartrate, isosorbide dinitrate, and isoproterenol HCl. The compositions of these tablets contain, in addition to the drug, various soluble excipients, such as lactose, powdered sucrose, dextrose, and mannitol. The solid dosage forms of the present invention may optionally be coated, and examples of suitable coating materials include, but are not limited to, cellulose polymers (such as cellulose acetate phthalate, hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, and hydroxypropyl methylcellulose acetate succinate), polyvinyl acetate phthalate, acrylic acid polymers and copolymers, and methacrylic resins (such as those commercially available under the trade name EUDRAGIT®), zein, shellac, and polysaccharides.

Powdered and granular compositions of a pharmaceutical preparation of the invention may be prepared using known methods. Such compositions may be administered directly to a patient or used in the preparation of further dosage forms, such as to form tablets, fill capsules, or prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these compositions may further comprise one or more additives, such as dispersing or wetting agents, suspending agents, and preservatives. Additional excipients (e.g., fillers, sweeteners, flavoring, or coloring agents) may also be included in these compositions.
Liquid compositions of the pharmaceutical composition of the invention which are suitable for oral administration may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

A tablet containing one or more compounds according to the present invention may be manufactured by any standard process readily known to one of skill in the art, such as, for example, by compression or molding, optionally with one or more adjuvant or accessory ingredient. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active agents.

Adjuvants or accessory ingredients for use in the compositions of the present invention can include any pharmaceutical ingredient commonly deemed acceptable in the art, such as binders, fillers, lubricants, disintegrants, diluents, surfactants, stabilizers, preservatives, flavoring and coloring agents, and the like. Binders are generally used to facilitate cohesiveness of the tablet and ensure the tablet remains intact after compression. Suitable binders include, but are not limited to: starch, polysaccharides, gelatin, polyethylene glycol, propylene glycol, waxes, and natural and synthetic gums. Acceptable fillers include silicon dioxide, titanium dioxide, alumina, talc, kaolin, powdered cellulose, and microcrystalline cellulose, as well as soluble materials, such as mannitol, urea, sucrose, lactose, dextrose, sodium chloride, and sorbitol. Lubricants are useful for facilitating tablet manufacture and include vegetable oils, glycerin, magnesium stearate, calcium stearate, and stearic acid. Disintegrants, which are useful for facilitating disintegration of the tablet, generally include starches, clays, celluloses, algins, gums, and crosslinked polymers. Diluents, which are generally included to provide bulk to the tablet, may include dicalcium phosphate, calcium sulfate, lactose, cellulose, kaolin, mannitol, sodium chloride, dry starch, and powdered sugar. Surfactants suitable for use in the composition according to the present invention may be anionic, cationic, amphoteric, or nonionic surface active agents. Stabilizers may be included in the compositions to inhibit or lessen reactions leading to decomposition of the active agents, such as oxidative reactions.

Solid dosage forms may be formulated so as to provide a delayed release of the active agents, such as by application of a coating. Delayed release coatings are known in the art, and dosage forms containing such may be prepared by any known suitable method. Such methods generally include that, after preparation of the solid dosage form (e.g., a tablet or caplet), a delayed release coating composition is applied.
Application can be by methods, such as airless spraying, fluidized bed coating, use of a coating pan, or the like. Materials for use as a delayed release coating can be polymeric in nature, such as cellulosic material (e.g., cellulose butyrate phthalate, hydroxypropyl methylcellulose phthalate, and carboxymethyl ethylcellulose), and polymers and copolymers of acrylic acid, methacrylic acid, and esters thereof.

Solid dosage forms according to the present invention may also be sustained release (i.e., releasing the active agents over a prolonged period of time), and may or may not also be delayed release. Sustained release compositions are known in the art and are generally prepared by dispersing a drug within a matrix of a gradually degradable or hydrolyzable material, such as an insoluble plastic, a hydrophilic polymer, or a fatty compound. Alternatively, a solid dosage form may be coated with such a material.

Compositions for parenteral administration include aqueous and non-aqueous sterile injection solutions, which may further contain additional agents, such as antioxidants, buffers, bacteriostats, and solutes, which render the compositions isotonic with the blood of the intended recipient. The compositions may include aqueous and non-aqueous sterile suspensions, which contain suspending agents and thickening agents. Such compositions for parenteral administration may be presented in unit-dose or multi-dose containers, such as, for example, sealed ampoules and vials, and may be stores in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water (for injection), immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets of the kind previously described.

The compositions according to the present invention may also be administered transdermally, wherein the active agents are incorporated into a laminated structure (generally referred to as a "patch") that is adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Typically, such patches are available as single layer "drug-in-adhesive" patches or as multi-layer patches where the active agents are contained in a layer separate from the adhesive layer.

Both types of patches also generally contain a backing layer and a liner that is removed prior to attachment to the skin of the recipient. Transdermal drug delivery patches may also be comprised of a reservoir underlying the backing layer that is
separated from the skin of the recipient by a semi-permeable membrane and adhesive layer. Transdermal drug delivery may occur through passive diffusion or may be facilitated using electrotransport or iontophoresis.

Compositions for rectal delivery of the compositions of the present invention include rectal suppositories, creams, ointments, and liquids. Suppositories may be presented as the active agents in combination with a carrier generally known in the art, such as polyethylene glycol. Such dosage forms may be designed to disintegrate rapidly or over an extended period of time, and the time to complete disintegration can range from a short time, such as about 10 minutes, to an extended period of time, such as about 6 hours.

Topical compositions may be in any form suitable and readily known in the art for delivery of active agents to the body surface, including dermally, buccally, and sublingually. Typical examples of topical compositions include ointments, creams, gels, pastes, and solutions. Compositions for topical administration in the mouth also include lozenges.

In certain embodiments, the compounds and compositions disclosed herein can be delivered via a medical device. Such delivery can generally be via any insertable or implantable medical device, including, but not limited to stents, catheters, balloon catheters, shunts, or coils. In one embodiment, the present invention provides medical devices, such as stents, the surface of which is coated with a compound or composition as described herein. The medical device of this invention can be used, for example, in any application for treating, preventing, or otherwise affecting the course of a disease or condition, such as those disclosed herein.

In another embodiment of the invention, the pharmaceutical composition comprising one or more compounds described herein is administered intermittently. Administration of the therapeutically effective dose may be achieved in a continuous manner, as for example with a sustained-release composition, or it may be achieved according to a desired daily dosage regimen, as for example with one, two, three, or more administrations per day. By "time period of discontinuance" is intended a discontinuing of the continuous sustained-released or daily administration of the composition. The time period of discontinuance may be longer or shorter than the period of continuous sustained-release or daily administration. During the time period of discontinuance, the level of the components of the composition in the relevant tissue is substantially below the maximum level obtained during the treatment. The
preferred length of the discontinuance period depends on the concentration of the
effective dose and the form of composition used. The discontinuance period can be at
least 2 days, at least 4 days or at least 1 week. In other embodiments, the period of
discontinuance is at least 1 month, 2 months, 3 months, 4 months or greater. When a
sustained-release composition is used, the discontinuance period must be extended to
account for the greater residence time of the composition in the body. Alternatively,
the frequency of administration of the effective dose of the sustained-release
composition can be decreased accordingly. An intermittent schedule of
administration of a composition of the invention can continue until the desired
therapeutic effect, and ultimately treatment of the disease or disorder, is achieved.

Administration of the composition according to the invention comprises
administering a single pharmaceutically active compound as described herein;
administering a pharmaceutically active compound as described herein with one or
more further pharmaceutically active compounds described herein; or administering
one or more pharmaceutically active compounds described herein in combination with
one or more further pharmaceutically active compounds (i.e., co-administration).
Accordingly, it is recognized that the pharmaceutically active compounds in the
compositions of the invention can be administered in a fixed combination (i.e., a
single pharmaceutical composition that contains both active materials). Alternatively,
the pharmaceutically active compounds may be administered simultaneously (i.e.,
separate compositions administered at the same time). In another embodiment, the
pharmaceutically active compounds are administered sequentially (i.e., administration
of one or more pharmaceutically active compounds followed by separate
administration or one or more pharmaceutically active compounds). One of skill in
the art will recognized that the most preferred method of administration will allow the
desired therapeutic effect.

Delivery of a therapeutically effective amount of a composition according to
the invention may be obtained via administration of a therapeutically effective dose of
the composition. Accordingly, in one embodiment, a therapeutically effective amount
is an amount effective to treat abnormal cell proliferation. In another embodiment, a
therapeutically effective amount is an amount effective to treat inflammation. In
specific embodiments, a therapeutically effective amount is an amount effective to:
inhibit viability of a cancerous cell line; inhibit formation of new cancerous cells;
and/or induce apoptosis of cancerous cells.
The active compounds are included in the pharmaceutical composition in an amount sufficient to deliver to a patient a therapeutic amount of a compound of the invention in vivo in the absence of serious toxic effects. The concentration of active compound in the drug composition will depend on absorption, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the medical professional administering or supervising the administration of the compositions, and that the dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed compositions including the inventive combinations. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

A therapeutically effective amount according to the invention can be determined based on the body weight of the recipient. Alternatively, a therapeutically effective amount can be described in terms of a fixed dose. The effective dosage range of pharmaceutically acceptable salts and prodrugs can be calculated based on the weight of the parent compounds to be delivered. If a salt or prodrug exhibits activity in itself, the effective dosage can be estimated as above using the weight of the salt or prodrug, or by other means known to those skilled in the art.

It is contemplated that the compositions of the invention comprising one or more compounds described herein will be administered in therapeutically effective amounts to a mammal, preferably a human. An effective dose of a compound or composition for treatment of any of the conditions or diseases described herein can be readily determined by the use of conventional techniques and by observing results obtained under analogous circumstances. The effective amount of the compositions would be expected to vary according to the weight, sex, age, and medical history of the subject. For example, in relation to NF-κB inhibiting compounds used in the inventive combinations (e.g., curcumin analogs), a dosage from about 0.5 to about 20 mg/kg body weight, preferably from about 1.0 to about 5.0 mg/kg, will have therapeutic efficacy.
Of course, other factors could also influence the effective amount of the composition to be delivered, including, but not limited to, the specific disease involved, the degree of involvement or the severity of the disease, the response of the individual patient, the particular compound administered, the mode of administration, the bioavailability characteristics of the preparation administered, the dose regimen selected, and the use of concomitant medication. The compound is preferentially administered for a sufficient time period to alleviate the undesired symptoms and the clinical signs associated with the condition being treated. Methods to determine efficacy and dosage are known to those skilled in the art. See, for example, Isselbacher et al. (1996) *Harrison's Principles of Internal Medicine* 13 ed., 1814-1882, herein incorporated by reference.

V. Articles of Manufacture

The present invention also includes an article of manufacture providing a composition comprising the compounds described herein. The article of manufacture can include a vial or other container that contains a composition suitable for use according to the present invention together with any carrier, either dried or in liquid form. In particular, the article of manufacture can comprise a kit including a container with a composition according to the invention. In such a kit, the composition can be delivered in a variety of combinations. For example, the composition can comprise a single dosage comprising all of the active ingredients in the inventive combination. Alternately, the composition can comprise multiple dosages, each comprising one or more of the active ingredients forming the inventive combination, the dosages being intended for administration in combination, in succession, or in other close proximity of time. For example, the dosages could be solid forms (e.g., tablets, caplets, capsules, or the like) or liquid forms (e.g., vials), each comprising a single active ingredient, but being provided in blister packs, bags, or the like, for administration in combination.

The article of manufacture further includes instructions for carrying out the methods of the invention. Such instructions may be in various forms, such as a label on the container, an insert included in a box in which the container is packaged, or a variety of computer readable formats. The instructions can also be printed on the box in which the vial is packaged. The instructions contain information such as sufficient dosage and administration information so as to allow the subject or a worker in the field to administer the pharmaceutical composition(s) providing the inventive combination of
active agents. It is anticipated that a worker in the field encompasses any doctor, nurse, 
technician, spouse, or other caregiver that might administer the composition(s). The 
pharmaceutical composition(s) can also be self-administered by the subject.

VI. Methods of Treatment

The susceptibility of cancer cells to chemotherapeutic -induced cell death can be 
dependent upon a balance between cell death and survival signaling. Increased 
survival signaling could counteract drug efficacy. For example, Taxol is known to 
induce phosphorylation and degradation of IKB, leading to the nuclear translocation 
of the NF-κB dimer, and this increased NF-κB activation may lead to Taxol 
resistance.

According to the present invention, it has been found that combinations of NF-
κB inhibiting compounds and p38 MAPK inhibiting compounds can provide a 
synergistic effect in treating and preventing cancers and inflammatory conditions.

Although not wishing to be bound by theory, it is believed that one or more of the 
MAPK pathways induced by treatment with NF-κB inhibitors (e.g., the curcumin analog EF24) can mediate a survival pathway to counteract induction of cell death. 
As more fully described below in the Experimental discussion, the present invention 
has illustrated that combinations of specific NF-κB inhibitors and p38 MAPK 
inhibitors exhibit synergy in cancer models indicating that p38 MAPK is a survival 
pathway induced by the administration of the NF-κB inhibitor.

The synergistic effect of the various combinations according to the invention can give rise to a great variety of useful treatments. For example, the combinations of 
the present invention can be used in the treatment of cancerous tissue and the tumors 
associated therewith. Specific, non-limiting types of benign tumors that can be 
treated according to the present invention include hemangiomas, hepatocellular 
adenoma, cavernous hemangiomas, focal nodular hyperplasia, acoustic neuromas, 
neurofibroma, bile duct adenoma, bile duct cystanoma, fibroma, lipomas, 
leiomyomas, mesotheliomas, teratomas, myxomas, nodular regenerative hyperplasia, 
trachomas, and pyogenic granulomas.

Representative, non-limiting cancers treatable according to the invention include 
breast cancer, skin cancer, bone cancer, prostate cancer, liver cancer, lung 
cancer, brain cancer, cancer of the larynx, gallbladder, pancreas, rectum, parathyroid, 
thyroid, adrenal, neural tissue, head and neck, colon, stomach, bronchi, kidneys, basal
cell carcinoma, squamous cell carcinoma of both ulcerating and papillary type, metastatic skin carcinoma, osteo sarcoma, Ewing's sarcoma, reticulum cell sarcoma, myeloma, giant cell tumor, small-cell lung tumor, gallstones, islet cell tumor, primary brain tumor, acute and chronic lymphocytic and granulocytic tumors, hairy-cell tumor, adenoma, hyperplasia, medullary carcinoma, pheochromocytoma, mucosal neuromas, intestinal ganglioneuromas, hyperplastic corneal nerve tumor, marfanoid habitus tumor, Wilm's tumor, seminoma, ovarian tumor, leiomyomater tumor, cervical dysplasia and in situ carcinoma, neuroblastoma, retinoblastoma, soft tissue sarcoma, malignant carcinoid, topical skin lesion, mycosis fungoide, rhabdomyosarcoma,

Kaposi's sarcoma, osteogenic and other sarcoma, malignant hypercalcemia, renal cell tumor, polycythemia vera, adenocarcinoma, glioblastoma multiforma, leukemias, lymphomas, malignant melanomas, epidermoid carcinomas, and other carcinomas and sarcomas.

The combinations of the present invention are also useful in the treatment of diseases characterized by inflammation. Diseases and conditions which have significant inflammatory components are ubiquitous and include, for example, skin disorders, bowel disorders, certain degenerative neurological disorders, arthritis, autoimmune diseases and a variety of other illnesses. In particular embodiments the combinations may be used to treat inflammatory bowel diseases (IBD), Crohn's disease (CD), ulcerative colitis (UC), chronic obstructive pulmonary disease (COPD), sarcoidosis, or psoriasis. The disclosed combinations may also be useful in the treatment of other inflammatory diseases, for example, allergic disorders, skin disorders, transplant rejection, poststreptococcal and autoimmune renal failure, septic shock, systemic inflammatory response syndrome (SIRS), adult respiratory distress syndrome (ARDS), envenomation, lupus erythematosus, Hashimoto's thyroiditis, autoimmune hemolytic anemias, insulin dependent diabetes mellitus, and rheumatic fever, pelvic inflammatory disease (PID), conjunctivitis, dermatitis, and bronchitis.

Subjects which can be treated include animal subjects, typically vertebrates, including both mammalian (e.g., human, cat, dog, cow, horse, sheep, pig, monkey, ape, etc.) and avian subjects (e.g., chicken, turkey, duck, goose, quail, pheasant, etc.).

In particular embodiments, the invention provides methods for enhancing the anti-cancer and/or anti-inflammatory activity of NF-κB inhibiting compounds, and particularly curcumin analogs. Curcumin analogs, such as the compound designated EF24, exhibit excellent anti-cancer and anti-inflammatory properties. However, the
The present invention has made the surprising discovery that co-administration of an NF-κB inhibiting compound, such as EF24, with a p38 MAPK inhibiting compound gives rise to a synergistic effect. For example, as described in the Examples below, the combination of a p38 inhibitor with EF24 synergistically increased the anti-cancer activity of the compound. In specific embodiments, the increased anti-cancer activity is exemplified as an increase in the activity of the curcumin analog for inhibiting growth of cancer cells. In further specific embodiments, the increased anti-cancer activity is exemplified as an increase in the activity for inducing apoptosis of cancer cells. Of course, this scope of increased anti-cancer activity should not be viewed as limiting the scope of the invention. Rather, it is believed that the surprising synergism exhibited by the inventive combinations would be conserved across the entire scope of activities of curcumin analogs specifically and also NF-κB inhibiting compounds generally.

EXPERIMENTAL

The present invention will now be described with specific reference to various examples. The following examples are not intended to be limiting of the invention and are rather provided as exemplary embodiments.

The combination of a NF-κB inhibiting compound (the curcumin analog EF24) and p38 MAPK inhibiting compounds (the pyridinyl imidazole compounds SB203580 - designated "SB80" - and SB202190 - designated "SB90") was evaluated in an A549 lung cancer model system. As a comparative, tests were also performed using curcumin as the NF-κB inhibiting compound. Curcumin and the analog EF24 were secured from the Department of Chemistry at Emory University. Synthesis of EF24 has previously been disclosed (such as in U.S. Patent No. 6,664,272 noted above). Stock solutions of curcumin and EF24 (0.01mM) were made in DMSO and stored in aliquots at -20°C. The compounds were diluted in incubation media immediately prior to each experiment. The pyridinyl imidazole compounds SB203580 (Promega; Madison, WI) and SB202 190 (Biosource; Camarillo, CA) were used as specific p38/β MAPK inhibitors. Additional materials used included the specific IKK-2 inhibitor IV (available from Calbiochem), and antibodies against total
and phospho-JNK (Thr^{183}/Tyr^{185}), total and phospho-p38 MAPK (Thr^{180}/Tyr^{182}), total and phospho-ERK (Thr^{202}/Tyr^{204}), and PARP (full length and cleaved fragments) (all available from Cell Signaling, Beverly, MA).

The human non-small cell lung cancer (NSCLC) cell line A549 were grown in RPMI-1640, 10% CellGro FBS and maintained at 37°C in an atmosphere containing 10% CO₂. The media was supplemented with 1% penicillin/streptomycin.

**EXAMPLE 1**

Growth Inhibition of Cancer Cells by EF24 and Curcumin

A549 lung cancer cells were plated at a density of 5,000 cells/well in 96-well plates and allowed to adhere overnight. The following day the cells were treated with EF24 (0.4 µM, 0.8 µM) or curcumin (8 µM, 15 µM) for 24, 48, or 72h. Curcumin concentrations were chosen in light of previous testing indicating EF24 is approximately 10 times more cytotoxic than curcumin.

To accurately obtain the IC₅₀ values, the sulforhodamine B assay was performed. Cells were fixed with 50 µl of 10% TCA at 4°C for 1 hr. The plates were washed with water and stained with 4% SRB (100 µl). Acetic acid (1%) was then used to wash the plates. Lastly, 10mM unbuffered Tris was added to solubilize the dye. Absorbance at 490 nm was recorded using a 96-well plate reader. The mean value and standard error for each treatment were determined and the % cell viability relative to control (0.5% DMSO) was calculated. The IC₅₀ is defined as the concentration of drug that kills 50% of the total cell population as compared to control cells at the end of the incubation period.

Growth inhibition results are shown in FIG. 1, wherein data is reported as the average of at least two independent experiments. In particular, IC₅₀ for EF24 was about 1 µM at 24h and between 0.4 and 0.8 µM after 48 and 72h, respectively. Treatment of A549 with curcumin caused growth inhibition with an IC₅₀ of >20 µM after 24h, between 15 and 20 µM after 48h, and approximately 15 µM at 72h. These results show that both EF24 and curcumin inhibit growth of A549 cells in a dose- and time-dependent manner, with EF24 providing the greatest inhibitory activity.
EXAMPLE 2
Immunoblot Analysis

The pathways modulated by treatment with EF24 were analyzed using an immunoblot analysis. Cells were lysed in 1% NP-40 lysis buffer supplemented with one protease inhibitor cocktail table (Roche, Indianapolis, IN) and protein (25-40 ug/lane) from the whole cell extracts were resolved by 12.5% SDS-PAGE, electrotransferred to nitrocellulose membranes, and blocked with 5% nonfat dry milk in TBS-Tween 20. The blots were then incubated with the indicated primary antibodies, followed by horseradish peroxidase-conjugated secondary antiserum. Immunoreactivity was visualized by enhanced chemiluminescence reagent (Amersham Biosciences, Piscataway, New Jersey). For sequential blotting with additional antibodies, the membranes were stripped using a strip buffer solution with BME (1:1000) and reprobed with the indicated antibodies.

EF24 is known to downregulate TNFα-induced NF-κB activation by negatively regulating the activity of the upstream kinase of IκB, IKK. Cross talk is known to exist between NFκB pathway and other important in regulating cell survival and proliferation including the three-tiered MAPK signaling pathways. Specifically, A549 cells were treated with increasing concentrations of EF24 or curcumin for 30' and immunoblotting was performed. The activation of ERK, p38 MAPK, and JNK were determined using phospho-specific antibodies for the Thr/Tyr activation motifs.

EF24 was found to induce the activation of each of the MAPKs in a dose-dependent manner, as illustrated in FIG. 2. ERK was activated in response to curcumin as well as p38 MAPK, however higher concentrations up to 50 and 100 µM were needed. At the time and doses used for this experiment, no phosphorylation of JNK was detected with curcumin treatment while activation of JNK was detected after 5µM treatment of EF24.

EXAMPLE 3
Effect of ERK or JNK Inhibition on EF24-Induced Cell Cytotoxicity

Since ERK participates in a mitogenic pathway that promotes cell survival and EF24 induces activation of ERK, tests were conducted to determine whether this pathway is important for the activity of EF24 and if inhibition of ERK would further augment the loss of cell viability of EF24-treated cells. A low dose of EF24 (0.4µM)
was chosen for this experiment since it only induces a low level of growth inhibition (15-25%) of A549 alone after 48h. The chemical inhibitor U0126 was used to selectively downregulate the activation of ERK by targeting the upstream kinase MEK. The concentrations of U0126 used in this study were determined to inhibit EF24-induced ERK phosphorylation. When comparing the percent of cell viability of EF24 alone, U0126 alone, and the combination of EF24 and U0126, no significant change in cell viability with inhibition of ERK was observed.

Similarly, the effect of JNK inhibition on EF24-induced cytotoxicity was evaluated. Several recent reports implicate JNK in the regulation of the apoptotic response in which activated JNK mediates the induction of cell death. Subsequently, it was hypothesized that inhibition of JNK would attenuate EF24-induced cell death. A treatment of EF24 and the pharmacological JNK inhibitor SP600125 was added simultaneously to A549 cells for 48h. JNK inhibition did not attenuate the loss of cell viability by EF24 but only caused a slightly additive drop in cell viability.

**EXAMPLE 4**

Enhancement of EF24-Induced Cytotoxicity of A549 Cells with p38 MAPK Inhibitors

The potential of p38 MAPK inhibition to affect the growth inhibitory effects of EF24 was evaluated. The same low dose of EF24 (0.4µM) was used as in the previous Examples. EF24 treatment was combined with increasing concentration of a pyridinyl imidazole p38 MAPK inhibitor (SB203580 or SB202190) that did not significantly inhibit A549 cell viability alone. A549 cells were plated at 5,000 cells/well in a 96 well plate. The following day the cells were dosed with the indicated treatments for 48 hours. The active agents used in each test were added simultaneously to the treatment media. Test results are shown in FIG. 3A - FIG. 3D.

Treatment with the p38 MAPK inhibitors alone (12.5 µM) had little effect on cell survival of A549 cell. As seen in FIG. 3C, administration of SB80 at this concentration affected only 5% of the cells. A549 cells treated with the same concentration of SB90 retained about 80-85% cell viability. The inhibition of p38 MAPK activity by SB80 was also demonstrated by evaluating the phosphorylation of MAPKAPK-2, an immediate downstream effector of p38 MAPK (Fig. 3D).

When SB80 was combined with EF24, the percentage of growth inhibition was noticeably greater than the additive effects of each compound alone (FIG. 3B).
The combination of SB90 and EF24 also had a dramatic effect on cell viability, particularly at an SB90 concentration of 12.5 \( \mu M \) (FIG. 3A). This combination of EF24 and p38 MAPK inhibitors was considered synergistic and specific to p38 MAPK inhibitors since the same synergistic cytotoxicity was not observed using the MEK or JNK inhibitors. Since SB80 has the least effect on A549 viability alone while still inhibiting p38 MAPK activity, this compound was used as the pharmacological p38 MAPK inhibitor for the rest of the experiments.

EXAMPLE 5

Dose Response Curve and Combination Index Analysis on Combination of EF24 and SB80

The Chou and Talalay combination index (CI) is a well-established index to determine the pharmacologic interaction of two drugs and is based on the multiple drug-effect equation derived from enzyme kinetic models. For the median-effect plots for each single agent, log \( \frac{fa}{fu} \) is plotted against log (D), where D represents the concentration of each single compound alone of the mixture of both while \( fa \) stands for the fraction affected (0 > I) and \( fu \) is the fraction unaffected (I > fa) at each concentration D. The CalcuSyn software (Biosoft, Ferguson, MO) was used to compute a CI for every fraction affected. Synergism is represented by a CKI, additivity by a CI = I, and antagonism with a CI > 1. Antagonism in this case is any effect less than an additive effect.

As the results from Example 4 illustrate, the combination of EF24 with p38 MAPK inhibitors provides a synergistic effect that exceeds a mere additive effect on growth inhibition. To further evaluate this synergistic effect, the dose response curve for EF24 was graphed by titrating increasing amounts of this compound into the media in combination with SB80 and determining the cell viability at each concentration after 48h. This is illustrated in FIG. 4A. As a comparative, the same dose response curve evaluation was conducted with curcumin, as shown in FIG. 4B.

These curves were compared to curves for the combination of EF24 or curcumin with SB80.

The IC50 was shown to be 0.75 \( \mu M \) for EF24 alone and 0.45 \( \mu M \) for the combination of EF24 and SB80. This represents an approximate 40% reduction in IC50, which is significant.
These graphs were used to analyze the CI values of EF24 and SB80 as described above. Synergism was observed for the combinations of EF24 and SB80 up to an EF24 concentration of 1 µM. CI values for the first two points on the graph were 0.3 < CI > 0.7, and CI values for the points up to 1 µM were 0.7 < CI > 0.85.

EXAMPLE 6
Effect of Genetic Inhibition of p38 MAPK on Sensitizing A549 Cancer Cells to EF24-induced Loss of Cell Viability

In order to determine if the inhibition of p38 MAPK is the true event that enhances EF24-induced cytotoxicity, a genetic inhibition of p38 MAPK was evaluated. A549 cells were doubly transfected with siRNA targeting p38 MAPK protein. 24h after the last transfections, total p38 MAPK levels were evaluated for the levels of reduction. There was a 50-70% knockdown of p38, which was deemed fairly specific based on ERK expression levels (see FIG. 5A and FIG. 5B). For analysis of cell viability, the cells were also double transfected and then treated with vehicle (0.5% DMSO) or the low dose of EF24 (0.4 µM) (FIG. 5C). Percent cell viability was analyzed after 48h and compared to a mock transfected control with a scramble siRNA. The combination of p38 siRNA and EF24 treatment significantly caused the loss of cell viability similar to that of the combination of EF24 and SB80.

EXAMPLE 7
Synergistic Inhibition of A549 Colony Formation by Combination of EF24 and SB80

To assess whether the combination of EF24 and SB80 was effective on relatively long term A549 cancer cell proliferation, a 10-day colony formation assay was conducted, as illustrated in FIG. 6A. The inhibitor SB80 was used instead of SB90 because the IC50 of SB80 was found to be 2-fold higher in cell viability assays (about 50 µM). Previous data determined the IC50 for inhibition of colony formation for EF24 was about 150nM. For the combination treatment for this experiment, 100nM of EF24 was used. To keep the EF24:SB80 ratio similar to that used in cell viability experiments (1:32.5), 5µM of SB80 was used. As expected, the combination proved effective in decreasing the number of colonies between all treatment groups by approximately 50% and also inhibiting the overall size of the colonies. This is illustrated in FIG. 6B.
EXAMPLE 8
Effect of Inventive Combination Treatment on
Induction of Apoptosis

Apoptosis was examined by flow cytometry of propidium iodide (PI)-stained cells. Unsynchronized A549 cells were exposed to the indicated drug treatment for 48h (0.4 μM EF24 and 12.5 μM SB80, alone or in combination). Attached and unattached cells were then collected, washed with 1%BSA/PBS and fixed with 75% cold ethanol for at least 1hr. PI (50μg/mL) was then used to stain the cells, and the DNA content of these stained cells was measured by a flow cytometer.

The data represents the cell cycle distribution for a representative experiment. FIG. 7A provides the cell cycle analysis of combined treatment of EF24 (0.4μM) and the p38 inhibitor SB80 (12.5μM). FIG. 7B provides quantification of the % of sub-G1 cells for three independent experiments. FIG. 7C provides Western blot analysis of A549 lysates treated with EF24 (0.4μM), SB203580 (12.5μM), curcumin (10μM) or the combination of two drugs after 48h. The amount of full length and cleaved PARP was evaluated using the specific antibody, and these results are provided in FIG. 7D.

A549 cells were plated in a 96 well plate at a density of 5,000 cells/well. The following day, the cells were treated with the indicated concentrations of EF24, SB80, Cure, or the combinations of EF24 or curcumin with SB203580 for 48h. The cellular stains DAPI, Yo-Pro-1, and PI were added to each well. Yo-Pro-1 positive cells are deemed early apoptotic, Pi-positive cells are necrotic, and Yo-Pro-1 and Pi-positive cells are late apoptotic. Cells that excluded both stains but had intact nuclear staining were deemed viable. A second concentration of EF24 (0.6μM) served as a control to ensure the assay could identify an increase in apoptosis. FIG. 7E shows the results of a cell viability assay that was conducted in which A549 was pretreated with the caspase inhibitor zVAD-fmk for 1hr before treatment with the combination of EF24 and SB80 at the same concentrations in previous experiments.

The treatment of 0.4μM EF24 or 12.5μM SB80 alone for 48h resulted in no significant difference in the percent of apoptotic cells from the vehicle-control. However, when these two agents were combined, a synergistic accumulation of cells in the sub-G1 fraction, widely accepted to correspond to cells undergoing apoptosis, was observed. Moreover, differentiating between apoptotic and necrotic cells using
double-staining techniques shows that the cells treated with the combination explicitly
undergo apoptosis (FIG 7C). The trend was also seen using western blot analysis
when examining combination drug-induced cleavage of PARP, another hallmark of
the induction of programmed cell death (FIG. 7D). Since PARP cleavage implicates
to the combination of EF24 and SB80 inducing caspase activation to elicit apoptosis,
testing was carried out to determine whether inhibiting global caspase activity could
attenuate the loss of cell viability induced by this combination (i.e., the zVAD-fmk
pretreatment described above). The pretreatment was successful in partially blocking
the loss of cell viability by EF24 and SB80 (FIG. 7E).

EXAMPLE 9
Scheduling of NF-κB Inhibitor and p38 Inhibitor Treatment

In the foregoing experiments, the combination of the NF-κB EF24 and the p38
inhibitors SB80 and SB90 consisted of simultaneous administration of the
compounds. To evaluate whether scheduling of doses affected observed synergy,
A549 cells were treated with either EF24 of SB90 for 30 minutes prior to the removal
of the media and the addition of the combination treatment. No significant reduction
of the synergy was observed when SB90 was pretreated compared to the combination
alone or pretreatment with EF24.

Many modifications and other embodiments of the invention will come to
mind to one skilled in the art to which this invention pertains having the benefit of the
teachings presented in the foregoing descriptions and the associated drawings.
Therefore, it is to be understood that the invention is not to be limited to the specific
embodiments disclosed and that modifications and other embodiments are intended to
be included within the scope of the appended claims. Although specific terms are
employed herein, they are used in a generic and descriptive sense only and not for
purposes of limitation.
THAT WHICH IS CLAIMED:

1. A pharmaceutical composition comprising a NF-κB (nuclear factor-kappa B) inhibiting compound and a p38 MAPK (mitogen activated protein kinase) inhibiting compound.

2. The composition of claim 1, wherein the NF-κB inhibiting compound is a compound that performs one or more of the following functions: inhibits activity of NF-κB, inhibits activation of the NF-κB pathway, increases the sensitivity of NF-κB to conventional chemotherapy, or inhibits phosphorylation or degradation of naturally occurring NF-κB inhibitors.

3. The composition of claim 1, wherein the NF-κB inhibiting compound is a curcumin analog.

4. The composition of claim 3, wherein the curcumin analog is a compound according to the structure of Formula (1)

\[
\text{Ar} - \text{Ar} - \text{Ar} 
\] (1)

wherein:

each Ar is optionally substituted and is independently a ring structure comprising 5-20 ring atoms and is selected from the group consisting of aryl, substituted aryl, heteroaryl having 1-3 heteroatoms selected from O, S, and N, substituted heteroaryl, heterocycle having 1-3 heteroatoms selected from O, S, and N, and substituted heterocycle;

any substituent on Ar is selected from the group of halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, azide, alkylcarbonyl, acyl, trialkylammonium, NH-aa, and O-aa, where aa is an amino acid;

A is selected from the group consisting of:
n is 1-8;
X is selected from the group consisting of -CH₂⁻, -CH₂⁻CH₂⁻, -CH₂⁻CH₂⁻CH₂⁻, -CH₂⁻CH₂⁻CH₂⁻⁻,
O, -O-NR₃⁻, S, SO, SO₂⁻, -S-S-, NR₃⁻, and -NR₃⁻NR₃⁻⁻;
Q is NH or NR₃⁻;
Vi₋₄ are each independently OH, OR₂⁻, or halogen;
R₁, R₂, and R₃ are independently H, alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, acyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl or dialkylaminocarbonyl;

the dashed lines indicate the presence of optional double bonds; and

L is the point of bonding of A to the compound structure;

as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

5. The composition of claim 3, wherein the curcumin analog is a compound according to the structure of Formula (2)

wherein:

each Ar is optionally substituted and is independently a ring structure comprising 5-20 ring atoms, selected from the group consisting of aryl, substituted aryl, heteroaryl having 1-3 heteroatoms selected from O, S, and N, substituted heteroaryl, heterocycle having 1-3 heteroatoms selected from O, S, and N, and substituted heterocycle;

any substituent on Ar is selected from the group of halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, CF₃, alkanyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, azide, alky carbonyl, acyl, trialkylammonium, NH-aa, and O-aa, where aa is an amino acid;

X is optional and is selected from the group consisting of -CH₂-, -CH₂-CH₂-, -CH₂-CH₂-CH₂-, O, -O-NR₃-, S, SO, SO₂-, -S-, NR₃, and -NR₃-NR₃-;
R₃ is H, alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, acyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl or dialkylaminocarbonyl;

Yᵢ is optional and is selected from the group consisting of halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, azide, alkylcarbonyl, acyl, trialkylammonium, NH-aa, and O-aa, where aa is an amino acid, or Yᵢ forms a fused ring structure with the central ring comprising X, the ring structure being carbocyclic, heterocyclic, aryl, or heteroaryl;

the dashed lines indicate the presence of optional double bonds;

as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

6. The composition of claim 3, wherein the curcumin analog is a compound according to the structure of Formula (3)

\[
\begin{align*}
 &\text{wherein:} \\
 &X \text{ is optional and is selected from the group consisting of } -\text{CH}_2-, -\text{CH}_2\text{-CH}_2-, -\text{CH}_2\text{-CH}_2\text{-CH}_2-, \text{ O, } -\text{O-NR}_3-, \text{ S, SO, SO}_2, -\text{S-S-}, \text{ NR}_3, \text{ and } -\text{NR}_3\text{-NR}_3^-; \\
 &R_3 \text{ is H, alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, acyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl or dialkylaminocarbonyl;}
\end{align*}
\]

each Yᵢ and Y₂ is optional and is independently selected from the group consisting of halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted
heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, azide, alkylcarbonyl, acyl, trialkylammonium, NH-aa, and O-aa, where aa is an amino acid; the dashed lines indicate the presence of optional double bonds; as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

7. The composition of claim 6, wherein:
   X is NR₃;
   R₁ is H, alkyl, substituted alkyl, or alkoxy;
   Y₁ is absent; and
   Y₂ is halo, hydroxyl, alkoxy, or CF₃.

8. The composition of claim 3, wherein the curcumin analog is a compound according to the structure of Formula (6)

   \[
   \begin{align*}
   \text{\textbf{F}} & \quad \text{O} \\
   \text{\textbf{F}} & \quad \text{\textbf{N}} \\
   \end{align*}
   \]

9. The composition of claim 1, wherein the p38 MAPK inhibiting compound is a compound having the structure of Formula (23)

   \[
   \begin{align*}
   \text{\textbf{Ar}} & \quad \text{Het} \\
   \text{\textbf{Ar}} & \quad \text{Z} \\
   \end{align*}
   \]

wherein:
Het indicates a 5-membered heterocycle with 1-3 ring carbons replaced with an atom selected from N, O, and S or a 6-membered heterocycle with 1-4 ring carbons replaced with an atom selected from N, O, and S;

each Ar is optionally substituted and is independently a ring structure comprising 5-20 ring atoms, selected from the group consisting of aryl, substituted aryl, heteroaryl having 1-3 heteroatoms selected from O, S, and N, substituted heteroaryl, heterocycle having 1-3 heteroatoms selected from O, S, and N, and substituted heterocycle;

a Z substituent may be present on any ring atom not bonded to an Ar group;

Z and any substituent on Ar are independently selected from the group of H, halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF$_3$, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkylsulfonyl, sulfinyl, alkylsulfinyl, sulfenyl, and trialkylammonium, or two Z groups may be combined to form a five or six-membered fused ring with Het, optionally including one or more heteroatoms in the fused ring, and the fused ring optionally including one or more substituents as described above;

as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

10. The composition of claim 1, wherein the p38 MAPK inhibiting compound is a compound having p38 MAPK inhibiting activity and is selected from the group of:

   a) 1-aryl-2-pyridinyl/pyrimidinyl heterocycles;
   b) 2-methoxypyrimidinylimidazoles;
   c) pyrazoles;
   d) oxazoles;
   e) thiazoles;
   f) compounds formed of a central heterocycle with vicinal groups selected from benzoimidazoles, benzothiazoles, and quinolines;
   g) compounds formed of a 6-membered heterocyclic cores selected from the group of pyridazines, pyrazines, and pyridines;
h) compounds formed of a fused bicyclic heterocyclic core that is an imidazopyrimidine;

i) compounds formed of ketone-containing compounds selected from benzophenones and pyrazoles;

j) cyclic ureas;

k) piperazine-based indole compounds;

l) urea-based compounds;

m) methylbenzamides;

n) N-p-tolylamides;

o) bis-aryl amides; and

p) bis-aryl benzamides.

11. The composition of claim 1, wherein the p38 MAPK inhibiting compound is:

A) a compound having the structure provided in Formula (25)

\[
\text{Het} \quad \text{A} \quad Z \\
\text{Z} \quad \text{Z} \\
\text{Z} \quad \text{Z} \\
\text{Z} \quad \text{Z}
\]

(25)

wherein:

Het indicates a 5-membered heterocycle with 1-3 ring carbons replaced with an atom selected from N, O, and S;

A is a heteroatom selected from N, O, and S;

each Z is optionally substituted and is independently selected from the group of halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF3, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, aroyl,
heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkylsulfonyl,
sulfinyl, alkylsulfinyl, sulfenyl, alkylsulfenyl, and trialklammonium, or two Z groups may be combined to form a five or six-membered fused ring with its respective base ring, optionally including one or more heteroatoms in the fused ring, and the fused ring optionally including one or more substituents as described above in relation to Z:

as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

B) a compound having the structure of Formula (56)

\[
\begin{align*}
\text{Ar} & \quad \text{X} \quad \text{Z} \\
R_1 & \quad \text{R}_2
\end{align*}
\]

(56)

wherein:

each Ar is optionally substituted and is independently a ring structure comprising 5-20 ring atoms and is selected from the group consisting of aryl, substituted aryl, heteroaryl having 1-3 heteroatoms selected from O, S, and N, substituted heteroaryl, heterocycle having 1-3 heteroatoms selected from O, S, and N, and substituted heterocycle;

\( R_1 \) and \( R_2 \) are optionally substituted and are independently selected from the group of halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF\textsubscript{3}, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkylsulfonyl, sulfinyl, alkylsulfinyl, sulfenyl, alkylsulfenyl, and trialklammonium;

\( X \) is -CH\textsubscript{2}, -CH\textsubscript{2}-CH\textsubscript{2}, -CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{2}, O, -0-NR\textsubscript{4}, S, SO, SO\textsubscript{2}, -S, S-, NR\textsubscript{4}, and -NR\textsubscript{4}-NR\textsubscript{4}:
R₄ is H, alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, acyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl or dialkylaminocarbonyl and

Z and any substituent on Ar, R₁, or R₂ are independently selected from the group of H, halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, aryl alkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkyl sulfonyl, sulfanyl, alkyl sulfanyl, sulfenyl, alkyl sulfenyl, and trialkylammonium;

as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof

C) a compound having the structure of Formula (85)

\[
\text{\begin{center}
\vspace{0.5cm}
\includegraphics[width=0.3\textwidth]{formula_image}
\vspace{0.5cm}
\end{center}}
\]

wherein:

Nc is a nitrogen atom that may be replaced by a carbon atom;

each Z is optionally substituted and is independently selected from the group of halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, aryl alkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkyl sulfonyl, sulfanyl, alkyl sulfanyl, sulfenyl, alkyl sulfenyl, and trialkylammonium, or two Z groups may be combined to form a five or six-membered fused ring with the respective base ring, optionally including one or more heteroatoms in the
fused ring, and the fused ring optionally including one or more substituents as described above;
as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof; or

D) a compound having the structure of Formula (98)

\[
\begin{align*}
\text{Ar} & \quad \text{N} \\
\text{N} & \quad \text{Ar}
\end{align*}
\]

(98)

wherein:
each Ar is optionally substituted and is independently a ring structure comprising 5-20 ring atoms and is selected from the group consisting of aryl, substituted aryl, heteroaryl having 1-3 heteroatoms selected from O, S, and N, substituted heteroaryl, heterocycle having 1-3 heteroatoms selected from O, S, and N, and substituted heterocycle; and

any substituents on Ar are independently selected from the group of halo, alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxyl, carbonyl, CF₃, alkenyl, alkynyl, aryl, substituted aryl, alkaryl, arylalkyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, amino, alkylamino, dialkylamino, carboxylic acid, carboxylic ester, carboxamide, nitro, cyano, amide, imide, azide, alkylcarbonyl, acyl, sulfonyl, alkylsulfonyl, sulfinyl, alkylsulfinyl, sulfonyl, alkylsulfenyl, and trialkylammonium;
as well as pharmaceutically acceptable esters, amides, salts, solvates, enantiomers, and prodrugs thereof.

12. The composition of claim 1, wherein the p38 MAPK inhibiting compound is a compound according to any of the structures provided in Formulas (26) - (55), (58) - (84), (86) - (97), and (99) - (153).

13. The composition of claim 1, wherein the p38 MAPK inhibiting compound is SB203580 or SB202190.
14. The composition of claim 1, comprising an NF-κB inhibiting compound according to the structure of Formula (6) and a p38 MAPK inhibiting compound according to any of the structures of Formulas (26) - (55), (58) - (84), (86) - (97), and (99) - (153).

15. The composition of claim 1, wherein the composition is provided as a single dosage unit.


17. The use of claim 16, wherein the NF-κB inhibiting compound is a curcumin analog.

18. The use of claim 16, wherein the method comprises administering the NF-κB inhibiting compound and the p38 MAPK inhibiting compound in a single composition.

19. The use of claim 16, wherein the method comprises administering the NF-κB inhibiting compound and the p38 MAPK inhibiting compound in separate compositions.


21. A p38 MAPK inhibiting compound for use in a method of enhancing the anti-cancer activity of a curcumin analog, the method comprising administering the p38 MAPK inhibiting compound in combination with the curcumin analog.

22. The use of claim 21, wherein the p38 MAPK inhibiting compound and the curcumin analog are administered in a single composition.

23. The use of claim 21, wherein the p38 MAPK inhibiting compound and the curcumin analog are administered in separate compositions.
24. The use of claim 21, wherein the anti-cancer activity is an activity for inhibiting growth of cancer cells.

25. The use of claim 21, wherein the anti-cancer activity is an activity for inducing apoptosis of cancer cells.
FIG. 1

FIG. 2
FIG. 4
FIG. 5
FIG. 6
**FIG. 7A**

**FIG. 7B**
FIG. 7C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Early Apoptosis</th>
<th>Late Apoptosis</th>
<th>Necrosis</th>
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<td>Control</td>
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<td>-</td>
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<td>Curc</td>
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<td>-</td>
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<td>Curc+SB80</td>
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<td>EF24 (0.6 µM)</td>
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% Cleaved PARP:

- 3.8
- 47.5
- 12.8
- 91.6
- 14.8
- 12.8

FIG. 7D
FIG. 7E
## A. CLASSIFICATION OF SUBJECT MATTER

| INV. | A61K31/12 | A61K31/4164 | A61P35/00 |

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

- A61K
- A61P

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

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

- EPO-Internal
- WPI Data
- EMBASE
- BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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- X Further documents are listed in the continuation of Box C.
- X See patent family annex.

### Detailed Category

- "A" document defining the general state of the art which is not considered to be of particular relevance.
- "E" earlier document but published on or after the international filing date.
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified).
- "O" document referring to an oral disclosure, use, exhibition or other means.
- "P" document published prior to the international filing date but later than the priority date claimed.

### Additional Information

Date of the actual completion of the international search: 21 October 2008

Date of mailing of the international search report: 03/11/2008

Name and mailing address of the ISA:

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel.: (+31-70) 340-2040 Fax: (+31-70) 340-3016

Authorized officer

Winger, Rudolf
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<td>WO 2006/109196 A (TRIPEP AB [SE]; SALLBERG MATTI [SE]; FRELIN LARS [SE]) 19 October 2006 (2006-10-19) cl aims 17-19</td>
<td>1, 2, 9-15</td>
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<td>X</td>
<td>EP 1 462 111 A (UNIVERSITEIT UTRECHT HOLDING B [NL]) 29 September 2004 (2004-09-29) cl aim 13; table 1</td>
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<td>Y</td>
<td>WO 01/40188 A (UNIV EMORY [US]; SNYDER JAMES P [US]; DAVIS MATTHEW C [US]; ADAMS BRIA) 7 June 2001 (2001-06-07) page 14, paragraph 1; cl aims</td>
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