(12) STANDARD PATENT (11) Application No. AU 2004274474 B2
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Thalidomide analogs as TNF-alpha modulators

(51) International Patent Classification(s)
C07D 209/44 (2006.01) *C07D 211/88* (2006.01)
C07D 209/46 (2006.01) *C07D 401/04* (2006.01)
C07D 211/84 (2006.01) *C07D 413/04* (2006.01)
C07D 211/86 (2006.01) *C07D 471/04* (2006.01)

(21) Application No: **2004274474** (22) Date of Filing: **2004.09.17**

(87) WIPO No: **WO05/028436**

(30) Priority Data

(31) Number	(32) Date	(33) Country
60/504,724	**2003.09.17**	**US**

(43) Publication Date: **2005.03.31**
(44) Accepted Journal Date: **2011.03.10**

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US 3794641 A

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 March 2005 (31.03.2005)

PCT

(10) International Publication Number
WO 2005/028436 A3

(51) International Patent Classification[7]: **A61K 31/454**, C07D 401/04, A61P 35/00, C07D 471/04, A61K 31/4439, C07D 413/04, 211/88, 209/46, 209/44, A61K 31/4035, 31/45, 31/536, C07D 211/86, 211/84

(21) International Application Number: PCT/US2004/030506

(22) International Filing Date: 17 September 2004 (17.09.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/504,724 17 September 2003 (17.09.2003) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report: 19 May 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2005/028436 A3

(54) Title: THALIDOMIDE ANALOGS AS TNF-ALPHA MODULATORS

(57) Abstract: Thalidomide analogs that modulate tumor necrosis factor alpha (TNF-α) activity and angiogenesis are disclosed. In particularly disclosed embodiments, the thalidomide analogs are isosteric sulfur-containing analogs. Also disclosed are methods of treating a subject with the analogs.

THALIDOMIDE ANALOGS

Cross-Reference to Related Applications

This application claims the benefit of U.S. Provisional Patent Application No. 60/504,724, filed September 17, 2003, which application is incorporated by reference herein.

Field

The present invention relates to thalidomide analogs, methods of synthesizing the analogs, and methods for using the analogs to modulate angiogenesis and tumor necrosis factor alpha activities in a subject. More particularly, the invention relates to sulfur-containing thalidomide analogs and methods of making and using the same.

Background

Thalidomide (*N*-α-phthalimidoglutarimide) is a glutamic acid derivative that was introduced onto the market as a sedative hypnotic in 1956, but was withdrawn in 1961 due to the development of severe congenital abnormalities in babies born to mothers using it for morning sickness. Interest in the agent was reawakened after thalidomide was found clinically effective in the treatment of erythema nodosum leprosum (ENL) and in the treatment of HIV wasting syndrome and various cancers. Mechanistic studies of its ENL activity demonstrated an anti-tumor necrosis factor alpha (anti-TNF-α) action. Specifically, thalidomide enhances the degradation of TNF-α RNA, and thereby lowers its synthesis and secretion. Further studies have defined it to be a co-stimulator of both CD8+ and CD4+ T cells, an inhibitor of angiogenesis via its inhibitory actions on basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), and an inhibitor of the transcription factor, NFκB.

TNF-α and family members play pivotal roles in a variety of physiological and pathological processes, which include cell proliferation and differentiation, apoptosis, the modulation of immune responses and induction of inflammation. TNF-α acts via two receptors, TNFR1 and 2. The former is expressed in all tissues and is the predominant signaling receptor for TNF-α. The latter is primarily expressed on immune cells and mediates more limited biological responses. The exposure of cells to

TNF-α can result in activation of a caspase cascade leading to cell death via apoptosis. Indeed, major cell surface molecules capable of initiating apoptosis are members of the TNF family of ligands and receptors. For example, death-inducing members of the TNF receptor family each contain a cytoplasmic 'death domain' (DD), which is a protein-protein interaction motif critical for engaging downstream components of the signal transduction machinery.

Recently, TRAIL, the tumor necrosis factor-related apoptosis-inducing ligand, has been shown to selectively induce apoptosis of tumor cells, but not most normal cells. It is indicated that TRAIL mediates thymocyte apoptosis and is important in the induction of autoimmune diseases. More often, however, TNF-α receptor binding induces the activation of transcription factors, AP-1 and NFκB, that thereafter induce genes involved in acute and chronic inflammatory responses. Overproduction of TNF-α has thus been implicated in many inflammatory diseases, such as rheumatoid arthritis, graft-versus-host disease and Crohn's disease, and it additionally exacerbates ENL, septic shock, AIDS and dementia associated with Alzheimer's disease (AD).

A number of thalidomide analogs optimized to reduce TNF-α synthesis have been designed and synthesized. Primarily, these analogs include structural modifications of the phthaloyl ring or glutarimide ring of thalidomide. In addition, following the demonstration that the anti-angiogenic property of thalidomide is associated with its hydroxylated, open-ring metabolites, syntheses of the hydroxylated and hydrolysis metabolites as inhibitors of angiogenesis or tumor metastasis have been reported. Although extensive studies exist regarding the structure-activity relationships between thalidomide and TNF-α, very little is known about the contribution of the four amide carbonyl groups of thalidomide to its biological activity.

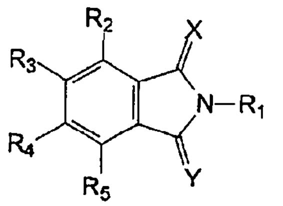
Summary

Thalidomide analogs having angiogenesis modulating activity and TNF-α modulating activity are disclosed. In some embodiments, the disclosed thalidomide analogs are sulfur-analogs of thalidomide, its open-ring metabolites and its derivatives (such as its hydroxylated derivatives) in which one or more carbonyl groups are replaced by thiocarbonyl groups. For example, in some embodiments, thalidomide analogs wherein at least one carbonyl group on the pthaloyl moiety or on the glutaramide moiety (or its open ring form) of a thalidomide or a thalidomide analog is replaced by a thiocarbonyl group. In particular embodiments, successive replacement of the carbonyl groups in thalidomide with thiocarbonyl groups provides thiothalidomide analogs having increased TNF-α inhibitory activity.

Surprisingly, the increase in TNF-α inhibition due to replacement of the carbonyl groups of thalidomide with thiocarbonyl groups is not associated with toxicity.

Improved methods for making thalidomide and thalidomide analogs are also disclosed, as are methods of converting thalidomide analogs into thiothalidomides. Due to their angiogenesis and TNF-α modulating activity, the disclosed thalidomide analogs, especially the disclosed thiothalidomides, can be used to treat a subject having a disease or condition related to angiogenesis or TNF-α activity, such as a tumor or unwanted neovascularization. Furthermore, the physical and toxicological properties of the disclosed thiothalidomide analogs make them suitable for potently and safely modulating angiogenesis and TNF-α activity without injection, for example, by oral administration. This is in contrast to many currently available agents used for such purposes.

In one specific embodiment, there is provided a compound having the formula:



wherein X and Y are independently CH_2, oxygen or sulfur, and at least one of X and Y is sulfur if R_1 does not include a sulfur atom; each of R_2-R_5 are independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy, substituted acyloxy, alkyl, substituted alkyl,

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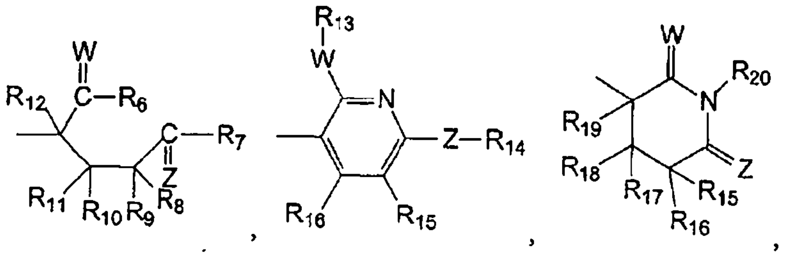
alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen, nitro or linked to form a five- or six-membered, unsubstituted or substituted, aliphatic, aromatic or heterocyclic ring, and R_1 is selected from the group consisting of:



wherein W and Z are each independently oxygen or sulfur; and further wherein at least two of X, Y, W and Z are sulfur; R_6 and R_7 are each independently hydroxyl, alkoxy or substituted alkoxy, each of R_8-R_{12} are independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy, substituted acyloxy, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen or nitro; R_{13} and R_{14} are each independently hydrogen, alkyl or substituted alkyl; R_{20} is hydrogen, hydroxyl, or alkyl; and R_{15}-R_{19} are each independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy, substituted acyloxy, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen or nitro; or a stereoisomer or pharmaceutically acceptable salt thereof.

In another specific embodiment, there is provided a pharmaceutical composition comprising the compound defined above.

In yet another specific embodiment, there is provided use of the compound defined above in the manufacture of a medicament for treating a disease treatable by modulation of TNF-α activity, or treating a disease treatable by modulation of angiogenesis, or achieving an anti-tumor effect, or inhibiting tumor metastasis, or treating a pathological angiogenesis, or treating a neurodegenerative disease.

In another specific embodiment, there is provided a method for treating a disease treatable by modulation of TNF-α activity, or treating a disease treatable by modulation of angiogenesis, or achieving an anti-tumor effect, or inhibiting tumor metastasis, or treating a pathological angiogenesis, or treating a neurodegenerative disease which comprises administering a therapeutically effective amount of the compound defined above to a subject in need thereof.

Brief Description of the Drawings

FIG. 1 is a bar graph showing the TNF-a inhibitory action of several disclosed thalidomide analogs in murine cells having a luciferase reporter element plus the 3'-UTR of human TNF-a relative to their action in cells lacking the 3'- UTR.

FIG. 2 is a bar graph showing the relative angiogenic modulating activity of 1, 3-Dioxo-2- (2-hydroxy-6-methoxypyridin-3-yl)-isoindoline hydrobromide at several concentrations.

FIG. 3 is a bar graph showing the relative angiogenic modulating activity of 2-(3-cyclohexenyl)-*H*-isoindol-1,3(2*H*)-dithione at several concentrations.

FIG. 4 is a bar graph showing the relative angiogenic modulating activity of 1-(2,6-Dithioxo-3-piperidinyl)-1*H*-isoindole-1,3(2*H*)-dione at several concentrations.

FIG. 5 is a bar graph showing the relative angiogenic modulating activity of 3-Camphanic amino-2,6-piperidinedione at several concentrations.

FIG. 6 is a bar graph showing the relative angiogenic modulating activity of Dithiophthalimide at several concentrations.

FIG. 7 is a bar graph showing the relative angiogenic modulating activity of 2-(1,3-Dihydro-1-oxo-3-thioxo-2*H*-isoindol-2-yl)-pentanedioic acid at several concentrations.

FIG. 8 is a bar graph showing the relative angiogenic modulating activity of 2-(2-Oxo-6-thioxo-3-piperidinyl)-1*H*-isoindole-1,3(2*H*)-dione at several concentrations.

FIG. 9 is a bar graph showing the relative angiogenic modulating activity of 2,3-Dihydro-3-thioxo-2-(2,6-dithioxo-3-piperidinyl)-1*H*-isoindol-1-one at several concentrations.

FIG. 10 is a bar graph showing the relative angiogenic modulating activity of 2-Acetoxy-*N*-(2,6-dioxopiperidin-3-yl)benzamide at several concentrations.

FIG. 11 is a bar graph showing the relative angiogenic modulating activity of 1,3-Dioxo-2-(2,6-dimethoxypyridin-3-yl)-isoindoline at several concentrations.

Detailed Description of Particularly Disclosed Embodiments

I. Abbreviations

TNF-α –tumor necrosis factor alpha

CDI – carboxyamidotriazole

ARE - adenylate/uridylate (AU)-rich element

UTR - untranslated region

THF – tetrahydrofuran

NMR – nuclear magnetic resonance

LR – Lawesson's Reagent

II. Terms

In order to facilitate an understanding of the embodiments presented, the following explanations are provided.

The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. The term "comprises" means "includes." Also, "comprising A or B" means including A or B, or A and B, unless the context clearly indicates otherwise. It is to be further understood that all molecular weight or molecular mass values given for compounds are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The term "subject" refers to animals, including mammals (for example, humans and veterinary animals such as dogs, cats, pigs, horses, sheep, and cattle).

An "R-group" or "substituent" refers to a single atom (for example, a halogen atom) or a group of two or more atoms that are covalently bonded to each other, which are covalently bonded to an atom or atoms in a molecule to satisfy the valency requirements of the atom or atoms of the molecule, typically in place of a hydrogen atom. Examples of R-groups/substituents include alkyl groups, hydroxyl groups, alkoxy groups, acyloxy groups, mercapto groups, and aryl groups.

"Substituted" or "substitution" refer to replacement of a hydrogen atom of a molecule or an R-group with one or more additional R-groups such as halogen, alkyl, alkoxy, alkylthio, trifluoromethyl, acyloxy, hydroxy, mercapto, carboxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, nitro, sulfato or other R-groups.

"Alkyl" refers to a cyclic, branched, or straight chain group containing only carbon and hydrogen, and unless otherwise mentioned typically contains one to twelve carbon atoms. This term is further exemplified by groups such as methyl, ethyl, n-propyl, isobutyl, t-butyl, pentyl, pivalyl, heptyl, adamantyl, and cyclopentyl.

Alkyl groups can either be unsubstituted or substituted. "Lower alkyl" groups are those that contain one to six carbon atoms.

"Acyl" refers to a group having the structure RCO-, where R may be alkyl, or substituted alkyl. "Lower acyl" groups are those that contain one to six carbon atoms.

"Acyloxy refers to a group having the structure RCOO-, where R may be alkyl or substituted alkyl. "Lower acyloxy" groups contain one to six carbon atoms.

"Alkenyl" refers to a cyclic, branched or straight chain group containing only carbon and hydrogen, and unless otherwise mentioned typically contains one to twelve carbon atoms, and contains one or more double bonds that may or may not be conjugated. Alkenyl groups may be unsubstituted or substituted. "Lower alkenyl" groups contain one to six carbon atoms.

"Alkynyl" refers to a cyclic, branched or straight chain group containing only carbon and hydrogen, and unless otherwise mentioned typically contains one to twelve carbon atoms, and contains one or more triple bonds. Alkynyl groups may be unsubstituted or substituted. "Lower alkynyl" groups are those that contain one to six carbon atoms.

"Alkoxy" refers to a group having the structure R-O-, where R may be alkyl or substituted alkyl. Examples of alkoxy groups include methoxy, ethoxy, propoxy and butoxy groups. "Lower alkoxy" groups are those that contain one to six carbon atoms.

The term "halogen" refers to fluoro, bromo, chloro and iodo substituents.

"Aryl" refers to a monovalent unsaturated aromatic carbocyclic group having a single ring (e.g., phenyl) or multiple condensed rings (e.g., naphthyl or anthryl), which can optionally be unsubstituted or substituted.

The term "amino" refers to an R-group having the structure $-NH_2$, which can be optionally substituted with, for example, lower alkyl groups, to yield an amino group having the general structure $-NHR$ or $-NR_2$.

"Nitro" refers to an R-group having the structure $-NO_2$.

The term "aliphatic" as applied to cyclic groups refers to ring structures in which any double bonds that are present in the ring are not conjugated around the entire ring structure.

The term "aromatic" as applied to cyclic groups refers to ring structures which contain double bonds that are conjugated around the entire ring structure, possibly through a heteroatom such as an oxygen atom or a nitrogen atom. Aryl groups, pyridyl groups and furan groups are examples of aromatic groups. The conjugated system of an aromatic group contains a characteristic number of electrons, for example, 6 or 10 electrons that occupy the electronic orbitals making up the conjugated system, which are typically un-hybridized p-orbitals.

"Pharmaceutical compositions" are compositions that include an amount (for example, a unit dosage) of one or more of the disclosed compounds together with one or more non-toxic pharmaceutically acceptable excipients, including carriers, diluents, and/or adjuvants, and optionally other biologically active ingredients. Such pharmaceutical compositions can be prepared by standard pharmaceutical formulation techniques such as those disclosed in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA (19th Edition).

A "therapeutically effective amount" of the disclosed compounds is a dosage of the compound that is sufficient to achieve a desired therapeutic effect, such as inhibition of angiogenesis or an anti-tumor or anti-metastatic effect, or inhibition of TNF-α activity. In some examples, a therapeutically effective amount is an amount sufficient to achieve tissue concentrations at the site of action that are similar to those that are shown to modulate angiogenesis or TNF-α activity in tissue culture, *in vitro*, or *in vivo*. For example, a therapeutically effective amount of a compound may be such that the subject receives a dosage of about 0.1 μg/kg body weight/day to about 1000 mg/kg body weight/day, for example, a dosage of about 1 μg/kg body weight/day to about 1000 μg/kg body weight/day, such as a dosage of about 5 μg/kg body weight/day to about 500 μg/kg body weight/day.

The term "stereoisomer" refers to a molecule that is an enatiomer, diasteromer or geometric isomer of a molecule. Stereoisomers, unlike structural isomers, do not differ with respect to the number and types of atoms in the molecule's structure but with respect to the spatial arrangement of the molecule's atoms. Examples of stereoisomers include the (+) and (-) forms of optically active molecules.

The term "modulate" refers to the ability of a disclosed compound to alter the amount, degree, or rate of a biological function, the progression of a disease, or amelioration of a condition. For example, modulating can refer to the ability of a compound to elicit an increase or decrease in angiogenesis, to inhibit TNF-α activity, or to inhibit tumor metastasis or tumorigenesis.

The term "angiogenic activity" refers to the ability of a disclosed compound or a particular concentration of a disclosed compound to stimulate angiogenesis. Angiogenic activity may be detected *in vivo* or *in vitro*. Angiogenic compounds or angiogenic concentrations of disclosed compounds stimulate angiogenesis, and such compounds and/or concentrations may be readily identified by those of ordinary skill in the art, using, for example, the methods described in the Examples that follow.

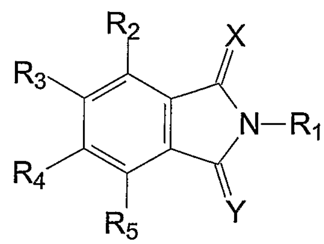
The term "anti-angiogenic activity" refers to the ability of a compound or a particular concentration of a disclosed compound to inhibit angiogenesis. Anti-angiogenic activity may be detected *in vivo* or *in vitro*. Anti-angiogenic or anti-angiogenic concentrations of disclosed compounds inhibit angiogenesis, and such compounds and/or concentrations may be readily identified by those of ordinary skill in the art, using, for example, the methods described in the Examples that follow.

III. Overview of Particularly Disclosed Embodiments

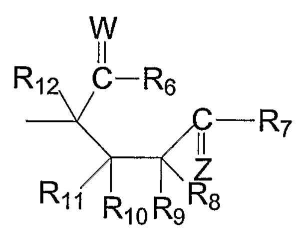
Disclosed are thalidomide analogs that modulate TNF-α activity and/or angiogenesis, and as such can be used to treat a wide variety of pathological conditions that are linked to angiogenesis and/or TNF-α activity. Pharmaceutically acceptable salts, stereoisomers, and metabolites of all of the disclosed compounds also are contemplated. In some embodiments, the thalidomide analogs are thiothalidomide derivatives in which carbonyl groups in corresponding non-sulfur-containing thalidomide derivatives are replaced by one or more thiocarbonyl groups.

In the structures that follow, all valency requirements are understood to be satisfied. Thus, for example, carbon atoms have four bonds to other atoms, even if all such bonds are not shown. As is understood by those of ordinary skill in the art, where all four bonds to a carbon atom are not shown, additional bonds to hydrogen atoms are implied. Further substitution of such implied hydrogen atoms is possible.

In other embodiments, the disclosed compounds include compounds having the chemical formula:

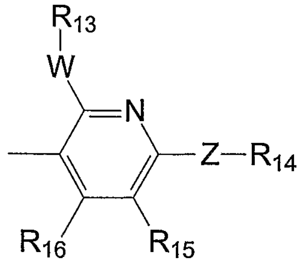


wherein X and Y are independently CH_2, oxygen or sulfur, and at least one of X and Y is sulfur if R_1 does not include a sulfur atom; each of R_2-R_5 are independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy, substituted acyloxy, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen, nitro or linked to form a five- or six-membered, unsubstituted or substituted, aliphatic, aromatic or heterocyclic ring, for example, hydrogen, lower alkyl, acyloxy, halogen, hydroxyl, amino or nitro such as hydrogen, acyloxy or hydroxyl; and R_1 is an unsubstituted or substituted, aliphatic or aromatic heterocyclic ring, an unsubstituted or substituted cycloalkenyl ring, or



wherein W and Z are each independently oxygen or sulfur, R_6 and R_7 are each independently hydroxyl, alkoxy or substituted alkoxy, and each of R_8-R_{12} are independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy, substituted acyloxy, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen or nitro, for example, hydrogen, lower alkyl, acyloxy, halogen, hydroxyl, amino or nitro such as hydrogen, acyloxy or hydroxyl.

In particular embodiments, R_1 is



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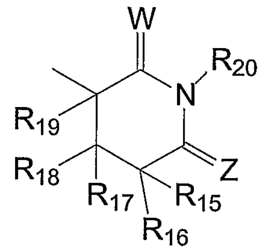

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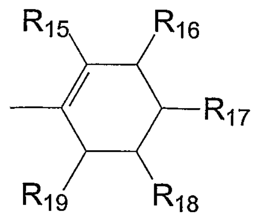

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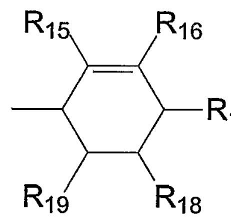

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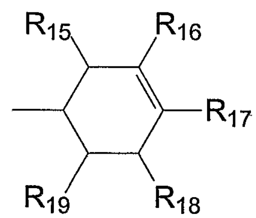
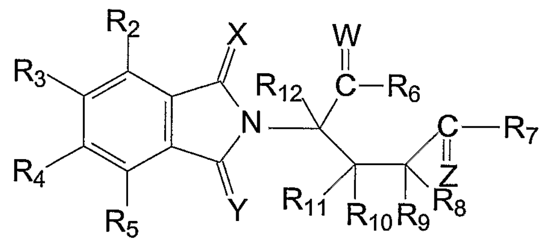


;

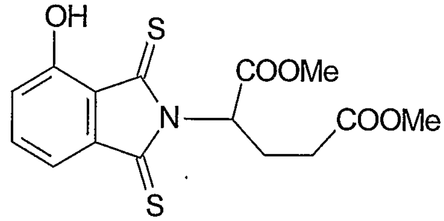
wherein W and Z are each independently oxygen or sulfur, R_{13} and R_{14} are each independently hydrogen, alkyl or substituted alkyl; R_{20} is hydrogen, hydroxyl, alkyl or substituted alkyl such as aryl substituted alkyl; and R_{15}-R_{19} are each independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy, substituted acyloxy, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen or nitro, for example, hydrogen, lower alkyl, acyloxy, halogen, hydroxyl, amino or nitro such as hydrogen, acyloxy or hydroxyl. In some embodiments, at least one of R_2, R_3, R_4, R_5, R_8, R_9, R_{10}, R_{11}, R_{15}, R_{16}, R_{17}, R_{18} and R_{19} is hydroxyl. In other embodiments, at least one of X, Y, W and Z is sulfur, at least two of X, Y, W and Z are sulfur, or at least three of X, Y, W and Z are sulfur. For example, in more particular embodiments, X or Y is sulfur, and both W and Z are oxygen if present; both X and Y are sulfur and both W and Z are oxygen if present; X and Y are both oxygen and W or Z is sulfur if present; both X and Y are sulfur and W or Z is sulfur if present; or X or Y are sulfur and both W and Z are sulfur if present. Alternatively, where W and Z are present the following are possible: X=S, Y=O, W=O, Z=O; X=O, Y=S, W=O, Z=O; X=O, Y=O, W=S, Z=O; X=O, Y=O, W=O, Z=S; X=S, Y=S, W=O, Z=O; X=S, Y=O, W=S, Z=O; X=S, Y=O, W=O, Z=S; X=O, Y=O, W=S, Z=S; X=O, Y=S, W=O, Z=S; X=O, Y=S, W=S, Z=O; X=S, Y=S, W=S, Z=O;

X=S, Y=S, W=O, Z=S; X=S, Y=O, W=S, Z=S; X=O, Y=S, W=S, Z=S; or X=S, Y=S, W=S, Z=S. In other particular embodiments X=S and Y=CH_2.

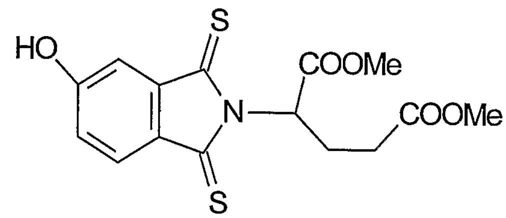
In more particular embodiments, the disclosed compounds have the formula



wherein X, Y, W and Z are independently sulfur or oxygen and at least one of X, Y, W and Z is sulfur, and R_2-R_{12} are as before. For example, in more particular embodiments, X or Y is sulfur, and both W and Z are oxygen if present; both X and Y are sulfur and both W and Z are oxygen if present; X and Y are both oxygen and W or Z is sulfur if present; both X and Y are sulfur and W or Z is sulfur if present; or X or Y are sulfur and both W and Z are sulfur if present. Alternatively, where W and Z are present the following are possible: X=S, Y=O, W=O, Z=O; X=O, Y=S, W=O, Z=O; X=O, Y=O, W=S, Z=O; X=O, Y=O, W=O, Z=S; X=S, Y=S, W=O, Z=O; X=S, Y=O, W=S, Z=O; X=S, Y=O, W=O, Z=S; X=O, Y=O, W=S, Z=S; X=O, Y=S, W=O, Z=S; X=O, Y=S, W=S, Z=O; X=S, Y=S, W=S, Z=O; X=S, Y=S, W=O, Z=S; X=S, Y=O, W=S, Z=S; X=O, Y=S, W=S, Z=S; or X=S, Y=S, W=S, Z=S. In more particular embodiments, at least one of R_2-R_5 and R_8-R_{11} is hydroxyl. Specific examples of such compounds include:

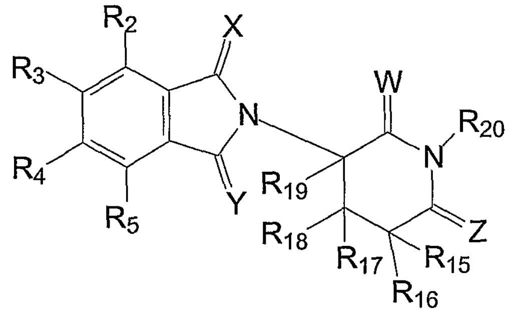


and

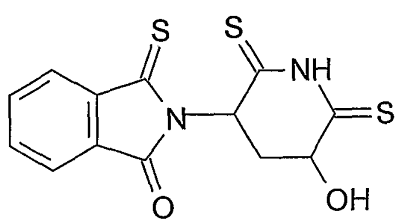


.

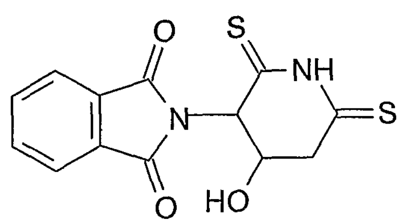
In other more particular embodiments, the disclosed compounds have the chemical formula:



wherein W, X, Y and Z each are independently sulfur or oxygen and at least one of W, X, Y and Z is sulfur; and R_2-R_5 and R_{15}-R_{20} are as before. For example, in more particular embodiments, X or Y is sulfur, and both W and Z are oxygen; both X and Y are sulfur and both W and Z are oxygen; X and Y are both oxygen and W or Z is sulfur; both X and Y are sulfur and W or Z is sulfur; or X or Y are sulfur and both W and Z are sulfur. Alternatively, the following are possible: X=S, Y=O, W=O, Z=O; X=O, Y=S, W=O, Z=O; X=O, Y=O, W=S, Z=O; X=O, Y=O, W=O, Z=S; X=S, Y=S, W=O, Z=O; X=S, Y=O, W=S, Z=O; X=S, Y=O, W=O, Z=S; X=O, Y=O, W=S, Z=S; X=O, Y=S, W=O, Z=S; X=O, Y=S, W=S, Z=O; X=S, Y=S, W=S, Z=O; X=S, Y=S, W=O, Z=S; X=S, Y=O, W=S, Z=S; X=O, Y=S, W=S, Z=S; or X=S, Y=S, W=S, Z=S. In more particular embodiments, at least one of R_2-R_5 and R_{15}-R_{19} is hydroxyl. Specific examples of such compounds include:

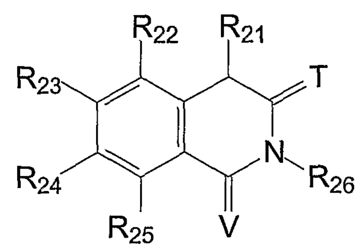


and

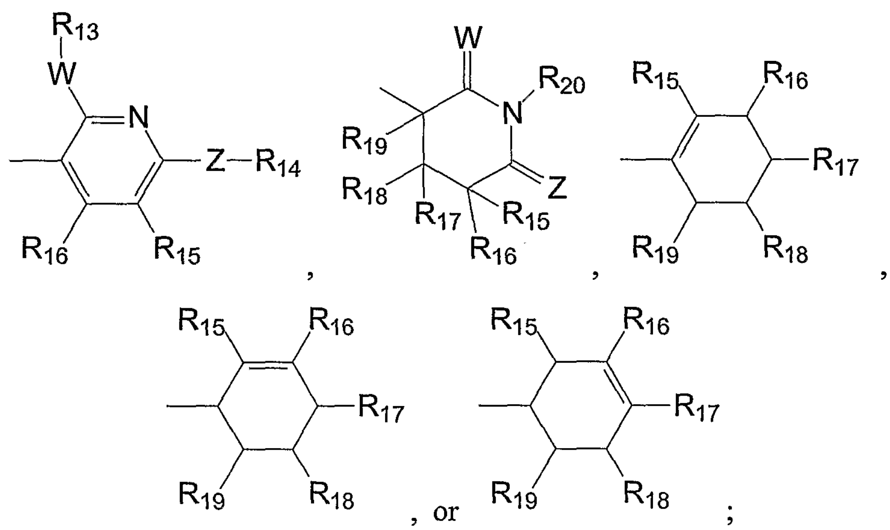


.

The disclosed compounds also include compounds having the formula



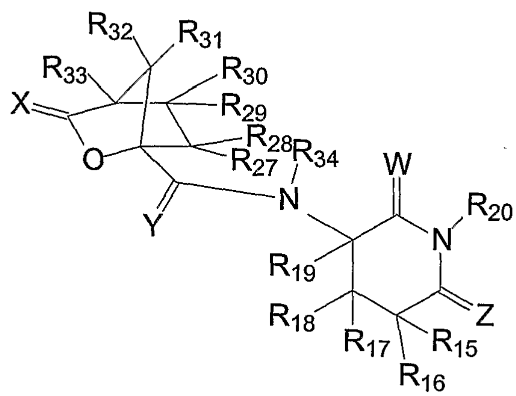
wherein T and V are independently oxygen or sulfur, R_{21}-R_{25} are independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy, substituted acyloxy, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen or nitro, for example, hydrogen, lower alkyl, acyloxy, halogen, hydroxyl, amino or nitro such as hydrogen, acyloxy or hydroxyl; and R_{26} is



wherein W, Z and R_{13}-R_{20} are as before. For example, in more particular embodiments, T or V is sulfur, and both W and Z are oxygen if present; both T and V are sulfur and both W and Z are oxygen if present; T and V are both oxygen and W or Z is sulfur if present; both T and V are sulfur and W or Z is sulfur if present; or T or V are sulfur and both W and Z are sulfur if present. Alternatively, where W and Z are present the following are possible: T=O, V=O, W=O, Z=O; T=S, V=O,

W=O, Z=O; T=O, V=S, W=O, Z=O; T=O, V=O, W=S, Z=O; T=O, V=O, W=O, Z=S; T=S, V=S, W=O, Z=O; T=S, V=O, W=S, Z=O; T=S, V=O, W=O, Z=S; T=O, V=O, W=S, Z=S; T=O, V=S, W=O, Z=S; T=O, V=S, W=S, Z=O; T=S, V=S, W=S, Z=O; T=S, V=S, W=O, Z=S; T=S, V=O, W=S, Z=S; T=O, V=S, W=S, Z=S; or T=S, V=S, W=S, Z=S. In some embodiments, at least one of R_{15}-R_{19} and R_{22}-R_{26} is hydroxyl.

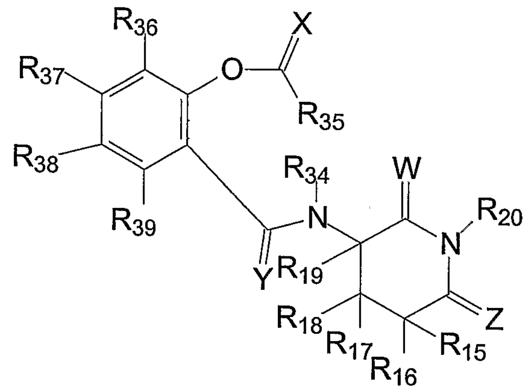
Still further, the disclosed compounds include compounds having the formula



wherein X, Y are each independently oxygen or sulfur; W, X and R_{15}-R_{20} are as before; R_{27}-R_{33} are each independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy, substituted acyloxy, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen or nitro, for example, hydrogen, lower alkyl, acyloxy, halogen, hydroxyl, amino or nitro such as hydrogen, acyloxy or hydroxyl; and R_{34} is hydrogen, alkyl or substituted alkyl. For example, in more particular embodiments, X or Y is sulfur, and both W and Z are oxygen; both X and Y are sulfur and both W and Z are oxygen; X and Y are both oxygen and W or Z is sulfur; both X and Y are sulfur and W or Z is sulfur; or X or Y are sulfur and both W and Z are sulfur. Alternatively, the following are possible: X=O, Y=O, W=O, Z=O; X=S, Y=O, W=O, Z=O; X=O, Y=S, W=O, Z=O; X=O, Y=O, W=S, Z=O; X=O, Y=O, W=O, Z=S; X=S, Y=S, W=O, Z=O; X=S, Y=O, W=S, Z=O; X=S, Y=O, W=O, Z=S; X=O, Y=O, W=S, Z=S; X=O, Y=S, W=O, Z=S; X=O, Y=S, W=S, Z=O; X=S,

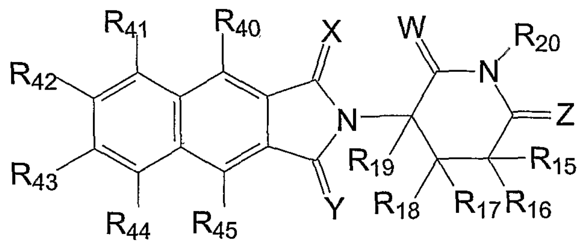
Y=S, W=S, Z=O; X=S, Y=S, W=O, Z=S; X=S, Y=O, W=S, Z=S; X=O, Y=S, W=S, Z=S; or X=S, Y=S, W=S, Z=S.

In addition, the disclosed compounds include compounds having the formula



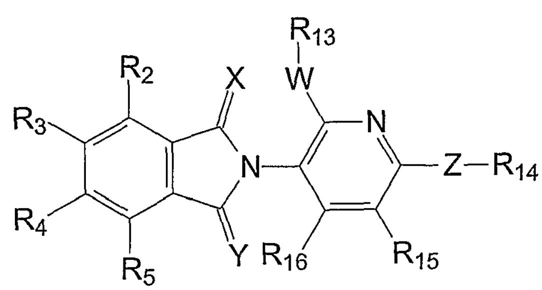
wherein X and Y are each independently oxygen or sulfur; W, Z, R_{15}-R_{20} and R_{34} are as before, R_{35} is alkyl or substituted alkyl, and R_{36}-R_{39} are each independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy, substituted acyloxy, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen or nitro, for example, hydrogen, lower alkyl, acyloxy, halogen, hydroxyl, amino or nitro such as hydrogen, acyloxy or hydroxyl. For example, in more particular embodiments, X or Y is sulfur, and both W and Z are oxygen; both X and Y are sulfur and both W and Z are oxygen; X and Y are both oxygen and W or Z is sulfur; both X and Y are sulfur and W or Z is sulfur; or X or Y are sulfur and both W and Z are sulfur. Alternatively, the following are possible: X=O, Y=O, W=O, Z=O; X=S, Y=O, W=O, Z=O; X=O, Y=S, W=O, Z=O; X=O, Y=O, W=S, Z=O; X=O, Y=O, W=O, Z=S; X=S, Y=S, W=O, Z=O; X=S, Y=O, W=S, Z=O; X=S, Y=O, W=O, Z=S; X=O, Y=O, W=S, Z=S; X=O, Y=S, W=O, Z=S; X=O, Y=S, W=S, Z=O; X=S, Y=S, W=S, Z=O; X=S, Y=S, W=O, Z=S; X=S, Y=O, W=S, Z=S; X=O, Y=S, W=S, Z=S; or X=S, Y=S, W=S, Z=S.

Other embodiments include compounds having the formula



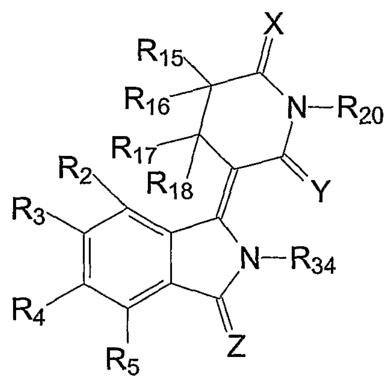
wherein X and Y each are independently oxygen or sulfur; W, Z and R_{15}-R_{20} are as before; and R_{40}-R_{45} are each independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy, substituted acyloxy, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen or nitro, for example, hydrogen, lower alkyl, acyloxy, halogen, hydroxyl, amino or nitro such as hydrogen, acyloxy or hydroxyl. For example, in more particular embodiments, X or Y is sulfur, and both W and Z are oxygen; both X and Y are sulfur and both W and Z are oxygen; X and Y are both oxygen and W or Z is sulfur; both X and Y are sulfur and W or Z is sulfur; or X or Y are sulfur and both W and Z are sulfur. Alternatively, the following are possible: X=O, Y=O, W=O, Z=O; X=S, Y=O, W=O, Z=O; X=O, Y=S, W=O, Z=O; X=O, Y=O, W=S, Z=O; X=O, Y=O, W=O, Z=S; X=S, Y=S, W=O, Z=O; X=S, Y=O, W=S, Z=O; X=S, Y=O, W=O, Z=S; X=O, Y=O, W=S, Z=S; X=O, Y=S, W=O, Z=S; X=O, Y=S, W=S, Z=O; X=S, Y=S, W=S, Z=O; X=S, Y=S, W=O, Z=S; X=S, Y=O, W=S, Z=S; X=O, Y=S, W=S, Z=S; or X=S, Y=S, W=S, Z=S.

The disclosed compounds further include compounds having the formula



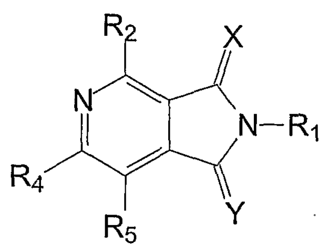
wherein X, Y, W and Z are independently oxygen or sulfur, and R_2-R_5 and R_{13}-R_{16} are as before. For example, in more particular embodiments, X or Y is sulfur, and both W and Z are oxygen; both X and Y are sulfur and both W and Z are oxygen; X and Y are both oxygen and W or Z is sulfur; both X and Y are sulfur and W or Z is sulfur; or X or Y are sulfur and both W and Z are sulfur. Alternatively, the following are possible: X=O, Y=O, W=O, Z=O; X=S, Y=O, W=O, Z=O; X=O, Y=S, W=O, Z=O; X=O, Y=O, W=S, Z=O; X=O, Y=O, W=O, Z=S; X=S, Y=S, W=O, Z=O; X=S, Y=O, W=S, Z=O; X=S, Y=O, W=O, Z=S; X=O, Y=O, W=S, Z=S; X=O, Y=S, W=O, Z=S; X=O, Y=S, W=S, Z=O; X=S, Y=S, W=S, Z=O; X=S, Y=S, W=O, Z=S; X=S, Y=O, W=S, Z=S; X=O, Y=S, W=S, Z=S; or X=S, Y=S, W=S, Z=S.

Also disclosed is a thalidomide analog compound having the formula

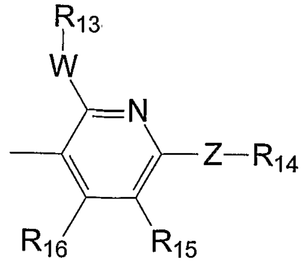


wherein X, Y and Z are independently oxygen or sulfur, and R_2-R_5, R_{15}-R_{20} and R_{34} are as before. For example, in more particular embodiments, X or Y is sulfur, and Z is oxygen; both X and Y are sulfur and Z is oxygen; X and Y are both oxygen and Z is sulfur. Alternatively, the following are possible: X=O, Y=O, Z=O; X=S, Y=O, Z=O; X=O, Y=S, Z=O; X=O, Y=O, Z=S; X=S, Y=S, Z=O; X=S, Y=O, Z=S; X=O, Y=S, Z=S; or X=S, Y=S, Z=S.

Also disclosed is a thalidomide analog compound having the formula



wherein X and Y are independently oxygen or sulfur, and R_1, R_2, R_4 and R_5 are as before. For example, in particular embodiments, R_1 is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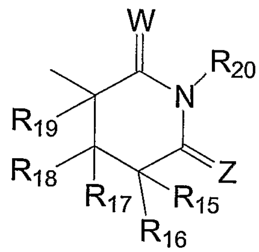
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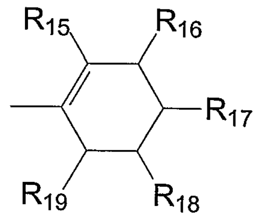
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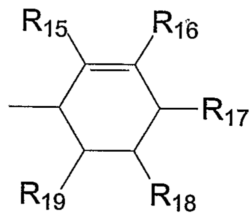
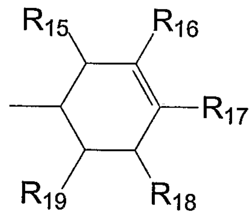
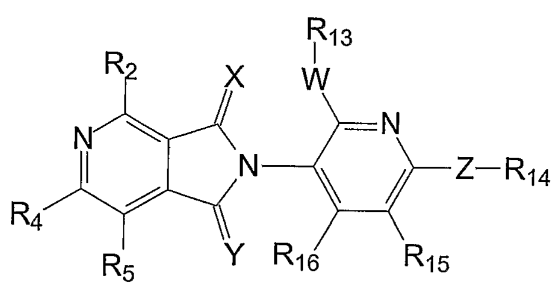
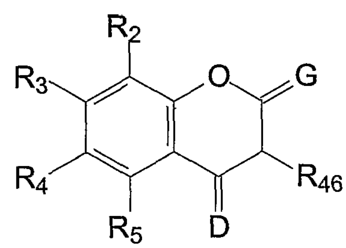

, or

;

wherein W, Z, and R_{13}-R_{20} are as before. For example, in more particular embodiments, X or Y is sulfur, and both W and Z are oxygen if present; both X and Y are sulfur and both W and Z are oxygen if present; X and Y are both oxygen and W or Z is sulfur if present; both X and Y are sulfur and W or Z is sulfur if present; or X or Y are sulfur and both W and Z are sulfur if present. Alternatively, where W and Z are present the following are possible: X=O, Y=O, W=O, Z=O; X=S, Y=O, W=O, Z=O; X=O, Y=S, W=O, Z=O; X=O, Y=O, W=S, Z=O; X=O, Y=O, W=O, Z=S; X=S, Y=S, W=O, Z=O; X=S, Y=O, W=S, Z=O; X=S, Y=O, W=O, Z=S; X=O, Y=O, W=S, Z=S; X=O, Y=S, W=O, Z=S; X=O, Y=S, W=S, Z=O; X=S, Y=S, W=S, Z=O; X=S, Y=S, W=O, Z=S; X=S, Y=O, W=S, Z=S; X=O, Y=S, W=S, Z=S; or X=S, Y=S, W=S, Z=S. In more particular embodiments, the compound has the formula:

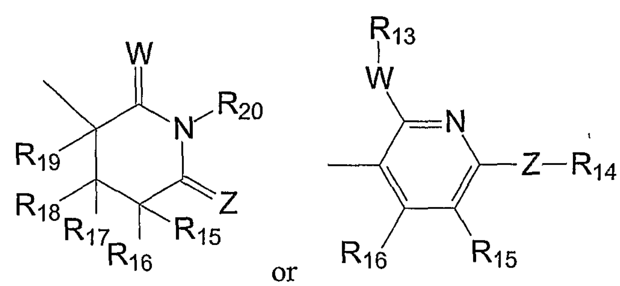


wherein X, Y are independently oxygen or sulfur, and W, Z, R_2, R_4, R_5, and R_{13}-R_{16} are as before. For example, in more particular embodiments, X or Y is sulfur, and both W and Z are oxygen; both X and Y are sulfur and both W and Z are oxygen; X and Y are both oxygen and W or Z is sulfur; both X and Y are sulfur and W or Z is sulfur; or X or Y are sulfur and both W and Z are sulfur. Alternatively, the following are possible: X=O, Y=O, W=O, Z=O; X=S, Y=O, W=O, Z=O; X=O, Y=S, W=O, Z=O; X=O, Y=O, W=S, Z=O; X=O, Y=O, W=O, Z=S; X=S, Y=S, W=O, Z=O; X=S, Y=O, W=S, Z=O; X=S, Y=O, W=O, Z=S; X=O, Y=O, W=S, Z=S; X=O, Y=S, W=O, Z=S; X=O, Y=S, W=S, Z=O; X=S, Y=S, W=S, Z=O; X=S, Y=S, W=O, Z=S; X=S, Y=O, W=S, Z=S; X=O, Y=S, W=S, Z=S; or X=S, Y=S, W=S, Z=S. In even more particular embodiments, at least one of R_2, R_4, R_5, R_{15} and R_{16} is hydroxyl.

Also disclosed is a compound having the formula:



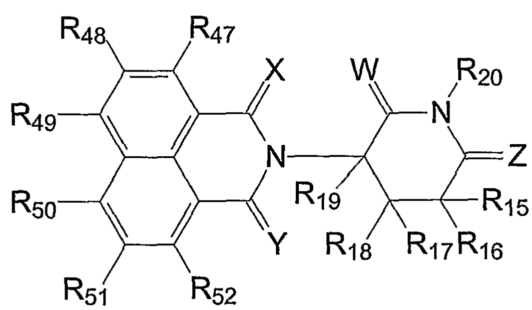
wherein G and D are each independently oxygen or sulfur, R_2-R_5 are as before, and R_{46} is



wherein W, Z and R_{13}-R_{20} are as before. For example, in particular embodiments, G or D is sulfur, and both W and Z are oxygen; both G and D are sulfur and both W and Z are oxygen; G and D are both oxygen and W or Z is sulfur; both G and D are sulfur and W or Z is sulfur; or G or D are sulfur and both W and Z are sulfur. Alternatively, the following are possible: G=O, D=O, W=O, Z=O; G=S, D=O, W=O, Z=O; G=O, D=S, W=O, Z=O; G=O, D=O, W=S, Z=O; G=O, D=O, W=O, Z=S; G=S, D=S, W=O, Z=O; G=S, D=O, W=S, Z=O; G=S, D=O, W=O, Z=S;

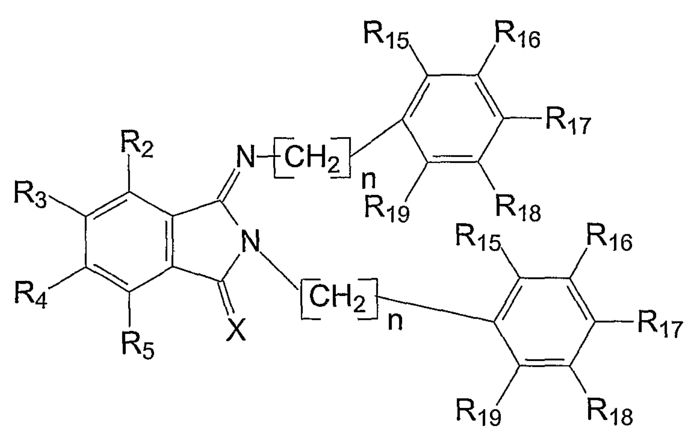
G=O, D=O, W=S, Z=S; G=O, D=S, W=O, Z=S; G=O, D=S, W=S, Z=O; G=S, D=S, W=S, Z=O; G=S, D=S, W=O, Z=S; G=S, D=O, W=S, Z=S; G=O, D=S, W=S, Z=S; or G=S, D=S, W=S, Z=S.

A method for modulating TNF-α activity in a subject also is disclosed. The method includes administering to the subject a therapeutically effective amount of one or more of any of the compounds disclosed above, or a compound having the formula:



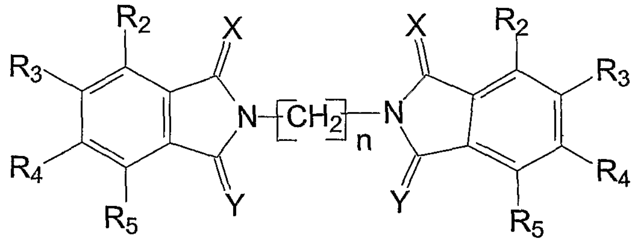
where X and Y are independently oxygen or sulfur; W, Z, R_{15}-R_{20} are as before; and R_{47}-R_{52} are each independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy, substituted acyloxy, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen or nitro, for example, hydrogen, lower alkyl, acyloxy, halogen, hydroxyl, amino or nitro such as hydrogen, acyloxy or hydroxyl;

or a compound having the formula



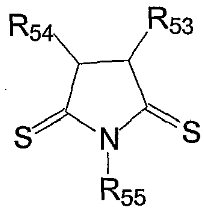
wherein n=1-5; X is oxygen or sulfur; and R_2-R_5 and R_{15}-R_{19} are as before;

or a compound having the formula:

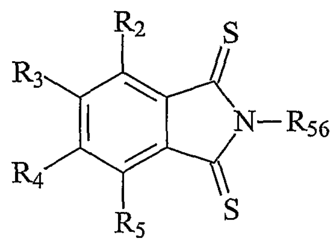


wherein each of X and Y are independently oxygen or sulfur, n=1-5, and R_2-R_5 are as before;

or a compound having the formula:



wherein R_{53} and R_{54} are independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy, substituted acyloxy, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen or nitro, for example, hydrogen, lower alkyl, acyloxy, halogen, hydroxyl, amino or nitro such as hydrogen, acyloxy or hydroxyl ;and R_{55} is hydrogen, alkyl, or substituted alkyl; or a compound having the formula:



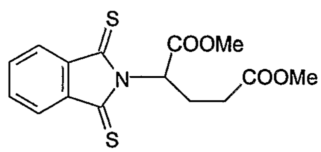
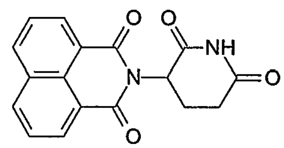
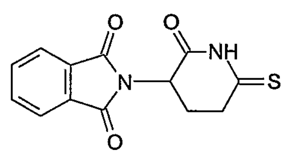
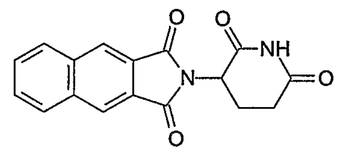
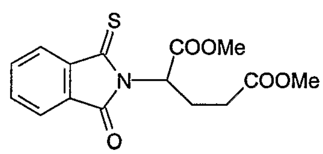
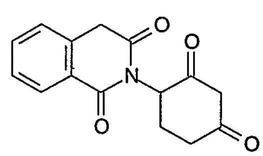
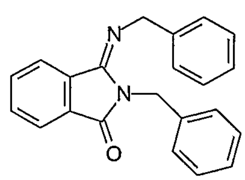
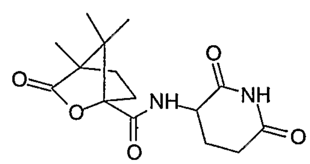
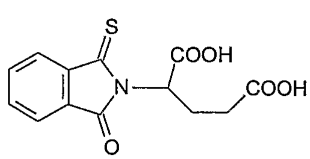
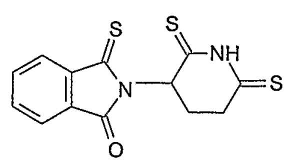
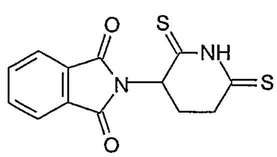
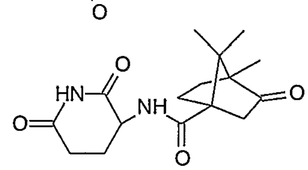
wherein R_2-R_5 are as before and R_{56} is hydrogen, alkyl or substituted alkyl;

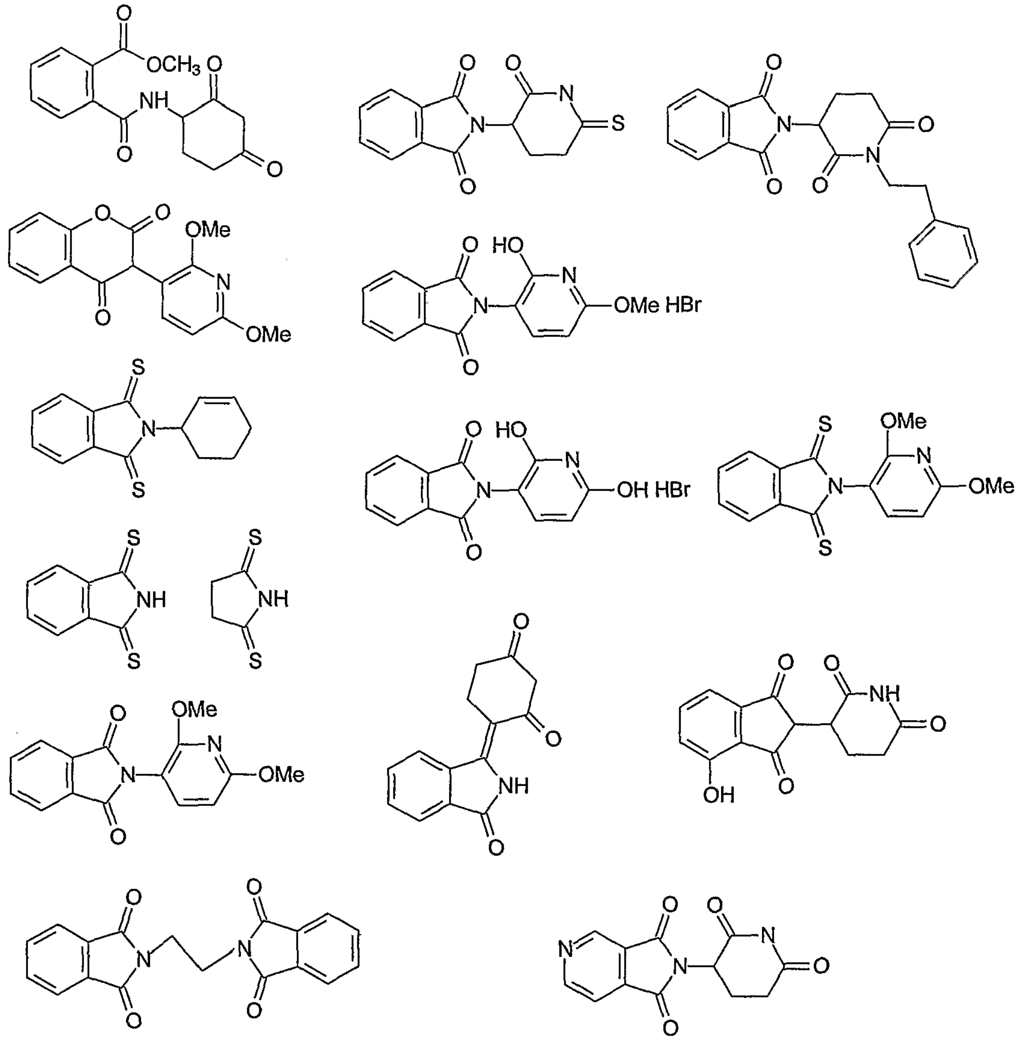
or pharmaceutically acceptable salts or stereoisomers thereof.

Novel thio-substituted analogs having the structures described with respect to the method above also are contemplated. For example, in more particular embodiments, X or Y is sulfur, and both W and Z are oxygen if present; both X and Y are sulfur and both W and Z are oxygen if present; X and Y are both oxygen and W or Z is sulfur if present; both X and Y are sulfur and W or Z is sulfur if present;

or X or Y are sulfur and both W and Z are sulfur if present. Alternatively, if W and Z are present, the following are possible: X=O, Y=O, W=O, Z=O; X=S, Y=O, W=O, Z=O; X=O, Y=S, W=O, Z=O; X=O, Y=O, W=S, Z=O; X=O, Y=O, W=O, Z=S; X=S, Y=S, W=O, Z=O; X=S, Y=O, W=S, Z=O; X=S, Y=O, W=O, Z=S; X=O, Y=O, W=S, Z=S; X=O, Y=S, W=O, Z=S; X=O, Y=S, W=S, Z=O; X=S, Y=S, W=S, Z=O; X=S, Y=S, W=O, Z=S; X=S, Y=O, W=S, Z=S; X=O, Y=S, W=S, Z=S; or X=S, Y=S, W=S, Z=S.

Particularly disclosed compounds and compounds that can be used in the disclosed methods include one or more compounds having the following structures:



Still further, a method for modulating angiogenesis in a subject is disclosed. The method includes administering to the subject a therapeutically effective amount of one or more of any of the disclosed compounds. Examples of compounds useful for the method are shown above. In some embodiments, where an anti-angiogenic compound or an anti-angiogenic concentration of a compound is utilized, the therapeutically effective amount of the compound can be administered to a subject with a tumor to achieve an anti-tumor effect, such as inhibition of tumorigenesis or tumor metastasis. In other embodiments, the therapeutically effective amount of the compound is administered to a subject with a pathological angiogenesis. Alternatively, where stimulation of angiogenesis is desired an angiogenic compound or an angiogenic concentration of a compound is administered to a subject to stimulate angiogenesis.

As angiogenesis inhibitors, the disclosed compounds are useful in the treatment of both primary and metastatic solid tumors, including carcinomas of breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, gallbladder and bile ducts, small intestine, urinary tract (including kidney, bladder and urothelium), female genital tract, (including cervix, uterus, and ovaries as well as choriocarcinoma and gestational trophoblastic disease), male genital tract (including prostate, seminal vesicles, testes and germ cell tumors), endocrine glands (including the thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas, sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma) and tumors of the brain, nerves, eyes, and meninges (including astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and meningiomas). Such compounds may also be useful in treating solid tumors arising from hematopoietic malignancies such as leukemias (i.e. chloromas, plasmacytomas and the plaques and tumors of mycosis fungoides and cutaneous T-cell lymphoma/leukemia) as well as in the treatment of lymphomas (both Hodgkin's and non-Hodgkin's lymphomas). In addition, these compounds may be useful in the prevention of metastases from the tumors described above either when used alone or in combination with radiotherapy and/or other chemotherapeutic agents.

Further uses of disclosed anti-angiogenic compounds/concentrations include the treatment and prophylaxis of autoimmune diseases such as rheumatoid, immune and degenerative arthritis. Such compounds can also be used to treat a pathological (i.e. abnormal, harmful or undesired) angiogenesis, for example, various ocular diseases such as diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, retrolental fibroplasia, neovascular glaucoma, rubeosis, retinal neovascularization due to macular degeneration, hypoxia, angiogenesis in the eye associated with infection or surgical intervention, and other abnormal neovascularization conditions of the eye; skin diseases such as psoriasis; blood vessel diseases such as hemagiomas, and capillary proliferation within atherosclerotic plaques; Osler-Webber Syndrome; myocardial angiogenesis; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; and wound granulation. Other uses include the treatment of diseases characterized by excessive or abnormal stimulation of endothelial cells, including but not limited to intestinal adhesions, Crohn's disease, atherosclerosis, scleroderma, and hypertrophic scars, such as keloids. Another use is as a birth control agent, by inhibiting ovulation and establishment of the placenta. The disclosed compounds are also useful in the treatment of diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochele minalia quintosa) and ulcers (Helicobacter pylori). The disclosed compounds are also useful to reduce bleeding by administration prior to surgery, especially for the treatment of resectable tumors.

Angiogenic compounds or angiogenic concentrations of disclosed compound can be used can be used to treat a variety of conditions that would benefit from stimulation of angiogenesis, stimulation of vasculogenesis, increased blood flow, and/or increased vascularity. Particular examples of conditions and diseases amenable to treatment using disclosed angiogenic compounds, or angiogenic concentrations of disclosed compounds, include any condition associated with an obstruction of a blood vessel, such as obstruction of an artery, vein, or of a capillary system. Specific examples of such conditions or disease include, but are not necessarily limited to, coronary occlusive disease, carotid occlusive disease, arterial occlusive disease, peripheral arterial disease, atherosclerosis, myointimal hyperplasia (such as due to vascular surgery or balloon angioplasty or vascular stenting), thromboangiitis obliterans, thrombotic disorders, vasculitis, and the like. Examples of conditions or diseases that may be prevented using the disclosed angiogenic compounds/concentrations include, but are not limited to, heart attack (myocardial infarction) or other vascular death, stroke, death or loss of limbs associated with decreased blood flow, and the like. Other therapeutic uses for angiogenesis stimulation according to the disclosure include, but are not necessarily limited to accelerating healing of wounds or ulcers; improving the vascularization of skin grafts or reattached limbs so as to preserve their function and viability; improving the healing of surgical anastomoses(such as in re-connecting portions of the bowel after gastrointestinal surgery); and improving the growth of skin or hair.

Yet further, a method for inhibiting TNF-α activity in a subject using the disclosed compounds is provided. The method includes administering a therapeutically effective amount of a disclosed compound to a subject to achieve a TNF-α inhibitory effect. The disclosed compounds having TNF-α inhibitory effects are useful for treating many inflammatory, infectious, immunological, and malignant diseases. These include but are not limited to septic shock, sepsis, endotoxic shock, hemodynamic shock and sepsis syndrome, post ischemic reperfusion injury, malaria, mycobacterial infection, meningitis, psoriasis and other dermal diseases, congestive heart failure, fibrotic disease, cachexia, graft rejection, cancer, tumor growth, undesirable angiogenesis, autoimmune disease, opportunistic infections in AIDS, rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, other arthritic conditions, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, systemic lupus erythrematosis, ENL in leprosy, radiation damage, and hyperoxic alveolar injury. In addition, the compounds can be used to treat other neurodegenerative diseases as exemplified by Alzheimer's disease, Parkinson's disease, head trauma, stroke and ALS.

The disclosed compounds can be used in combination with other compositions and procedures for the treatment of diseases. For example, a tumor can be treated conventionally with surgery, radiation or chemotherapy in combination with an anti-angiogenic compound/concentration and then, optionally the compound/concentration can be further administered to the subject to extend the dormancy of micrometastases and to stabilize and inhibit the growth of any residual primary tumor. Alternatively, an angiogenic compound or angiogenic concentration of a compound can be used in combination with other angiogenesis stimulating agents. For example, thermal energy (in the form of resistive heating, laser energy or both) to create thermally treated stimulation zones or pockets (optionally interconnected, at least initially, by small channels) in the tissue for the introduction of blood born growth and healing factors, along with stimulated capillary growth surrounding the thermally treated zones. Such stimulation zones allow increased blood flow to previously ischemic and/or nonfunctional tissue (such as cardiac tissue) with a concomitant increased supply of oxygen and nutrients ultimately resulting in a revitalization of the treated sections the tissue when used in combination with the angiogenic compositions/concentrations. In other embodiments, disclosed compounds exhibiting TNF-α inhibitory activity can be combined with other TNF-α inhibitory agents, for example, steroids such as dexamethasone and prednisolone. When used for treatment of a cancer, the compounds can be used in combination with chemotherapeutic agents and/or radiation and/or surgery.

Examples of other chemotherapeutic agents that can be used in combination with the disclosed compounds include alkylating agents, antimetabolites, natural products, kinase inhibitors, hormones and their antagonists, and miscellaneous other agents. Examples of alkylating agents include nitrogen mustards (such as mechlorethamine, cyclophosphamide, melphalan, uracil mustard or chlorambucil), alkyl sulfonates (such as busulfan), and nitrosoureas (such as carmustine, lomustine, semustine, streptozocin, or dacarbazine). Examples of antimetabolites include folic acid analogs (such as methotrexate), pyrimidine analogs (such as 5-FU or cytarabine), and purine analogs, such as mercaptopurine or thioguanine. Examples of natural products include vinca alkaloids (such as vinblastine, vincristine, or vindesine), epipodophyllotoxins (such as etoposide or teniposide), antibiotics (such as dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin, or mitocycin C), and enzymes (such as L-asparaginase). Examples of kinase inhibitors include small molecule inhibitors (such as Iressa, Tarceva, PKI-166, CI-1033, CGP-5923A, EKB-569, TAK165, GE-572016, CI-1033, SU5416, ZD4190, PTK787/ZK222584, CGP41251, CEP-5214, ZD6474, BIBF1000, VGA1102, SU6668, SU11248, CGP-57148, tricyclic quinoxalines, SU4984, SU5406, Gleevec, NSC680410, PD166326, PD1173952, CT53518, GTP14564, PKC412, PP1, PD116285, CGP77675, CGP76030, CEP-701, and CEP2583), ligand modulators (such as Bevacizumanb, MV833, Soluble Flt-1 and Flk-1, VEGF Trap, GFB 116, NM3, VEGF 121-diptheria toxin conjugate and Interfereon-α), and monoclonal antibodies against receptors (such as Cetuximab, ABX-EGF, Y10, MDX-447, h-R3, EMD 72000, herceptin, MDX-H210, pertuzumab, IMC-1C11, and MF1). Examples of hormones and antagonists include adrenocorticosteroids (such as prednisone), progestins (such as hydroxyprogesterone caproate, medroxyprogesterone acdtate, and magestrol acetate), estrogens (such as diethylstilbestrol and ethinyl estradiol), antiestrogens (such as tamoxifen), and androgens (such as testerone proprionate and fluoxymesterone). Examples of miscellaneous agents include platinum coordination complexes (such as cis-diamine-dichloroplatinum II, which is also known as cisplatin), substituted ureas (such as hydroxyurea), methyl hydrazine derivatives (such as procarbazine), vaccines (such as APC8024), AP22408, B43-genistein conjugate, paclitaxel, AG538, and adrenocrotical suppressants (such as mitotane and aminoglutethimide). In addition, the disclosed compounds can be combined with gene therapy approaches, such as those targeting VEGF/VEGFR (including antisense oligonucleotide therapy, Adenovirus-based Flt-1 gene therapy, Retrovirus-base Flk-1 gene therapy, Retrovirus-based VHL gene therapy, and angiozyme) and IGF-1R (including INX-4437). Examples of the most commonly used chemotherapy drugs that can be used in combination with the disclosed tricyclic compounds agent include Adriamycin, Alkeran, Ara-C, BiCNU, Busulfan, CCNU, Carboplatinum, Cisplatinum, Cytoxan, Daunorubicin, DTIC, 5-FU, Fludarabine, Hydrea, Idarubicin, Ifosfamide, Methotrexate, Mithramycin, Mitomycin, Mitoxantrone, Nitrogen Mustard, Taxol, Velban, Vincristine, VP-16, Gemcitabine (Gemzar), Herceptin, Irinotecan (Camptosar, CPT-11), Leustatin, Navelbine, Rituxan STI-571, Taxotere, Topotecan (Hycamtin), Xeloda (Capecitabine), Zevelin and calcitriol.

The disclosed compounds also can be combined with radiotherapy employing radioisotopes (such as ^{32}P, ^{90}Y, ^{125}I, ^{131}I, and ^{177}Lu), particle beams (such as proton, neutron and electron beams) and electromagnetic radiation (such as gamma rays, x-rays and photodynamic therapy using photosensitizers and visible or ultraviolet rays).

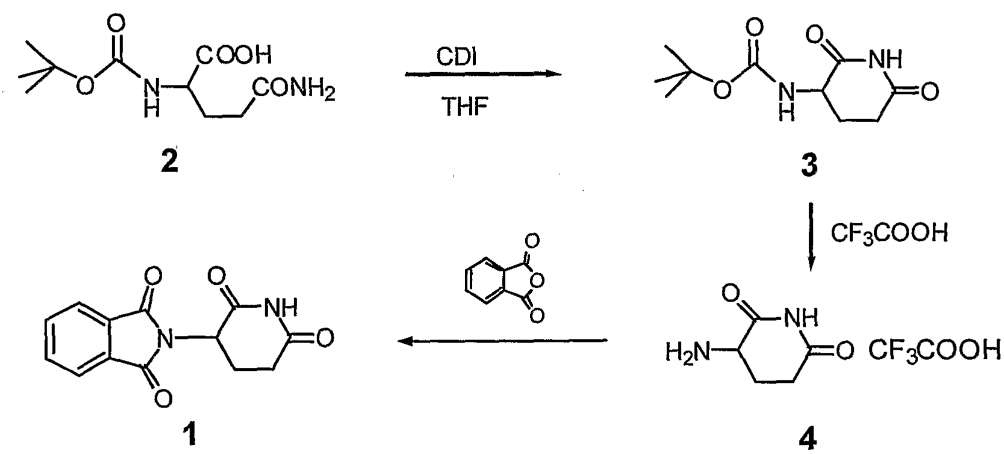
Additionally, the disclosed compounds can be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions. Therefore, also disclosed are pharmaceutical compositions including one or more of any of the compounds disclosed above and a pharmaceutically acceptable carrier. The composition may comprise a unit dosage form of the composition, and may further comprise instructions for administering the composition to a subject to inhibit angiogenesis, for example, instructions for administering the composition to achieve an anti-tumor effect or to inhibit a pathological angiogenesis. In particular embodiments, the pharmaceutical composition may comprise one or more of 1-Thioxo-3-oxo-2-(2-oxo-6-thioxopiperidin-3-yl)isoindoline, 1,3-Dioxo-2-(2,6-dithioxopiperidin-3-yl)isoindoline, 1-Thioxo-3-oxo-2-(2,6-dithioxopiperidin-3-yl)isoindoline, *N*-(2,6-dioxopiperidin-3-yl)-2,3-naphthalenedicarboxamide, 1,3-Dioxo-2-(2,6-dioxopiperidin-3-yl)-5-azaisoindoline, 1,3-Dioxo-2-(1-phenethyl-2,6-dioxopiperidin-3-yl)isoindoline, 2-Acetoxy-*N*-(2,6-dioxopiperidin-3-yl)benzamide, 2-(2-Oxo-6-thioxo-3-piperidinyl)-1*H*-isoindole-1,3(2*H*)-dione, Dimethyl 2-(1,3-dihydro-1-oxo-3-thioxo-2*H*-isoindol-2-yl)-pentanedioate, Dimethyl 2-(1,3-dihydro-1,3-dithioxo-2*H*-isoindol-2-yl)-pentanedioate, 2-(1,3-Dihydro-1-oxo-3-thioxo-2*H*-isoindol-2-yl)-pentanedioic acid, 2,3-Dihydro-3-thioxo-2-(2,6-dioxo-3-piperidinyl)-1*H*-isoindol-1-one, 2-(2, 6-Dithioxo-3-piperidinyl)-1*H*-isoindole-1,3(2*H*)-dione, 2,3-Dihydro-3-thioxo-2-(2-oxo-6-thioxo-3-piperidinyl)-1*H*-isoindol-1-one, 2,3-Dihydro-3-thioxo-2-(2,6-dithioxo-3-piperidinyl)-1*H*-isoindol-1-one, 2-(3-Cyclohexenyl)-1*H*-isoindol-1,3(2*H*)-dithione, 2-(3-Cyclohexenyl)-1*H*-isoindole-1,3(2*H*)-dione, 2-(3-Cyclohexenyl)-1*H*-isoindol-1,3(2*H*)-dithione, 2,3-Dihydro-3-thioxo-2-(3-cyclohexenyl)-1*H*-isoindol-1-one, 3-(2,6-Dioxopiperidin-3-yl)benzoxazine-2,4-dione, 1-(2,6-Dioxo-3-piperidinylidene)-3-oxoisoindoline, 6-Thioxo-2-piperidinone, 2,6-Piperidinedithione, monothiophthalimide, dithiophthalimide, N-phenethylphthalimide, 3-Benzylimino-2-benzyl-2,3-dihydroisoindol-1-one, 3-Camphanic amino-2,6-piperdinedione and 3-[2',6'-piperidinedion-3'-yl]-7-amino-2H-1,3-benzoxazine-2,4(3H)-dione; and a pharmaceutically acceptable carrier. In more particular embodiments, the disclosed compositions are compounded for oral administration, and such oral dosage forms can include one or more of any of the disclosed compounds including those compounds particularly disclosed by their IUPAC names above. Such pharmaceutical compositions may be used in methods for modulating angiogenesis or TNF-α activity in a subject by administering to the subject a therapeutically effective amount of the composition.

As is demonstrated in the Examples that follow, thionation of thalidomide analogs to replace carbonyl groups with thiocarbonyl groups can provide thalidomide analogs with increased TNF-α activity, increased angiogenic activity or increased anti-angiogenic activity. Thus, although in certain structures the compounds are shown with carbonyl groups, it is to be understood that thionated derivatives of such compounds are also part of the disclosure.

4. Examples

Example 1 - Improved Synthesis of Thalidomide

With reference to Scheme 1 below, *t*-Butoxycarbamate **2**, on reaction with carbodiimide in THF, gave imide **3**. Imide **3** was deprotected with trifluoroacetic acid in CH_2Cl_2 at room temperature to yield aminoglutarimide trifluoroacetate **4**. Without further purification, compound **4** was reacted with phthalic anhydride in refluxing THF in the presence of triethylamine to afford thalidomide **1** in the total yield of 24% from **2**. This procedure is much more practical and efficient than several prior reported synthetic routes for the preparation of thalidomide.



Scheme 1

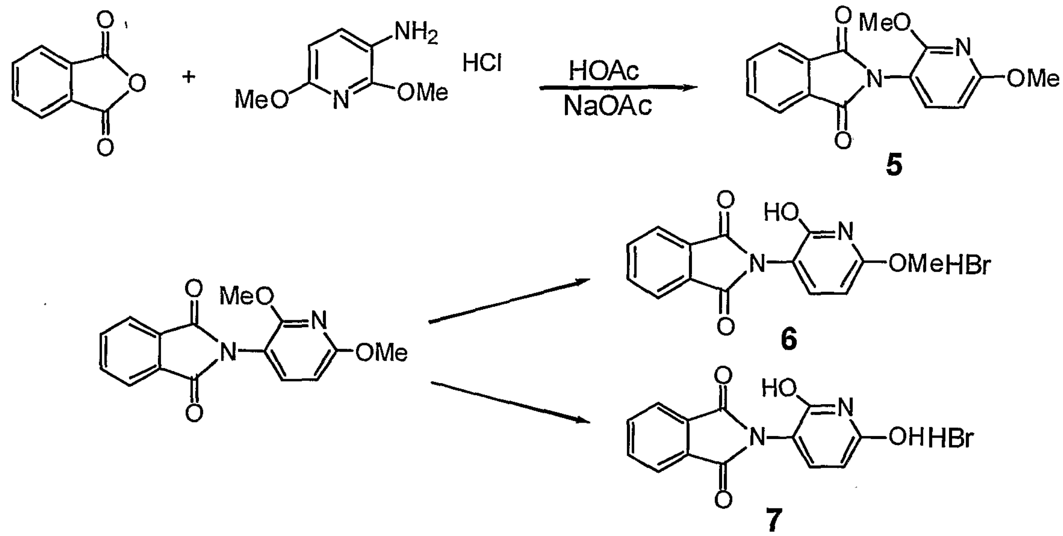
2,6-Dioxo-3-(t-butoxycarbonylamino)piperidine (**3**) was prepared and isolated as follows. A solution of *N*-(*t*-butoxycarbonyl)-L-glutamine (4.92 g) and carbonyl diimidazole (1.70 g) in THF (100 mL) was refluxed for 9 h. The solvent was removed and the crude product was recrystallized from hot EtOAc to give compound **3** (2.04 g, 45%) as white crystals: mp 214-215°C; ^1H NMR (DMSO-d_6) δ 4.22 (dd, J = 6.2 Hz, J = 11.0 Hz, 1H), 2.77-2.65 (m, 1H), 2,45 (m, 1 H), 1.96-1.87 (m, 2H), 1.40 (s, 9H); MS (CI/CH_4) 227 $[M-1]^+$.

2,6-Dioxo-3-aminopiperidine trifluoroacetate (**4**) was prepared and isolated as follows. Compound **3** (59 mg) was suspended in CH_2Cl_2 (5 mL). CF_3COOH (0.5 mL) was added. The reaction solution was stirred at room temperature for 4 h. The solvent was removed to give **4** (62 mg, 99%): ^1H NMR (DMSO-d_6) δ 11.42 (s, 1H), 8.70 (br, 2H), 4.31 (dd, J =5.4 Hz, J = 13 Hz), 2.88-2.72 (m, 2H), 2.25-2.09 (m, 2H).

Thalidomide (**1**) was prepared and isolated as follows. A mixture of **4**, phthalic anhydride and Et_3N in THF was refluxed for two days. The reaction mixture was concentrated and purification by column chromatography (eluent CH_2Cl_2/EtOAc=6:1) gave thalidomide (104 mg, 54%) as white crystals.

Example 2 - Synthesis of Aromatic Thalidomide Analogs

With reference to Scheme 2 below, dimethylether **5** was obtained by condensation of aminopyridine with phthalic anhydride in refluxing AcOH in the presence of sodium acetate. On standing with HBr in glacial AcOH solution (30 %) at room temperature for 18 h, selective ether cleavage of **5** was accomplished to give compound **6**. The structure of compound **6** was determined by mass spectroscopy, 1D NMR and 2D NMR. The molecular ion for compound **6** is 270 amu, demonstrating that only one methyl ether was cleaved. 2D NOESY showed that protons on the methoxy group correlated with H-5, indicating that the 2-methoxy was selectively cleaved and that the 6-methoxy remained. When the reaction temperature elevated to 70°C, both methyl ethers were cleaved with HBr/HOAc solution (30%) to give diol **7**.



Scheme 2

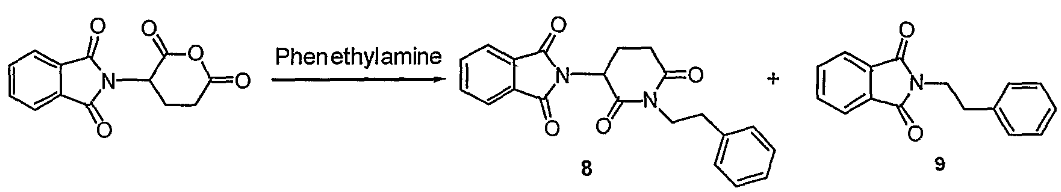
1,3-Dioxo-2-(2,6-dimethoxypyridin-3-yl)-isoindoline (**5**) was prepared and isolated as follows. A mixture of phthalic anhydride (0.89g, 6 mmol), 3-amino-2,6-dimethoxypyridine monohydrochloride (95%, 1 g, 5 mmol) and sodium acetate (0.49 g, 6 mmol) in glacial acetic acid (50 ml) was refluxed for 3 h. The solvent was removed under vacuum. The residue was dissolved in dichloromethane (200 ml) and washed with water (100 ml×3), dried over Na_2SO_4 and concentrated to give the crude product. The crude product was recrystallized with ethyl acetate to give **5** (1.345 g, 90%) as a pale pink crystals: mp 182-183°C; ^{1}H NMR ($CDCl_3$) δ 7.96-7.90 (m, 2H), 7.80-7.76 (m, 2H), 7.44 (d, J = 8.1 Hz, 1H), 6.42 (d, J = 8.1 Hz, 1H, 3.95 (s, 3 H), 3.91 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 166.5, 160.6, 156.1, 140.1, 132.8, 129.4, 121.4, 104.6, 99.3, 51.7, 51.5; MS (CI/CH_4) 285 $[M+1]^+$. Anal. Calcd for $C_{15}H_{12}N_2O_4$: C, 63.38; H, 4.25; N, 9.85. Found: C, 63.57; H, 4.18; N, 9.65.

1,3-Dioxo-2-(2-hydroxy-6-methoxypyridin-3-yl)-isoindoline hydrobromide (**6**) was prepared and isolated as follows. To a flask were added 2,6-dimethoxy-3-phthalimidopyridine (155 mg, 0.546 mmol) and hydrogen bromide solution in acetic acid (30%, 6 ml). The mixture was stirred at room temperature under N_2 for 18 h. Dry ether was added slowly until the solution became cloudy. White crystals were precipitated, filtered and washed with ether and ethyl acetate to afford **6** (127 mg, 67%) as white powdery crystals: mp 250°C; ^{1}H NMR (DMSO-d_6) δ 7.97-7.94 (m, 2H), 7.91-7.88 (m, 2H), 7.64 (d, J = 8.2 Hz, 1H), 6.25 (d, J = 8.2 Hz, 1H), 3.86 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.5, 162.1, 159.1 , 142.8, 135.4, 132.1, 123.7, 108.2, 96.4, 54.8; MS (CI/CH_4) 270 $[M]^+$.

1,3-Dioxo-2-(2,6-dihydroxypyridin-3-yl)-isoindoline hydrobromide (**7**) was prepared and isolated as follows. To a flask were added 2,6-dimethoxy-3-phthalimidopyridine (150 mg, 0.528 mmol) and hydrogen bromide solution in acetic acid (30%, 6 ml). The mixture was stirred at an 70°C oil bath under N_2 for 54 h. The mixture was cooled to room temperature, dry ether was added, and the supernatant liquid was decanted. Then ethyl acetate was added, solid precipitated, filtered and washed with ethyl acetate to afford **7** (126 mg, 71%) as a white solid: ^1H NMR (CD_3OD) δ 7.83-7.77 (m, 4H), 6.37 (d, J = 8.1 Hz, 1H), 6.37 (d, J = 8.1 Hz, 1H); MS (CI/CH_4) 256 $[M]^+$; HRMS (DEI) m/z calcd for $C_{13}H_8N_2O_4$ 256.0484, found 256.0483.

Example 3 – Synthesis of N-Substituted Thalidomide Analogs

With reference to Scheme 3 below, a mixture of *N*-phthaloyl-DL-glutamic anhydride and phenethylamine was heated in a 177°C oil bath. The reaction mixture was purified by chromatography on a silica gel column to afford *N*-phenethylthalidomide (**8**) and *N*-phenethylphthalimide (**9**).



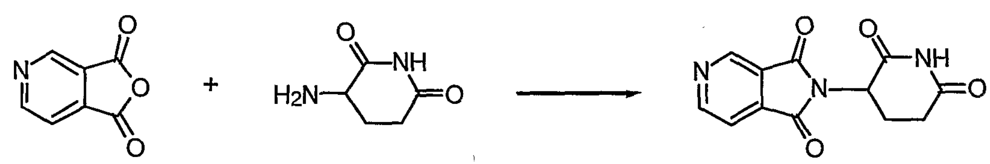
Scheme 3

1,3-Dioxo-2-(1-phenethyl-2,6-dioxopiperidin-3-yl)isoindoline (**8**) was specifically prepared and isolated as follows. A mixture of *N*-phthaloyl-DL-glutamic anhydride (300 mg, 1.13 mmol) and phenethylamine (139 mg, 1.13 mmol) was stirred in a 177°C oil bath for two hours. The reaction mixture was cooled down and purified by column chromatography, first using petroleum ether/dichloromethane (1:5) as an eluent to afford *N*-phenethyl phthalimide as a pale yellow solid [^1H NMR ($CDCl_3$) δ 7.78-7.77 (m, 2H), 7.65-7.62 (m 2 H), 7.22-7.16

(m, 5 H), 3.83 (t, 2H), 2.92 (t, 2 H)], and then using dichloromethane as an eluent to afford *N*-phenethyl thalidomide as a syrup that was then recrystallized from ether to provide white crystals [(139 mg, 34%): mp 122-123°C; ^{1}H NMR ($CDCl_3$) δ 7.84-7.81 (dd, J =3.1 Hz, J= 5.4 Hz, 2H), 7.72-7.69 (dd, J = 3.1 Hz, J = 5.4 Hz, 2 H), 7.20-7.14 (m, 5 H), 4.89 (dd, J = 5.4 Hz, J = 12.5 Hz, 1 H), 4.01-3.92 (m, 2 H), 2.90-2.63 (m, 5 H), 2.06-2.02 (m, 1 H); Anal. Calcd for $C_{21}H_{18}N_2O_4$: C, 69.60; H, 5.01; N, 7.73. Found: C, 69.40; H, 5.13; N, 7.74].

Example 4 – Synthesis of Azathalidomides

With reference to Scheme 4 below, azathalidomide was prepared from aminoglutarimide and commercial pyridine-3,4-dicarboxylic anhydride. Cbz-aminoglutarimide was deprotected by hydrogenolysis with catalyst palladium hydroxide on carbon (10%) to form aminoglutarimide. Pyridine-3,4-dicarboxyic anhydride was refluxed with aminoglutarimide in the presence of triethylamine to yield azathalidomide **11** in the total yield of 17% from Cbz-aminoglutarimide.



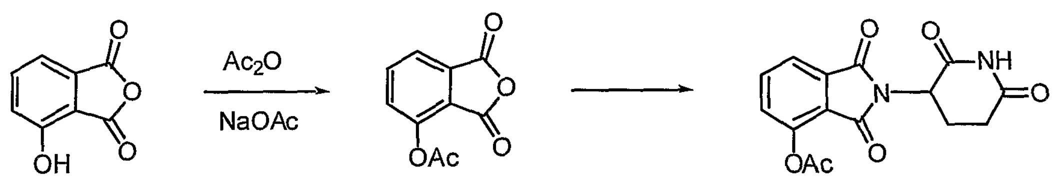
11

Scheme 4

1,3-Dioxo-2-(2,6-dioxopiperidin-3-yl)-5-azaisoindoline (**11**) was prepared specifically as follows. A mixture of Cbz-aminoglutarimide (302 mg) and palladium hydroxide on carbon (20%) in 2-propanol (20 ml) was stirred under H_2 for one day. The reaction mixture was filtered through celite and washed with 2-propanol and methanol. The combined filtrate was concentrated to afford crude 3-amino-1,6-dioxopiperidine as syrup. To the flask containing 3-amino-1,6-dioxopiperidine was added 3,4-pyridinedicarboxylic anhydride (205 mg), triethylamine (0.16 ml) and THF (10 ml). The mixture was refluxed for one and a half days. The solvent was removed under vacuum. The residue was purified by column chromatography using $CH2Cl_2$:MeOH (10:1) as eluent to afford azathalidomide (52 mg) in the yield of 17% from Cbz-aminoglutarimide as a pale purple solid: mp 233-235°C, ^{1}H NMR (DMSO) δ 11.18 (s, 1H), 9.21 (s, 1H), 9.17

(d, J = 4.8 Hz, 1 H), 7.98 (d, J = 4.8 Hz, 1 H), 5.23 (dd, J = 5.4 Hz, J = 12.8 Hz, 1 H), 2.96-2.85 (m, 2 H), 2.60-2.51 (m, 1 H), 2.12-2.07 (m, 1 H); MS (CI/CH_4) m/z 259 $[M]^+$; Anal. Calcd for $C_{12}H_9N_2O_4$: C, 55.60; H, 3.50; N, 16.21. Found: C, 55.36; H, 3.44; N, 15.94.

Example 5 – Synthesis of Acetoxythalidomide Analogs



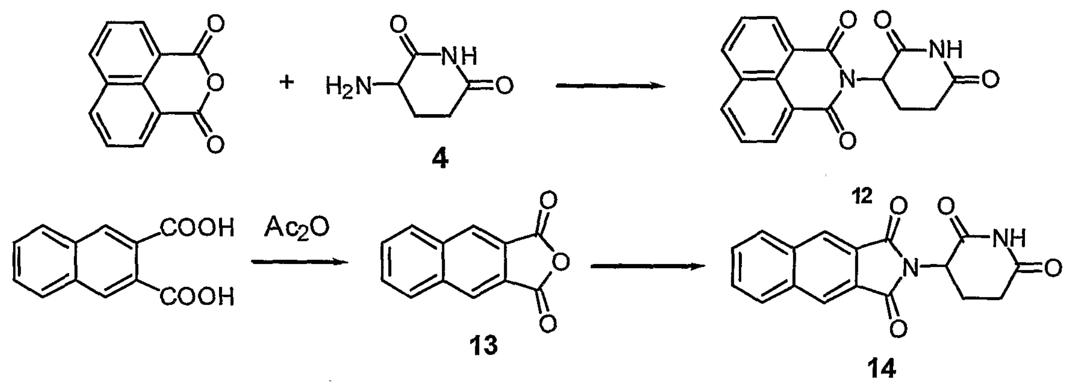
Scheme 5

With reference to scheme 5 above, acetoxythalidomide was prepared and isolated as follows. First, 3-Acetoxyphathalic anhydride was prepared by refluxing a mixture of 3-hydroxyphthalic anhydride (150 mg), acetic anhydride (2 mL), and NaOAc (150 mg) for 8 h. The reaction mixture was filtered. The filtrate was concentrated and washed with dry ether to give a pale yellow solid (127 mg, 68%). ^{1}H NMR (DMSO) δ 8.25 (d, J = 7.9 Hz, 1H), 8.18 (dd, J = 0.9 Hz, J = 7.5 Hz, 1H), 7.97 (dd, J = 0.9 Hz, J = 7.9 Hz, 1H), 2.59 (s, 3H).

1,3-Dioxo-2-(2,6-dioxopiperidin-3-yl)-4-acetoxyisoindoline was prepared and isolated as follows. A mixture of 3-acetoxyphthalic anhydride (40 mg), aminoglutarimide trifluoroacetate (47 mg), and NaOAc (32 mg) in acetic acid (2 mL) was refluxed for 5 h. The solvent was evaporated, water (10 mL) was added, and the resulting solution was stirred for several minutes. The solid was filtered out and recrystallized from ethyl acetate to give 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-acetoxyisoindoline as pale yellow crystals (35 mg, 66%): ^{1}H NMR (DMSO) δ 11.16 (s, 1H), 11.07 (s, 1H), 7.64 (t, J = 7.2 Hz, 1H), 7.22-7.31 (m, 2H), 5.05 (dd, J = 5.4 Hz, J = 12.5 Hz, 1H), 2.87-2.92 (m, 2H), 2.48 (s, 3H), 2.08-2.00 (m, 2H).

Example 6 – Synthesis of Benzothalidomides

With reference to Scheme 6 below, 1,8-Naphthalic anhydride on heating with amine **4** in the presence of triethylamine in THF gave **12**. Naphthalene-2,3-dicarboxylic acid was converted to the anhydride **13** which was reacted with aminoglutarimide trifluoroacetate **4** to afford benzothalidomide **14**. Spectral data, including mass spectra and NMR, as well as combustion analyses were in accord with the structures assigned to these products.



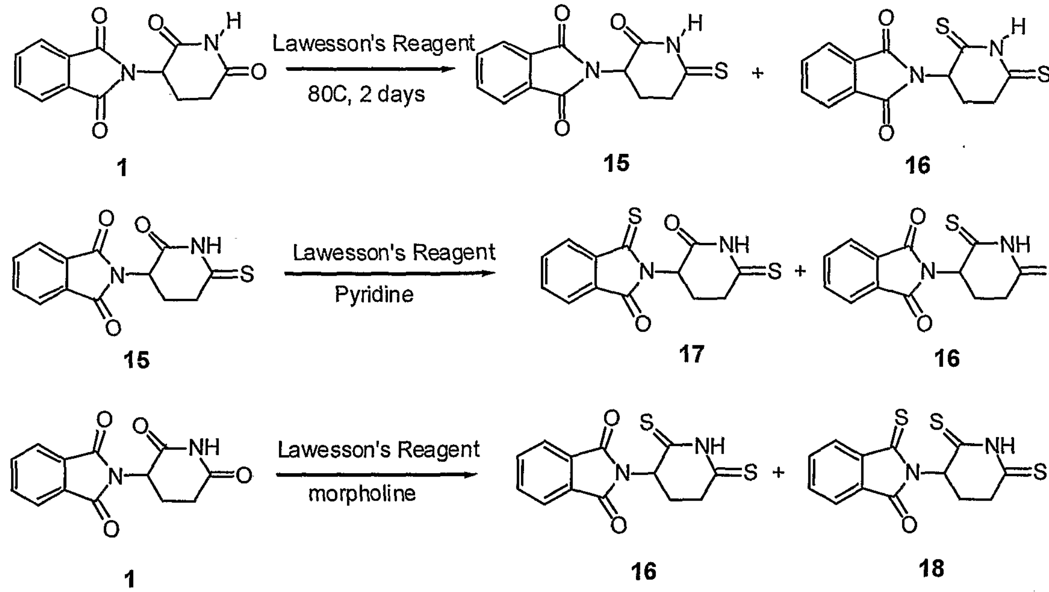
Scheme 6

Specifically, *N*-(2,6-dioxopiperidin-3-yl)-1,8-naphthalimide (**12**) was prepared and isolated as follows. A mixture of amine **4** (0.877 mmol), 1,8-naphthalic anhydride (174 mg, 0.879) and triethylamine (1.22 ml) in THF (10 ml) was refluxed for 20 h. The solvent was removed and the residue was suspended in acetic anhydride and refluxed for 20 minutes. Ethanol (5 ml) was added at 80°C and stirred for 30 min. On cooling the product was collected by filtration, and washed with EtOAc to give compound **12** (227 mg, 84%) as a pale green solid: mp > 300°C; ^{1}H NMR (DMSO-d$_6$) δ 11.03 (s, 1H), 8.61-8.47 (m, 4H), 7.92 (dd, J = 7.3 Hz, J = 13.5 Hz, 2H), 5.85 (dd, J = 5.4 Hz, J = 11.3 Hz, 1H), 3.01-2.88 (m, 1H), 2.73-2.61 (m, 2H), 2.08-1.99 (m, 1H). MS (DEI) m/z 309 $[M+1]^+$; HRMS (DEI) m/z calcd for $C_{17}H_{13}N_2O_4$ 309.0875, found 309.0874; Anal. Calcd for $C_{17}H_{12}N_2O_4$: C, 66.23; H, 3.92; N, 9.09. Found: C, 65.97; H, 3.99; N, 8.91.

N-(2,6-dioxopiperidin-3-yl)-2,3-naphthalenedicarboxamide (**14**) was prepared and isolated as follows. A mixture of 2,3-naphthalenedicarboxylic acid (199 mg, o.875 mmol) and acetic anhydride (2 mL) was refluxed for 30 min. The reaction mixture was cooled down, and the solid was collected by filter to afford anhydride **13** (0.133 g, 77%) as a white solid. To a solution of aminoglutarimide trifluoroacetate (163 mg) and triethylamine (1 mL) in THF (10 mL) was added anhydride **13** (133 mg). The mixture was refluxed for 16 h. The solvent was removed under vacuum, and the residue was dissolved in EtOAc, washed with saturated aqueous $NaHCO_3$ solution and H_2O, dried and concentrated. The residue was purified by flash chromatography to give compound **14** as a white solid (146 mg, 70%). mp > 300°C; ^{1}H NMR (DMSO-d_6) δ 11.3 (s, 1H), 8.60 (s, 2H), 8.30 (dd, J = 3.3 Hz, J = 6.1 Hz, 2H), 7.82 (dd, J = 3.2 Hz, J = 6.2 Hz, 2H), 5.24 (dd, J = 5.6 Hz, J = 13.0 Hz, 1H), 2.99-2.86 (m, 2H), 2.66-2.57 (m, 2H), 2.12-1.99 (m, 1H). MS (DEI) m/z 308 $[M]^+$; HRMS (DEI) m/z calcd for $C_{17}H_{12}N_2O_4$ 308.0797, found 308.0798; Anal. Calcd for $C_{17}H_{12}N_2O_4 \cdot 0.25H_2O$: C, 65.28; H, 4.03; N, 8.96 Found: C, 65.42; H, 3.93; N, 8.94.

Example 7- Synthesis of Sulfur Analogs of Thalidomide

With reference to Scheme 7 below, reaction of thalidomide **1** with Lawesson's reagent, when stirred in benzene at 80°C for 48 h, yielded thionamide **15** in a yield of 38%. In addition to monothiothalidomide, a trace of dithionimide **16** (1.6%) was also obtained. However, for the preparation of dithionimide, the yield proved to be very low (less than 2%) when the reaction of monothiothalidomide with Lawesson's reagent was performed between 80°C to 120°C. The situation changed greatly when organic base was added to the reaction mixture. Thus, thionation of monothiothalidomide **15** with Lawesson's reagent in toluene was carried out at 110°C in the presence of pyridine to give dithionimide **16** (45%) and dithionimide **17** (31%). The structures of these sulfur-substituted thalidomides were identified by mass spectra, 1DNMR and 2DNMR. Thalidomide was heated with Lawesson's reagent at 110°C in the presence of morpholine to afford dithionimide **16** and trithionimide **18**.



Scheme 7

1,3-Dioxo-2-(2-oxo-6-thioxopiperidin-3-yl)isoindoline (**15**) was synthesized and isolated as follows. A mixture of thalidomide (170 mg, 0.658 mmol) and Lawesson's reagent (293 mg, 0.724 mmol) in benzene (50 ml) was stirred in a 80°C oil bath for 2 days. The solvent was removed under vacuum. The residue was purified by column chromatography using CH_2Cl_2/petroluem ether (5:1) as eluent to afford compound **16** (3 mg, 1.6%) as a red solid and then, using CH_2Cl_2 as eluent, to afford compound **15** (68 mg, 38%) as a yellow solid: mp 225-226°C; ^{1}H NMR (DMSO-d_6) δ 12.83 (s, 1H), 8.00-7.92 (m, 4H), 5.32 (dd, J = 5.6 Hz, J = 12.9 Hz, 1H), 3.28-3.25 (m, 1H), 2.60-2.54 (m, 2H), 2.17-2.10 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 208.7(C-6'), 165.3(C-2'), 165.2(C-1 & C-3), 133.1(C-5 & C-6), 129.3 (C-3a, C-7a), 121.7 (C-4 & C-7), 46.9 (C-3'), 38.9 (C-5'), 21.79 (C-4'); MS (CI/CH_4) m/z 274 $[M]^+$; Anal. Calcd for $C_{13}H_{10}N_2O_3S$: C, 56.92; H, 3.67; N, 10.21 Found: C, 56.89; H, 3.78; N, 10.15.

1-Thioxo-3-oxo-2-(2-oxo-6-thioxopiperidin-3-yl)isoindoline (**16**) and 1,3-dioxo-2-(2,6-dithioxopiperidin-3-yl)isoindoline (**17**) were synthesized as follows. A mixture of **15** (146 mg, 0.533 mmol), Lawesson's reagent (108 mg, 0.267 mmol) and pyridine (21 μl) in toluene was stirred at 110°C under an atmosphere of N_2 for 12 h. Thereafter, more Lawesson's Reagent (108 mg, 0.267 mmol) and pyridine (21 μl) were added. The reaction mixture was stirred for a further 12 h. The solvent was removed under vacuum and the residue was purified by column chromatography (eluent CH_2Cl_2/petroleum ether = 2:1, 10:1, then CH_2Cl_2/EtOAc = 10:1) to afford **16** (30 mg, 45%) and **17** (21 mg, 31.5%). Starting material **15** (83 mg) was also recovered.

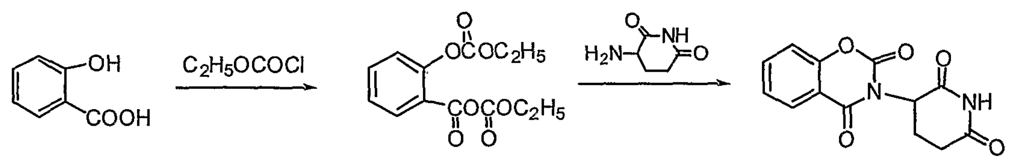
Compound **16**: (yellow solid): mp 263-265°C; ^{1}H NMR ($CDCl_3$) δ 7.78-7.74 (m, 2 H), 7.66-7.63 (m, 2 H), 5.00 (dd, J = 4.9 Hz, 11.9 Hz, 1 H), 3.43-3.35 (m, 1 H), 2.95-2.84 (m, 2 H), 2.08-2.06 (m, 1 H); MS (DEI) m/z 290 $[M]^+$; HRMS (DEI) m/z calcd for $C_{13}H_{10}N_2O_2S_2$ 290.0184, found 290.0185; Anal. Calcd for $C_{13}H_{10}N_2O_2S_2$: C, 53.77; H, 3.47; N, 9.65 Found: C, 53.38; H, 3.29; N, 9.50.

Compound **17**: (red solid): mp 240-242°C; ^{1}H NMR ($CDCl_3$) δ 9.44 (s, 1 H), 8.05-8.02 (m, 1 H), 7.86-7.76 (m, 3 H), 5.75-5.64 (m, 1 H), 3.57-3.52 (m, 1 H), 3.09-2.99 (m, 2 H), 2.19- 2.12 (m, 1 H). ^{13}C NMR (DMSO): 208.16, 207.98, 166.10, 165.39, 134.32, 133.11, 132.42, 124.30, 122.15, 121.11, 49.64, 21.29; MS(DEI) m/z 291 $[M+1]^+$; HRMS (DEI) m/z calcd for $C_{13}H_{11}N_2O_2S_2$ 291.0262, found 291.0264; Anal. Calcd for $C_{13}H_{10}N_2O_2S_2 \cdot 0.5H_2O$: C, 52.15; H, 3.70; N, 9.36 Found: C, 52.25; H, 3.44; N, 9.07.

1-Thioxo-3-oxo-2-(2,6-dithioxopiperidin-3-yl)isoindoline (**18**) was prepared and isolated as follows. A mixture of thalidomide (100 mg), Lawesson's reagent (157 mg) and morpholine (35 μl) in toluene (10 mL) was stirred at 105°C under the atmosphere of N_2 for 24 h. The solvent was removed under vacuum and the residue was purified by column chromatography, using CH_2Cl_2:petroleum ether (1:1) as eluent, to afford compound **18** (13 mg, 11%) as red crystals: mp 244°C; ^{1}H NMR ($CDCl_3$) δ 10.81 (s, 1H), 8.05-8.01 (m, 1H), 7.91-7.75 (m, 3H), 5.92 (m, 1H), 3.57-3.52 (m, 1H), 3.13-2.97 (m, 2H), 2.18-2.15 (m, 1H); MS(DEI) m/z 306 $[M]^+$; HRMS (DEI) m/z calcd for $C_{13}H_{10}N_2OS_3$ 305.9955, found 305.9951; Anal. Calcd for $C_{13}H_{10}N_2OS_2 \cdot 0.5H_2O$: C, 49.49; H, 3.51; N, 8.88 Found: C, 49.85; H, 3.24; N, 8.88. Then, CH_2Cl_2 was used as eluent to provide compound **16** (31 mg, 28%) as yellow crystals.

Example 8 - Synthesis of Benzoxazine-2,4-diones

With reference to Scheme 8 below, salicylic acid was treated with ethyl chloroformate, and then this reaction mixture was evaporated at reduced pressure to remove any unreacted ethyl chloroformate. Stirring the resulting residue with amine in the presence of triethylamine afforded substituted benzoxazine-2,4-diones

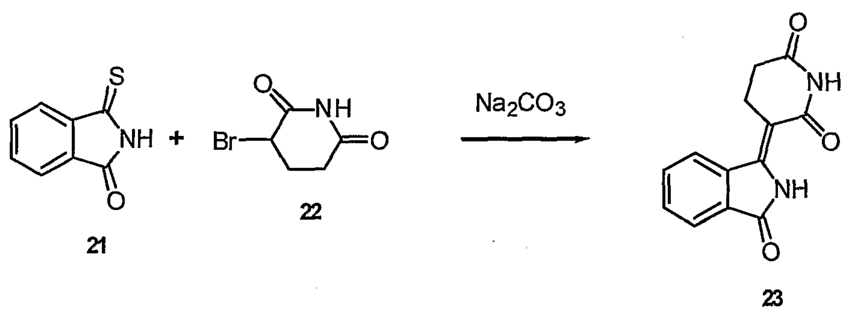


Scheme 8

3-(2,6-Dioxopiperidin-3-yl)benzoxazine-2,4-dione (**20**) was prepared and isolated as follows. To a cold ice/salt solution of salicylic acid (100 mg) and triethylamine (303 ml) in chloroform (10 mL) was added ethyl chloroformate (157 ml). The reaction mixture was allowed to warm to room temperature, and, thereafter, stirring was continued for 3h. The solvent was removed under vacuum to give crude **19**. Without further purification, crude compound **19** was dissolved in $CHCl_3$ and cooled with ice. To the ice cold solution was added amine (95 mg). The reaction mixture was allowed to warm to ambient temperature and stirred at room temperature overnight. The white solid precipitated, collected by filtration and washed with chloroform to give compound **20** (79 mg, 74%) as a white crystals: mp 264°C; ^1H NMR (DMSO-d_6) δ 11.18 (s, 1H), 8.07-7.85 (m, 2H), 7.50 (d, J = 8.5 Hz), 5.78-5.75 (m, 0.6H), 5.49-5.47 (m, 0.4H), 2.90-2.87 (m, 1H), 2.05 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 173.0 (0.6C), 172.9 (0.4C), 169.9 (0.6C), 169.6 (0.4C), 160.8 (0.6C), 159.8 (0.4C), 152.5 (1C), 148.4 (0.4C), 146.5 (0.6C), 137.2 (1C), 128.1 (0.6C), 127.6 (0.4C), 126.1 (1C), 116.8 (1C), 114.5 (0.4C), 113.9 (0.6C), 54.1 (0.4C), 51.4 (0.6C), 31.0 (1C), 21.2 (1C). MS(DEI) m/z 274 $[M]^+$; HRMS (DEI) m/z calcd for $C_{13}H_{10}N_2O_5$ 274.0590, found 274.0582; Anal. Calcd for $C_{13}H_{10}N_2O_5$: C, 56.94; H, 3.68; N, 10.22 Found: C, 56.51; H, 3.77; N, 9.95.

Example 9 – Synthesis of 1-(2,6-Dioxo-3-piperidinylidene)-3-oxoisoindoline

With reference to Scheme 9 below, monothiophthalimide (**21**) was stirred with 3-bromoglutarimide (**22**) in the presence of Na_2CO_3 in an Eschenmoser coupling reaction. Thus, compound **23** was formed by alkylation of monothiophthalimide with 3-bromoglutarimide, followed by elimination of sulfur.

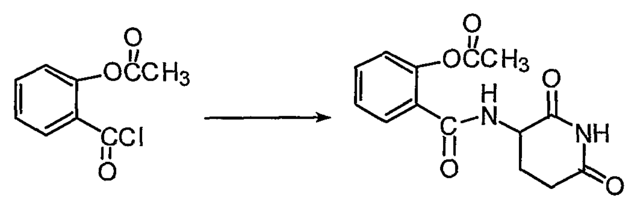


Scheme 9

1-(2,6-Dioxo-3-piperidinylidene)-3-oxoisoindoline (**23**) was specifically prepared and isolated as follows. A mixture of **21** (16 mg, 0.1 mmol), **22** (19 mg, 0.1 mmol), and potassium carbonate (100 mg) in anhydrous THF was refluxed for 7 h. Thin-layer chromatography (TLC) showed that the starting materials had disappeared. Ethyl acetate (20 ml) and water (10 ml) were added. The organic layer was separated, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by chromatography using petroleum ether/ethyl acetate (first 2:1 then 1:2) to give **23** (14 mg, 58%) as yellow crystals: mp 295°C; ^{1}HNMR (DMSO-d_6): 11.05 (s, 1 H), 10.29 (s, 1 H), 8.13 (d, J = 7.6 Hz, 1 H), 7.89 (d, J = 7.2 Hz, 1 H), 7.80 (m, 1 H), 7.73 (m, 1 H), 3.20 (t, J = 7.0 Hz, 2 H), 2.67 (t, J = 7.0 Hz, 2 H). ^{13}C NMR (DMSO-d_6): 172.6, 169.0, 167.3, 142.7, 136.1, 134.3, 131.7, 130.1, 126.4, 124.1, 104.6, 21.2, 11.7. MS(DEI) m/z 242 $[M]^+$; HRMS (DEI) m/z calcd for $C_{13}H_{10}N_2O_3$ 242.0691, found 242.0687.

Example 10 – Salicylamide Analogs

Reaction of commercial acetylsalicyloyl with aminoglutarimide trifluoroacetate was carried out to give acetylsalicylamide **24** according to Scheme 10 below.



24

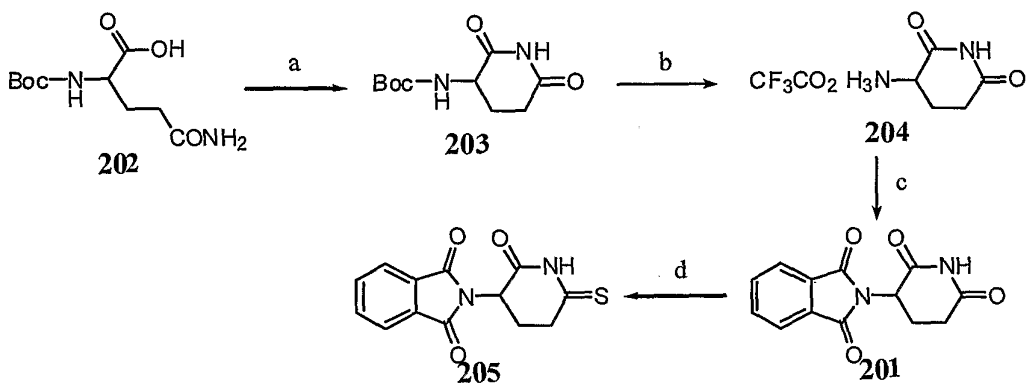
Scheme 10

More specifically, 2-acetoxy-*N*-(2,6-dioxopiperidin-3-yl)benzamide (**24**) was prepared as follows. To an ice cold solution of acetylsalicyloylchloride (252 mg) and triethylamine (0.58 mL) in chloroform (30 mL) was added 3-aminoglutaride trifluoroacetate (207 mg). The reaction temperature was allowed to warm to room temperature and stirring was continued overnight. The solvent was removed and recrystallization from ethyl acetate gave compound **24** as white crystals (0.36 g, 98%): ^{1}H NMR (DMSO-d_6) δ 11.00 (s, 1H), 8.73 (d, J = 8.3 Hz, 1H), 7.81 (dd, J = 1.6 Hz, J = 7.7 Hz, 1H), 7.72 (m, 1H), 7.54 (m, 1H), 7.38 (dd, J = 0.9 Hz, J = 8.1 Hz, 1H), 4.95-4.82 (m, 1H), 2.96-2.90 (m, 1H), 2.43 (s, 3H), 2.18-2.15 (m, 2H).

Example 11 –Synthesis of Thiothalidomides and Determination of Their TNF-α Inhibitory Activity

A series of thiothalidomides and analogs were designed to explore their action on inhibition of TNF-α. Monothiothalidomide **205** (same as compound **15** in Example 7) was prepared as shown in Scheme 11. *tert*-Butoxycarbonyl-L-glutamine **202** was refluxed with carbonyl diimidazole (CDI) in THF, and cyclized to afford imide **203** (Muller et al., "Amino-substituted thalidomide analogs: potent inhibitors of TNF-α production," *Bioorg. Med. Chem, Lett.* **9**, 1625-1630, 1999).

Imide **203** then was treated with trifluoroacetic acid in CH_2Cl_2 to remove the protective group to generate aminoglutarimide trifluoroacetate **204**. Without further purification, compound **204** was reacted with phthalic anhydride in refluxing THF in the presence of triethylamine to produce thalidomide **201** (same as compound **1** in Example 7) in the total yield of 31% from compound **202**. Thalidomide **201** was thionated with Lawesson's reagent (LR, Cava et al., "Thionation reaction of Lawesson's Reagents," *Tetrahedron*, **41**, 5061-5087, 1985, the entirety of which is incorporated herein by reference) to generate a single new product that had a structure identified as 6′-thiothalidomide **205** by mass spectrometry and 1D & 2D nuclear magnetic resonance spectroscopy. The position of the thiocarbonyl group was established from the heteronuclear multiple bond correlation (HMBC) cross peak of H-5′/C-6′.

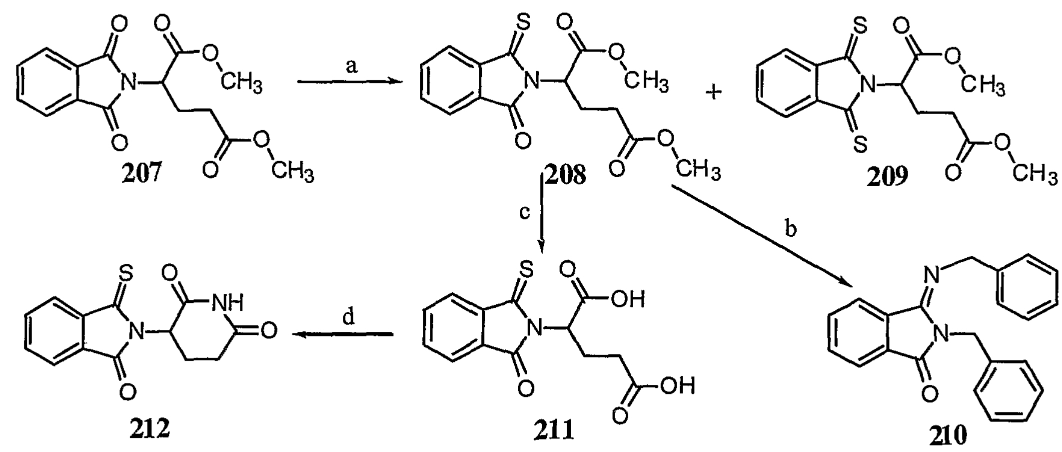


Scheme 11: Reagents: (a) CDI/THF; (b) CF_3COOH/CH_2Cl_2; (c) phthalic anhydride, Et_3N/THF; (d) Lawesson's reagent/toluene.

The synthesis of 3-thiothalidomide **212** is shown in Scheme 12 below. *N*-Phthaloyl-L-glutamic acid **206** was esterified to afford diester **207**. Compound **207** was thionated with LR at 110°C to give compound **208** as a major product. Concurrently, compound **209** was separated as a minor product by chromatography.

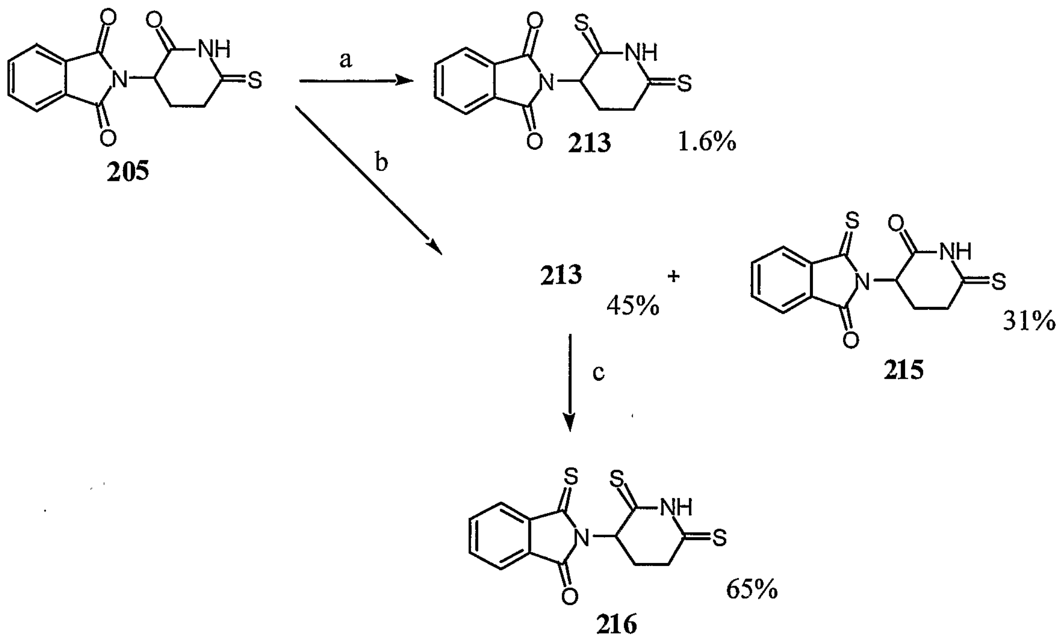
3-thiothalidomide, **212**, could not be prepared through the cyclization of compound **208** with ammonia or amine as ammonia reacts with the thioamide; reaction of compound **208** with benzylamine produced the unexpected compound **210**. In an alternative approach, compound **208** was hydrolyzed under acidic conditions to give diacid **211**. Compound **211** was then reacted with trifluoroacetamide to generate 3-thiothalidomide **212** in the presence of 1-hydroxybenzotriazole (HOBt) and 1-[3-

(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, Flaih et al., "An expeditious synthesis of cyclic imides," *Tetrahedron Lett.* **40**, 3697-3698, 1999).

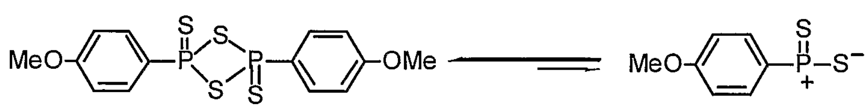


Scheme 12: Reagents: (a) Lawesson's reagent/toluene; (b) Benzylamine; (c) HCl/HOAc; (d) F_3CCONH_2, HOBt, EDCI, Et_3N/CH_2Cl_2.

In the synthesis of dithiothalidomide, one method involved the reaction of monothiothalidomide with LR at reflux in toluene. Under such conditions, 2',6'-dithiothalidomide was obtained in a yield of less than 2% (Scheme 13a). The yield was so low that improvement was desirable, and was undertaken by modifying the reaction conditions. It is believed that the mechanism underlying the reaction between LR and a carbonyl moiety is that a highly reactive dithiophosphine ylide **214**, rather than LR itself, likely is the active thionating agent (Scheme 4, Cava et al., "Thionation reaction of Lawesson's Reagents," *Tetrahedron*, **41**, 5061-5087, 1985, the entirety of which is incorporated herein by reference). The Lewis base may be able to increase the reactivity of LR as the base may drive the unfavorable equilibrium and elevate the concentration of the ylide **214**. When pyridine was used as a catalyst for thionation, monothiothalidomide **205** was thionated with LR to produce two dithiothalidomides, **213** (same as compound **16** in Example 7) and **215** (same as compound **17** in Example 7), in yields of 45% and 31%, respectively (Scheme 13 b,c). Dithiothalidomide **213** was further thionated with LR in the presence of the stronger base, morpholine, to give trithiothalidomide **216** (same as compound **18** in Example 7) in a yield of 65%.



Scheme 13: Reagents: (a) Lawesson's reagent/toluene; (b) Lawesson's reagent, pyridine/toluene; (c) Lawesson's reagent, morpholine/toluene.

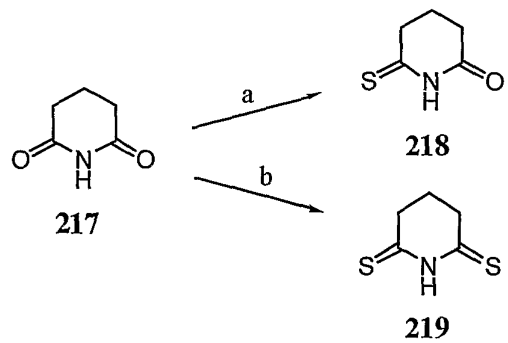


214

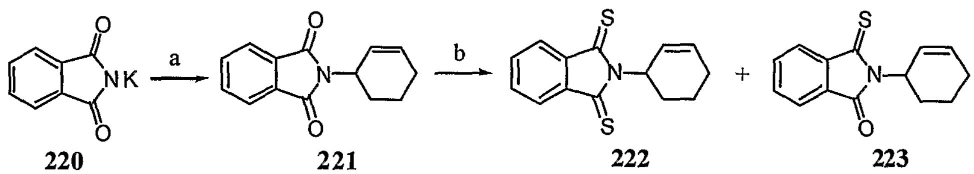
Scheme 14. The mechanism of catalysis for Lawesson's reagent.

Glutarimide **217** was thionated with LR in THF at room temperature to afford compound **218** as a major product. Glutarimide **217** also was refluxed with LR in toluene to produce dithioglutarimide **219** (Scheme 15). Reaction of potassium phthalimide with 3-bromocyclohexene in a Gabriel reaction gave compound **221**. Thereafter, thionation of compound **221** with LR afforded compounds **222** and **223**

(Scheme 16). Compounds **224** and **225** were prepared in a similar procedure to that used in the preparation of compounds **222** and **223**.

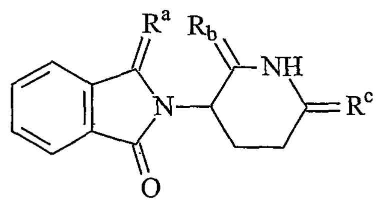


Scheme 15. Reagents: (a) Lawesson's reagent/THF, room temperature; (b) Lawesson's reagent, reflux/toluene

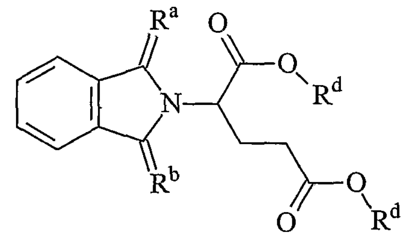


Scheme 16. Reagents: (a) 3-bromocyclohexene/DMF; (b) Lawesson's reagent/toluene.

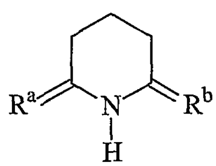
The structures of the thiothalidomide compounds of this Example are summarized below.



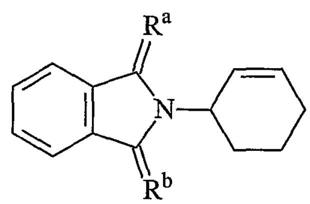
205: $R^a = O, R^b = O, R^c = S$
212: $R^a = S, R^b = O, R^c = O$
213: $R^a = O, R^b = S, R^c = S$
215: $R^a = S, R^b = O, R^c = S$
216: $R^a = S, R^b = S, R^c = S$



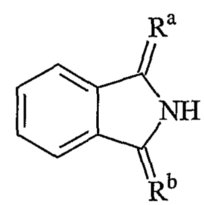
208: $R^a = S, R^b = O, R^d = Me$
209: $R^a = S, R^b = S, R^d = Me$
211: $R^a = S, R^b = O, R^d = H$



218: $R^a = S, R^b = O$
219: $R^a = S, R^b = S$



222: $R^a = S, R^b = S$
223: $R^a = S, R^b = O$



224: $R^a = S, R^b = S$
225: $R^a = S, R^b = O$

The action of the these thiothalidomide analogs in inhibiting TNF-α secretion was assessed in human peripheral blood mononuclear cells (PBMC) and the results are shown in Table 1. Freshly prepared PBMCs were utilized in all studies. Blood, 40 ml, was drawn from a volunteer, immediately mixed with 50 U/ml Na heparin and was diluted to 50 ml total volume with sterile PBS. Samples, 20 ml, of this preparation then were layered on 20 ml Ficoll-Paque and were centrifuged (800g, 20 min). The Ficoll/serum interface, containing PBMCs, was collected, diluted to 200 ml with PBS, and then was centrifuged (800g, 15 min) to pellet the cells. Thereafter, the recovered pellet was re-suspended in 37°C tissue culture medium (RPMI/1 mM Sodium pyruvate/10% heat inactivated FBS/ 2mM Glutamax) and placed on ice. Recovered cells were counted, pipetted (200 ul of 5 x 10^5/ml) into 96 well plates, and incubated for an hour (37°C, 5% CO_2). Thereafter, appropriate concentrations of test compounds or vehicle (10 ul DMSO) were added to duplicate wells. Following a further hour of incubation, a 10 ul sample of lipopolysaccharide (LPS)(100 ng/ml in supplemented medium) or vehicle was added to induce stimulated and unstimulated cells, respectively, and the cells were incubated overnight. Sixteen hours later, supernatants were collected for quantification of TNF-α levels by ELISA assay (Pierce-Endogen human TNF-α mini kit, Rockford, IL) and the use of specific capture and detection monoclonal antibodies, M303E and M302B (Pierce-Endogen), respectively. ELISA plates were read at λ=450 nm and TNF-α levels were determined from a six-point calibration curve that was run concurrently with the test samples. The effect of test drug concentrations on the cellular viability of PBMCs was assessed by MTS assay (Promega, Madison, WI) of the cells that provided the supernatant samples assayed for TNF-α levels, described above. It should be understood that this method can be used to test any of the disclosed compounds as a screening assay for readily determining their TNF-α modulating activity, and for selecting them for use in the disclosed method of treating a subject.

Table 1. Inhibition of LPS-induced TNF-α production in PBMC and cell viability

Compound	% Inhibition at 30 μM	IC_{50} (μM)	Cell viability at 30 μM	Cell viability at 3 μM	Cell viability at 0.3 μM
205	31	> 30	> 100	90	96
208	56	20	93	99	96
209	85	10	57	86	89
211	20	> 30	86	93	93
212	23	> 30	94	100	94
213	52	20	69	87	94
215	61	11	> 100	87	94
216	79	6	94	86	90
218	15	> 30	> 100	84	86
219	75	8	> 100	98	99
222	86	15	50	94	96
223	85	16	57	89	99
224	95	3	54	83	83
225	34	> 30	> 100	94	94

Thalidomide, **201**, entirely lacked activity at 30 μM. A concentration of 100 μM was required for significant activity (IC_{50} ~200 μM). The monothiothalidomides, 6′-thiothalidomide **205** and 3-thiothalidomide **212** showed only marginal activity at 30 μM with 31% and 23% inhibition of TNF-α secretion, respectively. In contrast, the dithiothalidomides, including 2′, 6′-dithiothalidomide **213** and 3, 6′-dithiothalidomide **215**, exhibited more potent inhibitory activities with IC_{50} values of 20 μM and 11 μM, respectively. However, assessment of cell viability by MTS assay showed that **213** induced increasing cytotoxicity at higher concentrations. Trithiothalidomide **216** inhibited TNF-α production with an IC_{50} of 6 μM, without accompanying toxicity. Compared with thalidomide, **201**, with an IC_{50} of ~200 μM for the inhibition of TNF-α synthesis, trithiothalidomide **216** is over 30-fold more active. Hence, successive replacement of a carbonyl with a thiocarbonyl group led to improved inhibitory activity compared to **201**, unassociated with toxicity. In this regard, the synthesized thiothalidomides possessed TNF-α lowering potency in the following decreasing order: trithiothalidomide **216** > dithiothalidomide **215** and **213** > monothiothalidomides **205** and **212** > thalidomide, **201**.

A comparison of the physical properties of thalidomide, **201**, and thiothalidomides shows that they have similar Van der Waals radii and bond angles, although the C=S bond is slightly longer than the C=O bond. Although not wishing to be bound by any particular theory, a possible explanation accounting for the elevated potency of the thiothalidomides is that their enhanced lipophilicity and loss of hydrogen bond acceptor capability potentially allows the attainment of higher intracellular drug levels. Interestingly, compounds **208**, **209** and **211** are thio analogs of hydrolysis metabolites of thalidomide. Assessment of their TNF-α inhibitory action determined that the monothio analog, **208**, has an IC_{50} of 20 μM without toxicity; demethylation (**211**) lowered potency. The dithio analog, **209**, proved 2-fold more potent still than **208**, but induced cellular toxicity at lower concentrations. Intriguingly, thio analogs **222** and **223,** with a simplified glutarimide ring, were found to be active TNF-α inhibitors, albeit with some toxicity at 30 μM, with IC_{50} values (15 μM and 16 μM respectively) that were greater than **212** (>30 μM) possessing a normal glutarimide ring.

In this regard, thalidomide is composed of two distinct moieties: the glutarimide and phthalimide rings. Thioglutarimides and thiophthalimides were thus synthesized and evaluated to assess the effect of thio-analogs of these two moieties on TNF-α levels. Monothioglutarimide **218** minimally inhibited TNF-α secretion at a concentration of 30 μM, however dithioglutarimide **219** exerted a potent inhibitory effect with an IC_{50} of 8 μM and a lack of toxicity. Surprisingly, such a simple structure, dithioglutarimide **219,** proved to be 25-fold more active than thalidomide **201**. In contrast, 2′,6′-dithiothalidomide **213**, a phthalimido substituted dithioglutarimide, is less active than dithioglutarimide **219,** and induces toxicity at high concentration. Monothiophthalimide **225** showed marginal TNF-α activity at a concentration of 30 μM without toxicity. Interestingly, however, dithiophthalimide **224** was found to possess potent activity with an IC_{50} of 3 μM. Although it was associated with toxicity at 30 μM, its inhibition of TNF-α occurred at an order of magnitude lower concentration that was well tolerated.

As described, compounds **215**, **216** and **219** potently inhibited TNF-α secretion without toxicity. As a consequence, additional studies were undertaken to elucidate the mechanism underpinning this action. Gene and protein expressions are controlled at the level of transcription, post-transcription, RNA stability, and translation under different physiological stimuli. Recently, post-transcriptional pathways have been recognized to provide a major means of regulating eukaryotic gene expression. In this regard, TNF-α and other cytokines and protooncogenes are known to be regulated at the post-transcriptional level. Multiple proteins, including the four cloned proteins AUF1, HuR, TTP and HuD have been shown to bind to a region of the mRNA that contains adenylate/uridylate (AU)-rich elements (AREs) in the 3'-untranslated region (UTR). These proteins mediate RNA turnover and decay, and hence translational efficiency. The stability of TNF-α mRNA is largely regulated at its 3'-UTR, which contains a well characterized ARE. Although AREs are found in a number of different cytokine and protooncogene RNAs, the pathways by which they induce degradation are highly specific for a given ARE indicating some cellular specificity. When the AREs from different cytokines are complexed with AUF1, different binding affinities are observed. Notably, however, the highest affinity for AUF1 is to human and then mouse TNF-α.

To determine the involvement of the 3'-UTR in the action of the thalidomide analogs, their ability to inhibit reporter gene activity in cells containing the TNF-α 3'-UTR versus a control vector was assessed. The results are shown in FIG. 1. This cell-based assay utilized two stably transfected cell lines derived from the mouse macrophage line, RAW264.7. One line, designated "luciferase only" expressed a luciferase reporter construct without any UTR sequences. The other line, designated "luciferase + TNF-α UTR" expressed a luciferase reporter construct with the entire 3'-UTR of human TNF-α inserted directly downstream of the luciferase coding region. Compounds were added in a concentration-dependent manner, and at the end of the incubation period (16 h, 37°C, 5% CO_2) the media was removed, cells were lysed and luciferase activity was assayed with Steady-glo luciferase assay reagent (Promega) according to the supplier's directions. Background was subtracted and data from this assay was expressed as a ratio of the +3'-UTR to –3'-UTR (control) values, and was expressed as a percent as shown in FIG. 1. In this manner, compounds that show a differential effect on the two cell lines, with and without a 3'-UTR, are highlighted. The action of compounds **215**, **216** and **219** in cells (mouse macrophage cell line, RAW264.7) possessing a luciferase reporter element plus the 3'-UTR of human TNF-α compared to cells lacking the 3'-UTR are shown in FIG. 1. Compounds **215**, **216** and **219** exerted differential effect on the two cell lines in a dose-dependent manner, consistent with their ability to inhibit TNF-α production via the 3'-UTR. All agents lowered luciferase reporter activity in cells stably expressing the 3'-UTR. Thalidomide lacked activity at 50 μM.

As TNF-α protein levels changed without significant alterations in mRNA levels (data not shown), protein expression is presumably regulated via translational control (at the post-transcriptional level). There is precedence for translational (protein) control through either the 3'- or 5'-UTR regions of a number of critical proteins that are current drug targets. For example, levels of the beta-amyloid precursor protein (APP) that is central to the development of AD can be regulated by either UTR. Turnover and translation of APP mRNA is regulated by a 29-nucleotide instability element within the 3'-UTR, located 200 nucleotides downstream from the stop codon. This 3'-UTR element acts as an mRNA destabilizer whose function can be inhibited by the presence of growth factors. In contrast, different cytokines, including TNF-α, and iron can up regulate APP protein synthesis at the level of its 5'-UTR; where, interestingly, the anticholinesterase, phenserine, that is currently in clinical trials for AD, lowers APP protein levels with concurrent maintenance of mRNA steady-state levels through translational modification within the same 5'-UTR element. A further example is that of the human immunodeficiency virus 1 (HIV-1) Trans-activating transduction (tat) protein, which binds trans-activation-responsive region (TAR) RNA. Tat is brought into contact with the transcription machinery after binding the TAR element, which is a 59-residue stem-loop RNA found at the 5' end of all HIV-1 transcripts. Finally, thalidomide (**201**) has been reported to lower cyclooxygenase-2 (Cox-2) biosynthesis via its 3'-UTR that appears to likewise contain an ARE that can regulate Cox-2 mRNA stability. The studies of analogs **215**, **216** and **219** confirm regulation of TNF-α protein levels by thalidomide (**201**) via its 3'-UTR, but whether or not the 5'-UTR contains a similar element that is accessible to pharmacological manipulation remains to be determined, as does action against Cox-2.

In summary, disclosed thiothalidomide analogs include analogs that are more potent inhibitors of TNF-α production in LPS-induced human PBMCs than thalidomide **201**. The isosteric replacement of successive carbonyl groups by a thiocarbonyl leads to an increasing inhibition with the number of moieties replaced (trithiothalidomide **216** > dithiothalidomide **215** and **213** > monothiothalidomides **205** and **212** > thalidomide **201**).

TNF-α has been validated as a drug target for two drugs on the market; Remicade (Cetocor, Malvern, PA; Schering-Plough, Orange, NJ) and Enbrel (Amgen, Thousand Oaks, CA; Wyeth-Ayerst, Princeton, NJ). However, both of these drugs are large macromolecules and hence require injection. In contrast, the small molecule drugs disclosed herein offer a means to potently and safely inhibit TNF-α without injection, for example, by oral administration.

Synthesis and Characterization Details

General. Melting points were determined with a Fisher-Johns apparatus and are uncorrected. ^{1}H NMR, ^{13}C NMR and 2D NMR were recorded on a Bruker AC-300 spectrometer. Mass spectra and high resolution mass spectra (HRMS) were recorded on a VG 7070 mass spectrometer and a Agilent Technologies 5973N GC-MS (CI). All exact mass measurements show an error of less than 5 ppm. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

3-(*tert*-Butoxycarbonylamino)-2,6-piperidinedione (203). A mixture of *N*-(*tert*-butoxycarbonyl)-L-glutamine (4.92 g, 20 mmol) and carbonyl diimidazole (3.24 g, 20 mmol) in THF (100 mL) was refluxed for 16 h. Thereafter, solvent was removed and the crude product was recrystallized from hot EtOAc to give compound **203** (2.04 g, 45%) as white crystals: mp 214-215°C; ^{1}H NMR (DMSO-d_6) δ 4.22 (dd, J = 6.2 Hz, J = 11.0 Hz, 1H), 2.77-2.65 (m, 1H), 2,45 (m, 1 H), 1.96-1.87 (m, 2H), 1.40 (s, 9H); MS (CI/CH_4) *m/z* 227 $[M-1]^+$.

2-(2-Oxo-6-thioxo-3-piperidinyl)-1*H*-isoindole-1,3(2*H*)-dione (205). Compound **203** (1.14 g, 5 mmol) was suspended in CH_2Cl_2 (100 mL). To the mixture was added CF_3COOH (10 mL) and this then was stirred at room temperature for 4 h. The solvent was evaporated to give crude **204** (1.25 g): ^{1}H NMR (DMSO-d_6) δ 11.42 (s, 1H), 8.70 (br, 2H), 4.31 (dd, J =5.4 Hz, J = 13 Hz), 2.88-2.72 (m, 2H), 2.25-2.09 (m, 2H). A mixture of crude **204** (1.25g) and phthalic anhydride (0.89 g, 6 mmol) and Et_3N (1.39 ml, 10 mmol) in THF (150 mL) was refluxed for two days. The reaction mixture was concentrated and the residue was crystallized from ethyl acetate to give thalidomide (**201**) (0.89 g, 69%) as white crystals; mp 276°C (lit.276-279°C). A mixture of thalidomide **201** (258 mg, 1 mmol) and Lawesson's reagent (222 mg, 0.55 mmol) in toluene (50 ml) was stirred at reflux for 12 h; thereafter, solvent was removed under vacuum. The resulting residue was purified by column chromatography using CH_2Cl_2 as the eluent to afford compound **205** (200 mg, 73%) as a yellow solid: mp 225-226°C; ^{1}H NMR (DMSO-d_6) δ 12.83 (s, 1H, NH), 8.00-7.92 (m, 4H, Ph), 5.32 (dd, J = 5.6 Hz, J = 12.9 Hz, 1H, H-3′), 3.28-3.25 (m, 2H, H-5′), 2.60-2.54 (m, 1H, H-4′), 2.17-2.10 (m, 1H, H-4′); ^{13}C NMR (DMSO-d_6) δ 208.7(C-6′), 165.3(C-2′), 165.2(C-1 & C-3), 133.1(C-5 & C-6), 129.3 (C-3a, C-7a), 121.7 (C-4 & C-7), 46.9 (C-3′), 38.9 (C-5′), 21.79 (C-4′); MS (CI/CH_4) *m/z* 274 (M^+); Anal. ($C_{13}H_{10}N_2O_3S$) C, H, N.

Dimethyl 2-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-pentanedioate (207). To a solution of *N*-phthaloyl-L-glutamic acid (200 mg, 0.72 mmol) in methanol (10 mL) was added, dropwise, thionyl chloride (1 mL). The reaction mixture was refluxed for 6 h. The solvent was removed under reduced pressure, dissolved in ethyl acetate (100 mL), and then washed with saturated aqueous Na_2CO_3 solution (2×30 mL) and water (2×30 mL). The ethyl acetate layer was dried over Na_2SO_4 and then evaporated, leaving an oil, which upon purification by silica gel chromatography, using CH_2Cl_2:EtOAc (1:1) as the eluent, gave compound **7** (161 mg, 73%) as an oil; ^{1}H NMR ($CDCl_3$) δ 7.87-7.84 (m, 2H), 7.75-7.72 (m 2H), 4.91 (dd, J = 5 Hz, J = 9 Hz, 1H), 3.73 (s, 3H), 3.62 (s, 3H), 2.67-2.56 (m, 1H), 2.51-2.44 (m, 1H), 2.41-2.35 (m, 2H).

Dimethyl 2-(1,3-dihydro-1-oxo-3-thioxo-2*H*-isoindol-2-yl)-pentanedioate (208) and Dimethyl 2-(1,3-dihydro-1,3-dithioxo-2*H*-isoindol-2-yl)-pentanedioate (209). A mixture of compound **207** (144 mg, 0.47 mmol) and LR (191 mg, 0.47 mmol) in toluene was stirred in a 110°C oil bath for 10 h. The solvent was then evaporated and the residue was purified by column chromatography, (silica gel) using CH_2Cl_2 as the eluent, to obtain compound **209** (17 mg, 11%) as a dark red oil. Thereafter, using

CH_2Cl_2:EtOAc (10:1) as the eluent the more polar component **208** (105 mg, 70%) was obtained as a red oil.

Compound **208**: ^{1}H NMR ($CDCl_3$) δ 7.98-7.96 (m, 1H), 7.81-7.70 (m, 3H), 5.53 (dd, J = 5.1 Hz, J = 10 Hz, 1H), 3.70 (s, 3H), 3.59 (s, 3H), 2.76-2.56 (m, 2H), 2.40-2.33 (m, 2H); MS (CI/CH_4) *m/z* 321 (M^+).

Compound **209**: ^{1}H NMR ($CDCl_3$) δ 7.87-7.84 (m, 2H), 7.73-7.68 (m. 2H), 6.09 (dd, J = 5 Hz, J = 10 Hz, 1H), 3.70 (s, 3H), 3.58 (s, 3H), 2.81-2.63 (m, 2H), 2.40-2.24 (m, 2H); MS (DEI) *m/z* 337 (M^+); HRMS (DEI) calcd for $C_{15}H_{15}NO_4S_2$ 337.0442 (M^+), found 337.0449.

2-(1,3-Dihydro-1-oxo-3-thioxo-2*H*-isoindol-2-yl)-pentanedioic acid (211). Compound **208** (350 mg, 1.09 mmol) was stirred with a 1:1 mixture of acetic acid glacial and conc. HCl in a 100°C oil bath for 2.5 h. Ethyl acetate (100 mL) and ice water (30 mL) were added. The ethyl acetate layer was separated, washed with ice water, dried over Na_2SO_4 and concentrated. The resulting syrup was crystallized with ether to afford compound **211** as red crystals (253 mg, 79%); mp 157°C; ^{1}H NMR (DMSO-d_6) δ 8.04-7.96 (m, 1H), 7.91-7.74 (m, 3H), 5.43 (dd, J = 5.1 Hz, J = 9.6 Hz, 1H), 2.42-2.33 (m, 2H), 2.30-2.26 (m, 2H); MS (DEI) *m/z* 293 (M^+); HRMS (DEI) calcd. for $C_{13}H_{11}NO_5S$ 293.0358 (M^+), found 293.0363; Anal. ($C_{13}H_{11}NO_5S$) H, N; C: calcd, 53.24; found, 53.88.

2,3-Dihydro-3-thioxo-2-(2,6-dioxo-3-piperidinyl)-1*H*-isoindol-1-one (212). A mixture of compound **208** (81 mg, 0.276 mmol), trifluoroacetamide (57 mg, 0.50 mmol), 1-hydroxybenzotriazole (145 mg, 1.07 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (200 mg, 1.04 mmol) and triethylamine (0.21 mL, 1.51 mmol) in CH_2Cl_2 (1.5 mL) was stirred at ambient temperature for 3 days. Water (10 mL) and CH_2Cl_2 (10 mL) were added. The dichloromethane layer was separated, washed with water, dried over Na_2SO_4 and evaporated under reduced pressure. Purification by chromatography, with EtOAc:CH_2Cl_2 (1:10) as the eluent, gave compound **212** (48 mg, 63%) as a red solid: mp 255°C; ^{1}H NMR ($CDCl_3$) δ 8.00-7.98 (m, 1H), 7.80-7.71 (m, 3H), 5.63 (br, 1H), 2.98-2.70 (m, 3H), 2.18-2.15 (m, 1H); MS (CI/CH_4) *m/z* 274 (M^+); Anal. ($C_{13}H_{10}N_2O_3S$) C, H, N.

2-(2, 6-Dithioxo-3-piperidinyl)-1*H*-isoindole-1,3(2*H*)-dione (213) and 2,3-dihydro-3-thioxo-2-(2-oxo-6-thioxo-3-piperidinyl)-1*H*-isoindol-1-one (215). The mixture of **205** (146 mg, 0.533 mmol), LR (108 mg, 0.267 mmol) and pyridine (21 μl) in toluene was stirred at 110°C under an atmosphere of N_2 for 12 h. Thereafter, additional LR (108 mg, 0.267 mmol) and pyridine (21 μl) were added, and the reaction mixture was stirred for a further 12 h.. The solvent was removed under vacuum and the residue was purified by column chromatography with CH_2Cl_2:petroleum ether (2:1, 10:1) and then CH_2Cl_2:EtOAc (10:1) as eluents to afford **213** (30 mg, 45%), **215** (21 mg, 31.5%) and starting material **205** (83 mg).

Compound **213** (yellow solid): mp 263-265°C; ^{1}H NMR ($CDCl_3$) δ 7.78-7.74 (m, 2 H), 7.66-7.63 (m, 2 H), 5.00 (dd, J = 4.9 Hz, 11.9 Hz, 1 H), 3.43-3.35 (m, 1 H), 2.95-2.84 (m, 2 H), 2.08-2.06 (m, 1 H); MS (DEI) *m/z* 290 (M^+); HRMS (DEI) calcd for $C_{13}H_{10}N_2O_2S_2$ 290.0184 (M^+), found 290.0185; Anal. ($C_{13}H_{10}N_2O_2S_2$) C, H, N.

Compound **215** (red solid): mp 240-242°C; ^{1}H NMR ($CDCl_3$) δ 9.44 (s, 1 H), 8.05-8.02 (m, 1 H), 7.86-7.76 (m, 3 H), 5.75-5.64 (m, 1 H), 3.57-3.52 (m, 1 H), 3.09-2.99 (m, 2 H), 2.19- 2.12 (m, 1 H). ^{13}C NMR (DMSO): 208.16, 207.98, 166.10, 165.39, 134.32, 133.11, 132.42, 124.30, 122.15, 121.11, 49.64, 21.29; MS (DEI) *m/z* 291 (MH^+); HRMS (DEI) calcd for $C_{13}H_{11}N_2O_2S_2$ 291.0262 (M^+), found 291.0264; Anal. ($C_{13}H_{10}N_2O_2S_2 \cdot 0.5H_2O$) C, H, N.

2,3-Dihydro-3-thioxo-2-(2,6-dithioxo-3-piperidinyl)-1*H*-isoindol-1-one (216). A mixture of compound **213** (29 mg, 0.1 mmol), LR (22 mg, 0.054 mmol) and morpholine (9 μl, 0.1 mmol) in toluene (10 mL) was stirred at reflux under an atmosphere of N_2 for 16 h. The solvent was removed under vacuum and the residue was purified by column chromatography using CH_2Cl_2:petroleum ether (1:1) as the eluent to afford compound **216** (20 mg, 65%) as a red solid: mp 244°C; ^{1}H NMR ($CDCl_3$) δ 10.81 (s, 1H), 8.05-8.01 (m, 1H), 7.91-7.75 (m, 3H), 5.92 (m, 1H), 3.57-3.52 (m, 1H), 3.13-2.97 (m, 2H), 2.18-2.15 (m, 1H); MS(DEI) *m/z* 306 (M^+); HRMS (DEI) calcd for $C_{13}H_{10}N_2OS_3$ 305.9955 (M^+), found 305.9951; Anal. ($C_{13}H_{10}N_2OS_3 \cdot 0.5H_2O$) C, H, N.

6-Thioxo-2-piperidinone (218). The mixture of glutarimide (0.45 g, 4 mmol) and LR (0.809 g, 2 mmol) in THF (30 mL) was stirred at room temperature for 2 days. The solvent was evaporated under vacuum and the residue was purified by column chromatography using petroleum ether:EtOAc (1:1) as the eluent to give compound **218** as a yellow solid (0.361g, 70%): mp 135°C; ^{1}H NMR ($CDCl_3$) δ 2.96 (t, J = 5.7 Hz, 2 H), 2.58 (t, J = 5.8 Hz, 2 H), 1.96 (m, 2 H); MS (CI/CH_4) *m/z* 129 (M^+); Anal. (C_5H_7NOS) C, H, N.

2,6-Piperidinedithione (219). A mixture of glutarimide (0.34 g, 3 mmol) and LR (1.22 g, 3 mmol) in toluene (30 mL) was stirred at reflux for 3 h. The solvent was evaporated under vacuum and the residue was purified by column chromatography using petroleum ether:EtOAc (20:1) as the eluent to give compound **219** as a yellow solid (0.286 g, 66%): mp 103°C; ^{1}H NMR ($CDCl_3$) δ 3.02 (t, J = 6.3 Hz, 4H), 1.98 (t, J = 6.3 Hz, 2H); MS (CI/CH_4) *m/z* 145 (M^+); Anal. ($C_5H_7NS_2$) C, H, N.

2-(3-Cyclohexenyl)-1*H*-isoindole-1,3(2*H*)-dione (221). A mixture of potassium phthalimide (1.85 g, 3 mmol) and 3-bromocyclohexene (1.79 g, 3 mmol) in DMF (15 mL) was stirred in a 100°C oil bath for 12 h. The cooled reaction mixture was poured into ice water. The solid was collected by filtration and purified by flash chromatography with CH_2Cl_2 as the eluent to afford compound **221** (1.6 g, 72%) as pink crystals; mp 114°C; ^{1}H NMR ($CDCl_3$) δ 7.73-7.69 (m, 2H), 7.62-7.58 (m, 2H), 5.85-5.82 (m, 1H), 5.47-5.44 (m, 1H), 4.80-4.78 (m, 1H), 2.14-2.00 (m, 3H), 1.86-1.78 (m, 2H), 1.64-1.58 (m, 1H).

2-(3-Cyclohexenyl)-1*H*-isoindol-1,3(2*H*)-dithione (222) and 2,3-dihydro-3-thioxo-2-(3-cyclohexenyl)-1*H*-isoindol-1-one (223). A mixture of compound **221** (68 mg, 0.3 mmol) and LR (121 mg, 0.3 mmol) in toluene was refluxed under N_2 for 10 h. The solvent was removed under vacuum and the residue was purified by column chromatography using petroleum ether as the eluent to obtain compound **222** (37 mg, 48%) as a dark green solid. Then, using CH_2Cl_2:petroleum ether (1:1) as the eluent, the more polar component **223** (23 mg, 32%) was obtained as a red solid.

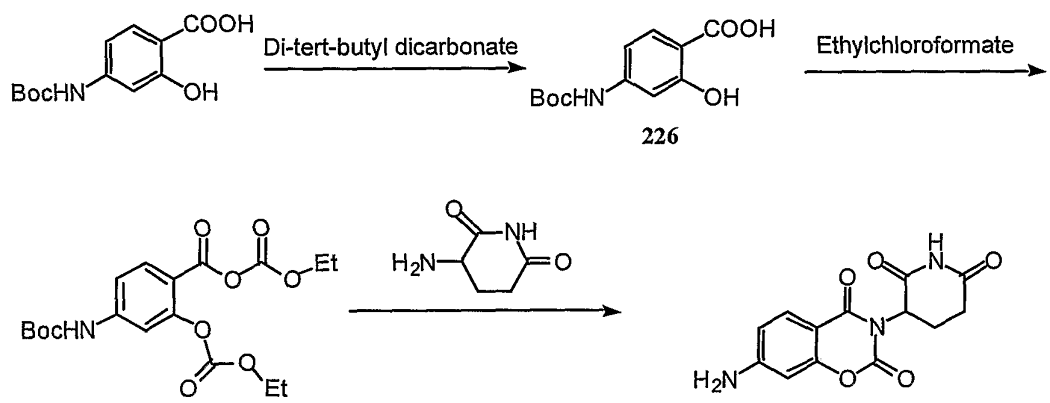
Compound **222**: mp 93°C; ^{1}H NMR ($CDCl_3$) δ 7.65-7.60 (m, 2H), 7.49-7.42 (m, 2H), 5.92-5.88 (m, 1H), 5.66-5.63 (m, 1H), 5.47-5.43 (m, 1H), 2.40-2.35 (m, 1H), 1.99-1.95 (m, 2H), 1.75-1.59 (m, 3H); MS (CI/CH_4) *m/z* 259 (M^+); Anal. ($C_{14}H_{13}NS_2$) C, H, N.

Compound **223**: mp 67-68°C; ^{1}H NMR ($CDCl_3$) δ 7.94- 7.91 (m, 1H), 7.73-7.64 (m, 3H), 5.92-5.88 (m, 1H), 5.60-5.51 (m, 2H), 2.27-2.10 (m, 3H), 1.96-1.76 (m, 2H), 1.81-1.70 (m, 1H); MS (CI/CH_4) *m/z* 243 (M^+); Anal. ($C_{14}H_{13}NOS$) C, H, N.

Dithiophthalimide (225). A mixture of phthalimide (436 mg, 3.40 mmol) and Lawesson's reagent (1.199 g, 3.40 mmol) in toluene (50 ml) was refluxed (oil bath 120°C) under nitrogen for 5 hours. The solvent was removed under vacuum and the residue was directly chromatographed (silica gel, petroleum ether: methylenedichloride / 2: 3) to give dithiophthalimide as black red needle crystals (240 mg, 39.4%):^{1}HNMR($CDCl_3$) δ 9.80 (br, 1H), 7.95 (d, 2H), 7.80 (d, 2H); MS (CI / CH_4) m/z 179 (M^+).

Example 12-Synthesis and TNF-α Inhibitory Activity of 3-[2',6'-piperidinedion-3'-yl]-7-amino-2H-1,3-benzoxazine-2,4(3H)-dione

3-[2',6'-piperidinedion-3'-yl]-7-amino-2H-1,3-benzoxazine-2,4(3H)-dione was prepared as shown below in Scheme 17.



Scheme 17

4-(t-Butoxycarbonyl amido)salicylic acid **(226)** was prepared as follows. To a mixture of 4-aminosalicylic acid (306 mg, 2 mmol) and di-t-butyl dicarbonate (655 mg, 3 mmol) in H_2O was added NaOH (2N in H_2O) at 0 °C. This reaction mixture was allowed to warm to room temperature and then was stirred for 5 hours. 2N HCl was added dropwise until the mixture was neutralized. The reaction mixture was then extracted with EtOAc, dried and evaporated to give product (336 mg , 66%) as a dark gray solid: ^{1}HNMR (DMSO-d_6) δ 11.50 (s, 1H), 7.65 (d, 1H), 6.23 (d, 1H), 6.07 (s, 1H), 1.70 (s, 9H).

2-[(Ethoxycarbonyl)oxy]-4-(t-butoxycarbonyl amido)-benzoic anhydride with ethyl hydrogencarbonate (**227**) was prepared as follows. 4-t-Butoxycarbonyl amidosalicylic acid (**226**) (101 mg, 0.399 mmol) in THF (10 ml) was cooled with dry ice in acetone. Et_3N (0.166 ml) was added, and then ethyl chloroformate (108 mg, 1.135 mmol) was added dropwise over a period of 30 min. The reaction mixture was stirred at the same temperature for 5 hours, and then was allowed to warm to room temperature. Thereafter, the reaction mixture was stirred continuously overnight. After evaporation of solvent, the residue was partitioned between water and ethyl ether. The ether solution was washed with brine, dried over Na_2SO_4 and evaporation of solvent gave product (111 mg, 70%) as a yellow gum: ^{1}HNMR ($CDCl_3$) δ 7.75 (d, 1H), 6.48 (d, 1H), 6.38 (s, 1H), 4.38 (m, 4H), 1.35 (m, 6H).

3-[2',6'-piperidinedion-3'-yl]-7-amino-2H-1,3-benzoxazine-2,4(3H)-dione (**228**) was prepared as follows. A mixture of **227** (32.8 mg, 0,0826 mmol), aminoglutarimide (20 mg, 0.0826 mmol) and Et_3N (25. 0 mg, 0.248 mmol in 2 ml THF) was stirred at room temperature overnight. Evaporation of solvent gave a residue which was stirred with a mixture of EtOAc and a saturated aqueous solution of $NaHCO_3$. The precipitated white solid was collected by filtration as the product: ^{1}HNMR (DMSO-d_6) δ 11.3 (br, 1H), 7.85 (d, 1H), 6.80 (d, 1H), 6.60 (s, 1H), 3.15 (t, 2H), 2.15 (t, 2H).

Evaluation of compound **228** in the TNF-α assay described above in Example 11 showed that it possessed potent inhibitory action on TNF-α, having an EC_{50} of 0.4 μM.

Example 13 – Angiogenesis Modulating Activity

Angiogenesis is the formation of new blood vessels from pre-existing vessels. Angiogenesis is prominent in solid tumor formation and metastasis, and is part of the wound healing process. Pathological angiogenesis sometimes occurs in inappropriate anatomic locations, such as the retina or cornea, in response to disease and injury. Inhibition of angiogenesis could avoid the progression of conditions of inappropriate angiogenesis.

Tumor formation, for example, requires a network of blood vessels to sustain the nutrient and oxygen supply for continued growth. Tumors in which angiogenesis is important include most solid tumors and benign tumors, such as acoustic neuroma, neurofibroma, trachoma, and pyogenic granulomas. Inhibition of angiogenesis could halt the growth of these tumors and the resultant damage due to the presence of the tumor.

There is a direct correlation between tumor microvessel density and the incidence of metastasis. Tumor cells themselves can produce factors that stimulate the proliferation of endothelial cells and new capillary growth. Angiogenesis is important in two stages of tumor metastasis. The first stage where angiogenesis stimulation is important is in the vascularization of the primary tumor, which allows tumor cells to enter the blood stream and to circulate throughout the body. After the tumor cells have left the primary site, and have settled into the secondary, metastatic site, angiogenesis must occur before the metastasis can grow and expand. Therefore, inhibiting angiogenesis could lead to the reduction or elimination of metastasis of tumors and possibly contain the neoplastic growth at the primary site. These observations have led to the investigation of anti-angiogenic agents as possible therapeutic options for various cancers.

The angiogenesis modulating activity of representative compounds was assessed in a rat aortic ring microvessel growth assay. Briefly, twelve-well tissue culture plates were coated with 250 μl of Matrigel (Becton-Dickinson, Bedford, MA) and allowed to gel for 30 min at 37°C and 5% CO_2. Thoracic aortas were excised from 8- to 10-week-old male Sprague Dawley rats. After careful removal of fibroadipose tissues, the aortas were cut into 1-mm-long cross-sections, placed on Matrigel-coated wells, and covered with an additional 250 μl of Matrigel. After the second layer of Matrigel had set, the rings were covered with EGM-II and incubated overnight at 37°C and 5% CO2. EGM-II consists of endothelial cell basal medium (EBM-II; Clonetics, San Diego, CA) plus endothelial cell growth factors provided as the EGM-II Bulletkit (Clonetics). The culture medium was subsequently changed to EBM-II supplemented with 2% fetal bovine serum, 0.25 μg/ml amphotericin B, and 10 μg/ml gentamicin. Aortic rings were treated daily with either the vehicle (0.5% DMSO), carboxyamidotriazole (CAI, 12 μg/ml), thalidomide or thalidomide analogs (0.1-20 μg/ml) for 4 days and photographed on the 5th day using a x2.5 objective. CAI, a known antiangiogenic agent, was used at higher than clinically achievable concentration as a positive control. Experiments were repeated four times using aortas from four different rats. The area of angiogenic sprouting, reported in square pixels, was quantified using Adobe PhotoShop. Further details of the method are provided in Luzzio et al., *J Med Chem.*; **46**:3793-9, 2003, which is incorporated by reference herein. It should be understood that this method can be used as an assay to rapidly select compounds having a desired angiogenic or anti-angiogenic effect, for example, for use in the disclosed methods of treating a subject.

Bar graphs showing the results of the angiogenesis assay for several compounds are shown in FIGS. 2-11. For convenience, the structures of the assayed compounds also are presented in these figures.

FIG. 2 shows the angiogenic modulating activity of 1,3-Dioxo-2-(2-hydroxy-6-methoxypyridin-3-yl)-isoindoline hydrobromide at several concentrations. This compound exhibited anti-angiogenic activity in the rat aortic ring assay at all concentrations tested.

FIG. 3 shows the angiogenic modulating activity of 2-(3-cyclohexenyl)-*H*-isoindol-1,3(2*H*)-dithione at several concentrations. This compounds exhibited anti-angiogenic activity at higher concentrations and angiogenic activity at lower concentrations.

FIG. 4 shows the angiogenic modulating activity of 1-(2,6-Dithioxo-3-piperidinyl)-1*H*-isoindole-1,3(2*H*)-dione at several concentrations. This compound exhibited anti-angiogenic activity at all concentrations tested.

FIG. 5 shows the angiogenic modulating activity of 3-Camphanic amino-2,6-piperidinedione at several concentrations. This compound exhibited potent angiogenic activity at all concentrations tested, making this compound promising for treating conditions where increased angiogenesis is desired, for example, as an aid to wound healing.

FIG. 6 shows the angiogenic modulating activity of Dithiophthalimide at several concentrations. This compound exhibited angiogenic activity at all concentrations tested.

FIG. 7 shows the angiogenic modulating activity of 2-(1,3-Dihydro-1-oxo-3-thioxo-2*H*-isoindol-2-yl)-pentanedioic acid at several concentrations. This compound exhibited angiogenic activity at all concentrations tested.

FIG. 8 shows the angiogenic modulating activity of 2-(2-Oxo-6-thioxo-3-piperidinyl)-1*H*-isoindole-1,3(2*H*)-dione at several concentrations. This compound showed anti-angiogenic activity at higher concentration, and some angiogenic activity at lower concentrations.

FIG. 9 shows the angiogenic modulating activity of 2,3-Dihydro-3-thioxo-2-(2,6-dithioxo-3-piperidinyl)-1*H*-isoindol-1-one at several concentrations. This compound exhibited potent anti-antigiogenic activity at higher concentrations and angiogenic activity at lower concentrations.

FIG. 10 shows the angiogenic modulating activity of 2-Acetoxy-*N*-(2,6-dioxopiperidin-3-yl)benzamide at several concentrations. At all concentrations tested, this compound exhibited potent angiogenic activity.

FIG. 11 shows the angiogenic modulating activity of 1,3-Dioxo-2-(2,6-dimethoxypyridin-3-yl)-isoindoline at several concentrations. This compound exhibited angiogenic activity at all concentrations tested.

In summary, the disclosed compounds exhibit a range of angiogenic modulating activities ranging from potent inhibition of angiogenesis (anti-angiogenic activity) to potent stimulation of angiogenesis (angiogenic activity). Some compounds exhibit both angiogenic and anti-angiogenic activity in a dose-dependent manner. Those compounds (or particular concentrations thereof) having angiogenic activity are useful for treating conditions or diseases where increasing angiogenesis is desirable (for example, wound healing) and those compounds (or particular concentrations thereof) having anti-angiogenic activity are useful for treating conditions or diseases where decreasing angiongenesis is desirable (for example, cancers, diabetic retinopathy or corneal neovascularization). Persons of ordinary skill in the art can use the assay described above (or other known angiogenic/anti-angiogenic activity assays) to readily determine amounts of the disclosed compounds that therapeutically effective for stimulating or inhibiting angiogenesis as appropriate for a given subject's condition.

Example 14 - Synthesis of 3-Camphanic amino-2,6-piperidinedione

A mixture of (+)-camphanic chloride (19 mg, 00868 mmol), aminoglutarimide (21 mg, 0.0868 mmol) and Et_3N (24 µl) in $CHCl_3$ (1 ml) was stirred at room temperature for 16 hours. The solution was diluted with $CHCl_3$,washed with saturated aqueous solution of $NaHCO_3$, dried over Na_2SO_4, concentrated and purified by chromatography (silica gel, CH2Cl2 ; EtOAc = 10 : 1) to give product (16 mg, 60.0% yield) as a colorless gel: ^{13}CNMR ($CDCl_3$) δ 172.6, 169.4, 168.6, 165.5, 90.3, 58.3, 53.3, 48.2, 47.6, 29.2, 28.2, 26.9, 22.7, 14.6, 7.6; MS (CI / CH_4) m/z 308 (M^+). This compound exhibited angiogenic activity in the assay of Example 13.

Example 15 – Synthesis of 3-Benzylimino-2-benzyl-2,3-dihydroisoindol-1-one

A solution of Dimethyl 2-(1,3-dihydro-1-oxo-3-thioxo-2*H*-isoindol-2-yl)-pentanedioate (compound **208** of Example 11, 100mg, 0.311 mmol) and benzylamine was stirred in a 50 °C oil bath for 5 hours. The reaction mixture was partitioned between water and ethyl acetate. The organic layer was washed with water, dried, and concentrated. The residue was purified by chromatography (silica gel, CH_2Cl_2) to give product as white crystals (60 mg, 59.0%): ^{1}HNMR ($CDCl_3$) δ 7.10 – 7.90 (m, 10 H), 5.18 (s, 2H), 4.95 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 167.8, 151.3, 140.6, 138.2, 133.5, 133.3, 132.1, 130.4, 130.1, 129.1, 128.8, 128.7, 128.5, 128.4, 127.9, 127.6, 127.5, 127.2, 126.0, 124.1, 53.9, 42.5; FAB-MS m/z 327 (MH^+). This compound exhibited angiogenic activity in the assay of Example 13.

Example 16 - Thionation

Although many of the disclosed compounds are illustrated without thionyl groups in their structures, it is to be understood that any of the carbonyl groups shown in the structures of the disclosed compounds may be converted into thiocarbonyl groups, and that such thio-derivatives are part of this disclosure. Thionation may be accomplished by any known method. Particular methods of thionation include use of phosphorus pentasulfide, hydrogen sulfide, *O,O*-diethyldithiophosphonic acid, boron sulfide, silicon disulfide and elemental sulfur in HMPA. However, a particularly convenient method of thionation is the use of 2,4-bis(*p*-methoxyphenyl)-1,3-dithiadiphosphetane-2,4-disulfide and its derivatives (generically "Lawesson's Reagents"). These reagents are described in Cava and Levinson, "Thionation Reactions of Lawesson's Reagents," *Tetrahedron*, **41**: 5061-5087, 1985, which is incorporated by reference herein.

Example 17 – Pharmaceutical Compositions

The disclosed pharmaceutical compositions can be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions (e.g., eye or ear drops, throat or nasal sprays, etc.), transdermal patches, and other forms known in the art.

Pharmaceutical compositions can be administered systemically or locally in any manner appropriate to the treatment of a given condition, including orally, parenterally, rectally, nasally, buccally, vaginally, topically, optically, by inhalation spray, or via an implanted reservoir. The term "parenterally" as used herein includes, but is not limited to subcutaneous, intravenous, intramuscular, intrasternal, intrasynovial, intrathecal, intrahepatic, intralesional, and intracranial administration, for example, by injection or infusion. For treatment of the central nervous system, the pharmaceutical compositions may readily penetrate the blood-brain barrier when peripherally or intraventricularly administered.

Pharmaceutically acceptable carriers include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins (such as human serum albumin), buffers (such as phosphates), glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol, and wool fat.

Tablets and capsules for oral administration can be in a form suitable for unit dose presentation and can contain conventional pharmaceutically acceptable excipients. Examples of these include binding agents such as syrup, acacia, gelatin, sorbitol, tragacanth, and polyvinylpyrrolidone; fillers such as lactose, sugar, corn starch, calcium phosphate, sorbitol, or glycine; tableting lubricants, such as magnesium stearate, talc, polyethylene glycol, or silica; disintegrants, such as potato starch; and dispersing or wetting agents, such as sodium lauryl sulfate. Oral liquid preparations can be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or can be presented as a dry product for reconstitution with water or other suitable vehicle before use.

The pharmaceutical compositions can also be administered parenterally in a sterile aqueous or oleaginous medium. The composition can be dissolved or suspended in a non-toxic parenterally-acceptable diluent or solvent, e.g., as a solution in 1,3-butanediol. Commonly used vehicles and solvents include water, physiological saline, Hank's solution, Ringer's solution, and sterile, fixed oils, including synthetic mono- or di-glycerides, etc. For topical application, the drug may be made up into a solution, suspension, cream, lotion, or ointment in a suitable aqueous or non-aqueous vehicle. Additives may also be included, for example, buffers such as sodium metabisulphite or disodium edeate; preservatives such as bactericidal and fungicidal agents, including phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorhexidine, and thickening agents, such as hypromellose.

The dosage unit involved depends, for example, on the condition treated, nature of the formulation, nature of the condition, embodiment of the claimed pharmaceutical compositions, mode of administration, and condition and weight of the patient. Dosage levels are typically sufficient to achieve a tissue concentration at the site of action that is at least the same as a concentration that has been shown to be active *in vitro*, *in vivo*, or in tissue culture. For example, a dosage of about 0.1 μg/kg body weight/day to about 1000 mg/kg body weight/day, for example, a dosage of about 1 μg/kg body weight/day to about 1000 μg/kg body weight/day, such as a dosage of about 5 μg/kg body weight/day to about 500 μg/kg body weight/day can be useful for treatment of a particular condition.

The compounds can be used in the form of pharmaceutically acceptable salts derived from inorganic or organic acids and bases, including, but not limited to: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate. Base salts include, but are not limited to, ammonium salts, alkali metal salts (such as sodium and potassium salts), alkaline earth metal salts (such as calcium and magnesium salts), salts with organic bases (such as dicyclohexylamine salts), N-methyl-D-glucamine, and salts with amino acids (such as arginine, lysine, etc.). Basic nitrogen-containing groups can be quaternized, for example, with such agents as C1-8 alkyl halides (such as methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides), dialkyl sulfates (such as dimethyl, diethyl, dibutyl, an diamyl sulfates), long-chain halides (such as decyl, lauryl, myristyl, and stearyl chlorides, bromides, and iodides), aralkyl halides (such as benzyl and phenethyl bromides), etc. Water or oil-soluble or dispersible products are produced thereby.

Pharmaceutical compositions can be included in a kit accompanied by instructions for intended use, for example instructions required by a pharmaceutical regulatory agency, such as the Food and Drug Administration in the United States.

In view of the many possible embodiments of the invention that exist, it should be recognized that the illustrated embodiments are only examples of the invention and should not be taken as a limitation on the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention, all that comes within the scope and spirit of these claims.

It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

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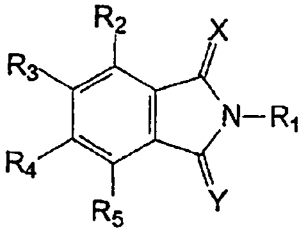
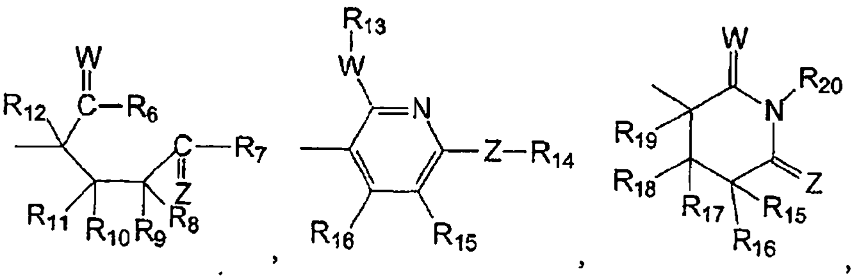
The claims defining the invention are as follows:

1. A compound having the formula:



wherein X and Y are independently CH_2, oxygen or sulfur, and at least one of X and Y is sulfur if R_1 does not include a sulfur atom; each of R_2-R_5 are independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy, substituted acyloxy, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen, nitro or linked to form a five- or six-membered, unsubstituted or substituted, aliphatic, aromatic or heterocyclic ring, and R_1 is selected from the group consisting of:



wherein W and Z are each independently oxygen or sulfur; and further wherein at least two of X, Y, W and Z are sulfur; R_6 and R_7 are each independently hydroxyl, alkoxy or substituted alkoxy, each of R_8-R_{12} are independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy, substituted acyloxy, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen or nitro; R_{13} and R_{14} are each independently hydrogen, alkyl or substituted alkyl; R_{20} is hydrogen, hydroxyl, or alkyl; and R_{15}-R_{19} are each independently hydrogen, hydroxyl, acyl, substituted acyl, acyloxy,

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substituted acyloxy, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, aryl, substituted aryl, amino, substituted amino, halogen or nitro; or a stereoisomer or pharmaceutically acceptable salt thereof.

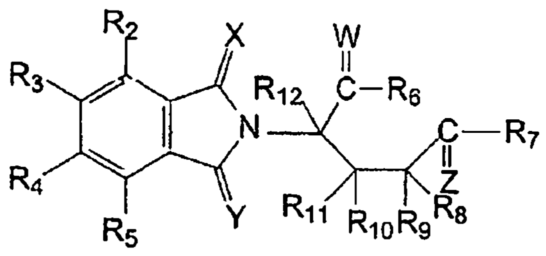
2. The compound of claim 1, wherein X, W and Z are each S and Y is O.

3. The compound of claim 1, wherein X = S, Y = O, W = S, Z = O; or X = S, Y = O, W = O, Z = S.

4. The compound of claim 1, wherein X = O, Y = O, W = S and Z = S.

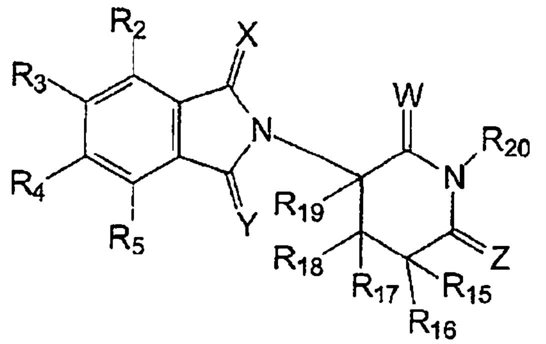
5. The compound of claim 1, wherein at least one of W and Z is sulfur, or wherein at least one of X and Y is sulfur.

6. The compound of any preceding claim, wherein the compound has the formula:



wherein X, Y, W and Z are independently sulfur or oxygen and at least two of X, Y, W and Z is sulfur, or a stereoisomer or pharmaceutically acceptable salt thereof.

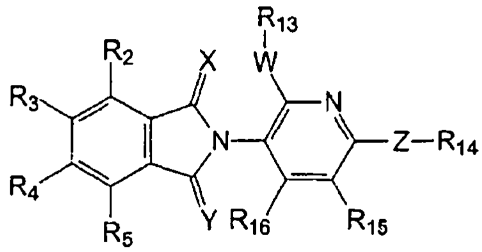
7. The compound of any one of claims 1 to 6, wherein the compound has the formula:



wherein W, X, Y and Z each are independently sulfur or oxygen and at least two of W, X, Y and Z is sulfur, or a stereoisomer or pharmaceutically acceptable salt thereof.

8. The compound of claim 7, wherein at least one of R_2-R_5 and R_{15}-R_{19} is hydroxyl, or wherein each of R_2-R_5 and R_{15}-R_{19} is independently hydrogen, lower alkyl, acyloxy, halogen, hydroxyl, amino or nitro.

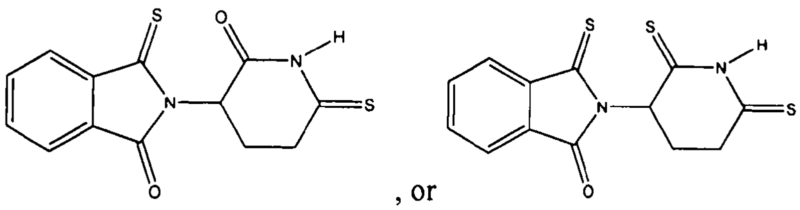
9. The compound of claim 1, wherein the compound satisfies the formula:



;

or a stereoisomer or pharmaceutically acceptable salt thereof.

10. The compound of claim 1, wherein the compound has the structure;



, or

11. The compound of claim 1, wherein the compound is 2,3-dihydro-3-thioxo-2-(2-oxo-6-thioxo-3-piperidinyl)-1*H*-isoindol-1-one or 2,3-Dihydro-3-thioxo-2-(2,6-dithioxo-3-piperidinyl)-1*H*-isoindol-1-one.

12. The compound of claim 1, wherein the compound is 1-Thioxo-3-oxo-2-(2-oxo-6-thioxopiperidin-3-yl)isoindoline, 1,3-Dioxo-2-(2,6-dithioxopiperidin-3-yl)isoindoline, 1-Thioxo-3-oxo-2-(2,6-dithioxopiperidin-3-yl)isoindoline, Dimethyl 2-(1,3-dihydro-1,3-dithioxo-2*H*-isoindol-2-yl)-pentanedioate, 2-(2, 6-Dithioxo-3-piperidinyl)-1*H*-isoindole-1,3(2*H*)-dione, 2,3-Dihydro-3-thioxo-2-(2-oxo-6-thioxo-3-piperidinyl)-1*H*-isoindol-1-one, or 2,3-Dihydro-3-thioxo-2-(2,6-dithioxo-3-piperidinyl)-1*H*-isoindol-1-one.

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13. A compound of any one of claims 1 to 12 for pharmaceutical use.

14. A pharmaceutical composition comprising a compound as defined in any one of claims 1 to 12 and a pharmaceutically acceptable carrier.

15. The composition of claim 14 for oral administration.

16. A compound of any one of claims 1 to 12 or the composition of claims 14 or 15, for use in treating a disease treatable by modulation of TNF-α activity, or in treating a disease treatable by modulation of angiogenesis, or in achieving an anti-tumor effect, or in inhibiting tumor metastasis, or in a treating pathological angiogenesis, or in treating a neurodegenerative disease.

17. Use of a compound of any one of claims 1 to 12 in the manufacture of a medicament for treating a disease treatable by modulation of TNF-α activity, or treating a disease treatable by modulation of angiogenesis, or achieving an anti-tumor effect, or inhibiting tumor metastasis, or treating a pathological angiogenesis, or treating a neurodegenerative disease.

18. A method for treating a disease treatable by modulation of TNF-α activity, or treating a disease treatable by modulation of angiogenesis, or achieving an anti-tumor effect, or inhibiting tumor metastasis, or treating a pathological angiogenesis, or treating a neurodegenerative disease which comprises administering a therapeutically effective amount of a compound of any one of claims 1 to 12, or a pharmaceutical composition of claim 14 or 15 to a subject in need thereof.

19. A compound as defined in claim 1, or a pharmaceutical composition comprising the compound, or uses or methods involving the compound or the pharmaceutical composition, substantially as herein described with reference to the Examples and/or Figures.

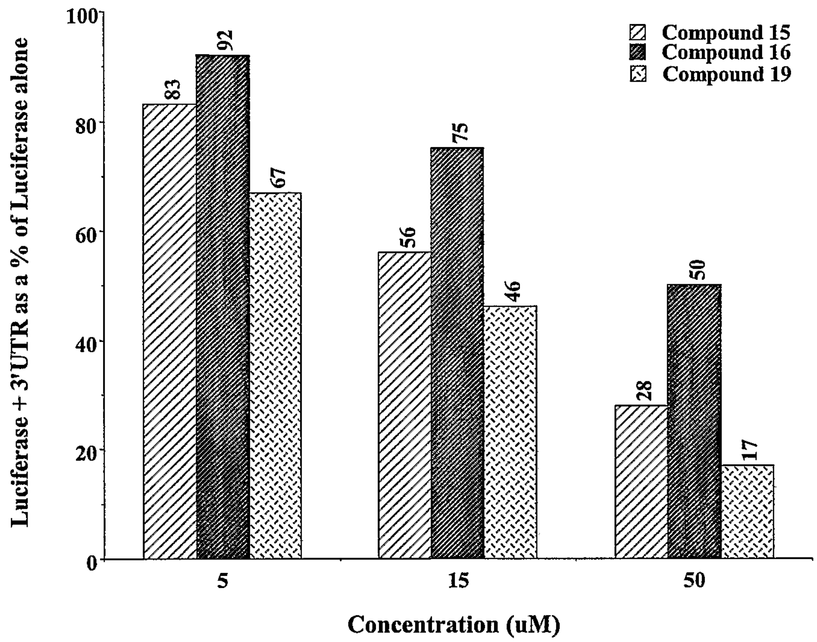
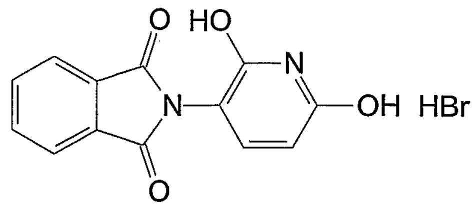

Luciferase + 3'UTR as a % of Luciferase alone
100
80
60
40
20
0
Compound 15
Compound 16
Compound 19
83
92
67
56
75
46
28
50
17
5
15
50
Concentration (uM)

FIG. 1


O
HO
N
N
OH HBr
O

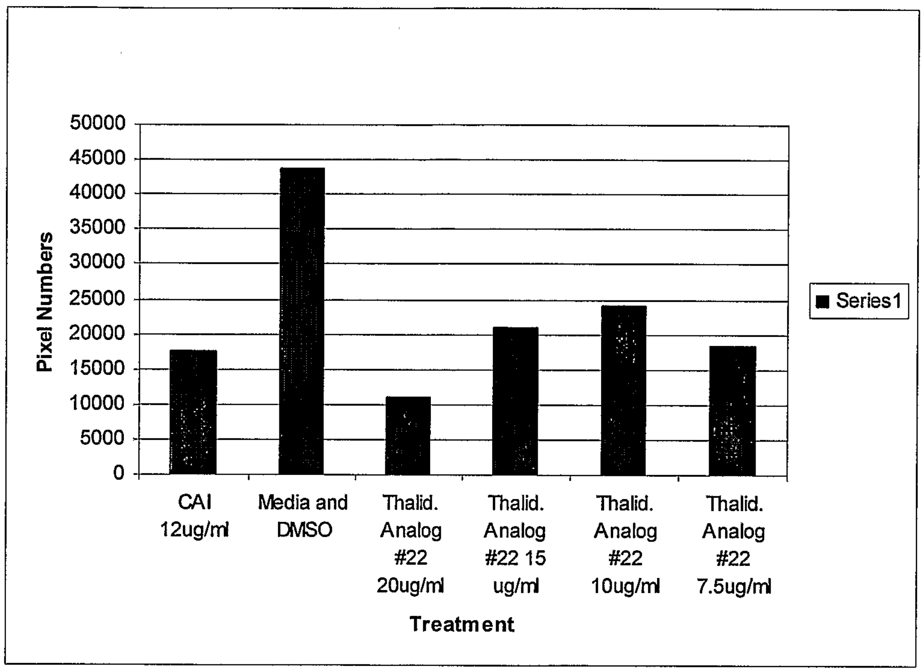
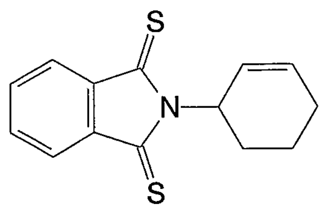

50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Pixel Numbers
Series1
CAI 12ug/ml
Media and DMSO
Thalid. Analog #22 20ug/ml
Thalid. Analog #22 15 ug/ml
Thalid. Analog #22 10ug/ml
Thalid. Analog #22 7.5ug/ml
Treatment

FIG. 2


S
N
S

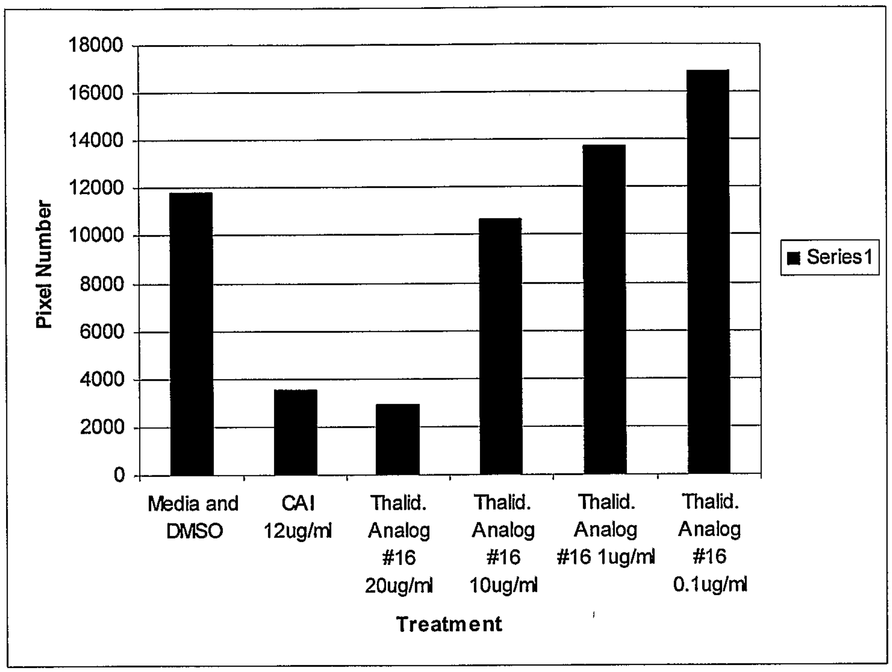
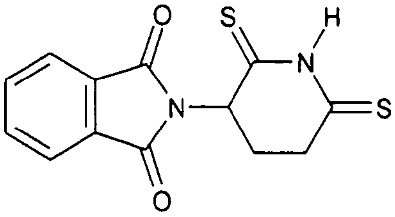

18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Pixel Number
Series1
Media and DMSO
CAI 12ug/ml
Thalid. Analog #16 20ug/ml
Thalid. Analog #16 10ug/ml
Thalid. Analog #16 1ug/ml
Thalid. Analog #16 0.1ug/ml
Treatment

FIG. 3


O
S
H
N
N
S
O

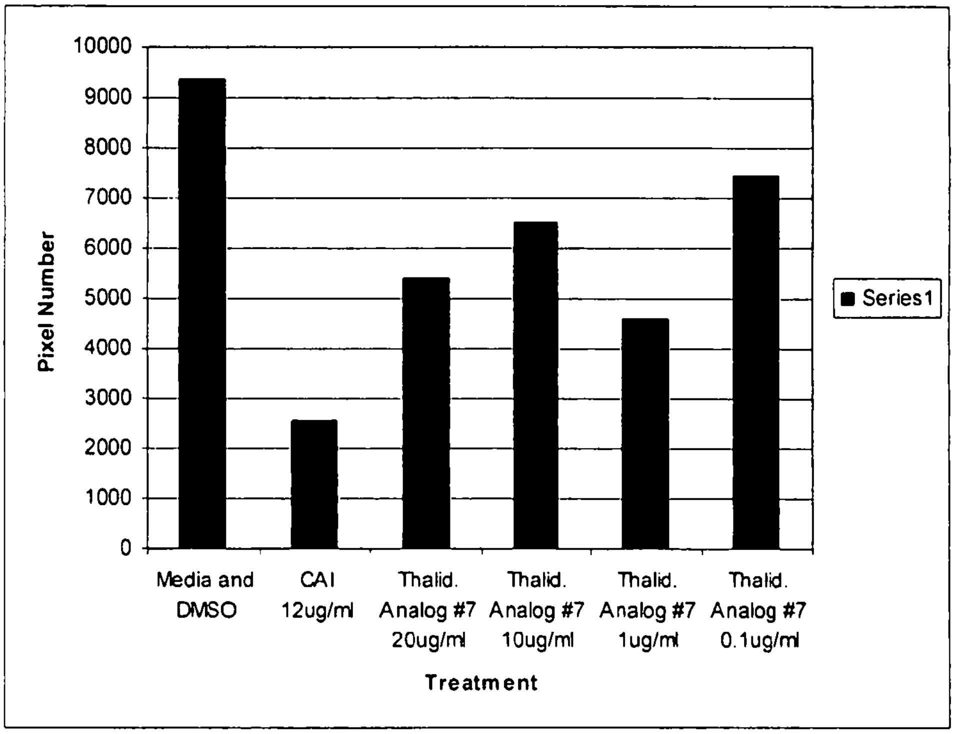
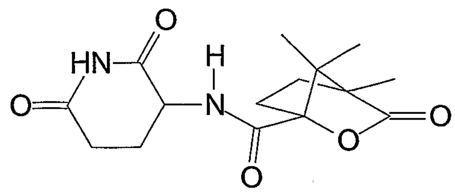

10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Pixel Number
Series1
Media and DMSO
CAI 12ug/ml
Thalid. Analog #7 20ug/ml
Thalid. Analog #7 10ug/ml
Thalid. Analog #7 1ug/ml
Thalid. Analog #7 0.1ug/ml
Treatment

FIG. 4



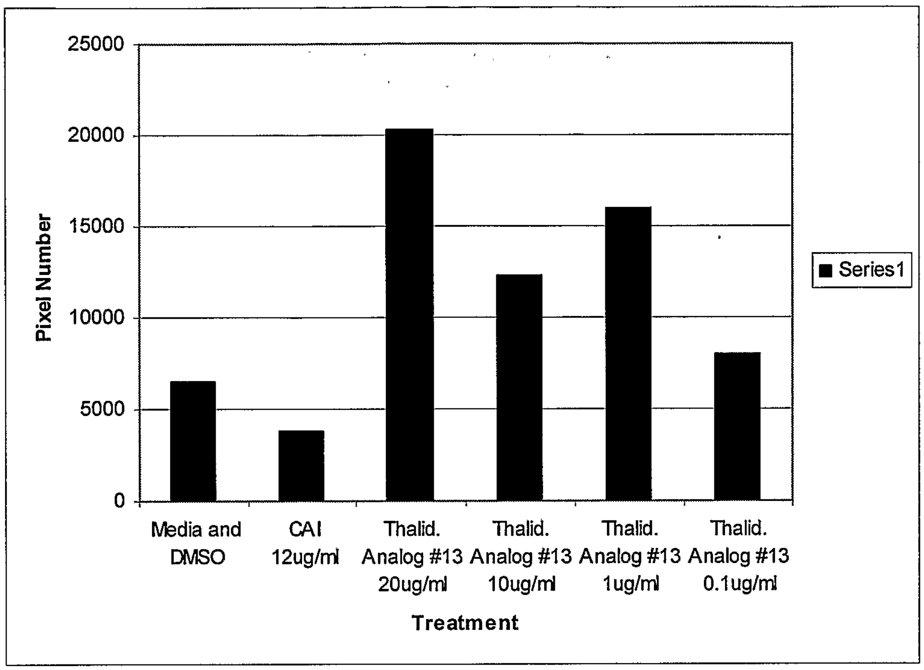
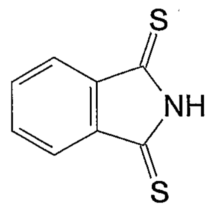

25000
20000
15000
10000
5000
0
Pixel Number
Series1
Media and DMSO
CAI 12ug/ml
Thalid. Analog #13 20ug/ml
Thalid. Analog #13 10ug/ml
Thalid. Analog #13 1ug/ml
Thalid. Analog #13 0.1ug/ml
Treatment

FIG. 5


S
NH
S

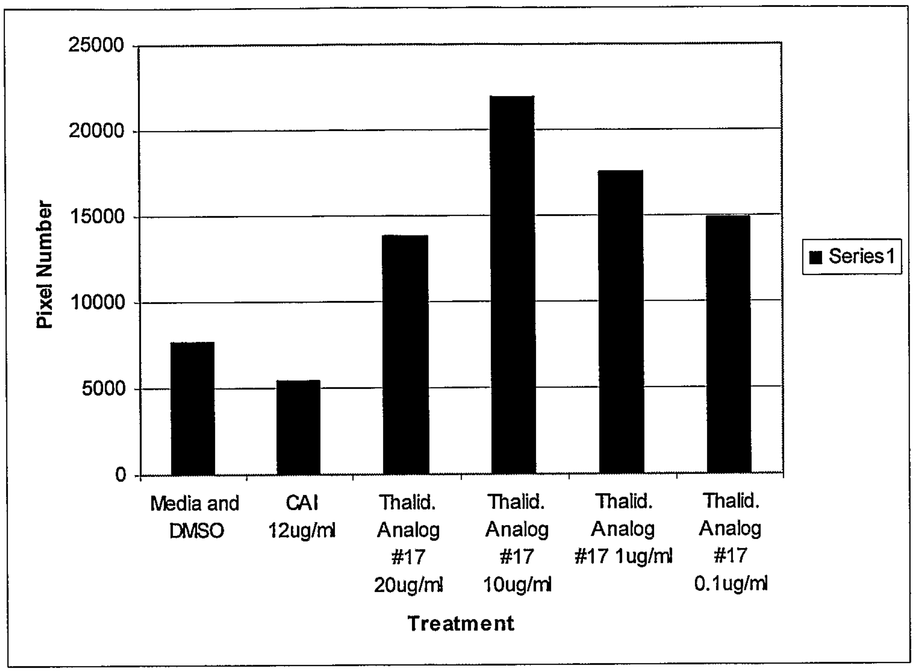
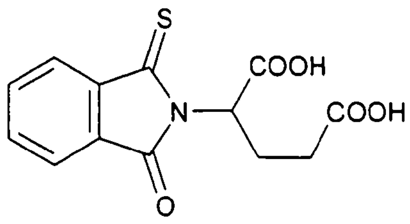

25000
20000
15000
10000
5000
0
Pixel Number
Series1
Media and DMSO
CAI 12ug/ml
Thalid. Analog #17 20ug/ml
Thalid. Analog #17 10ug/ml
Thalid. Analog #17 1ug/ml
Thalid. Analog #17 0.1ug/ml
Treatment

FIG. 6


S
N
COOH
COOH
O

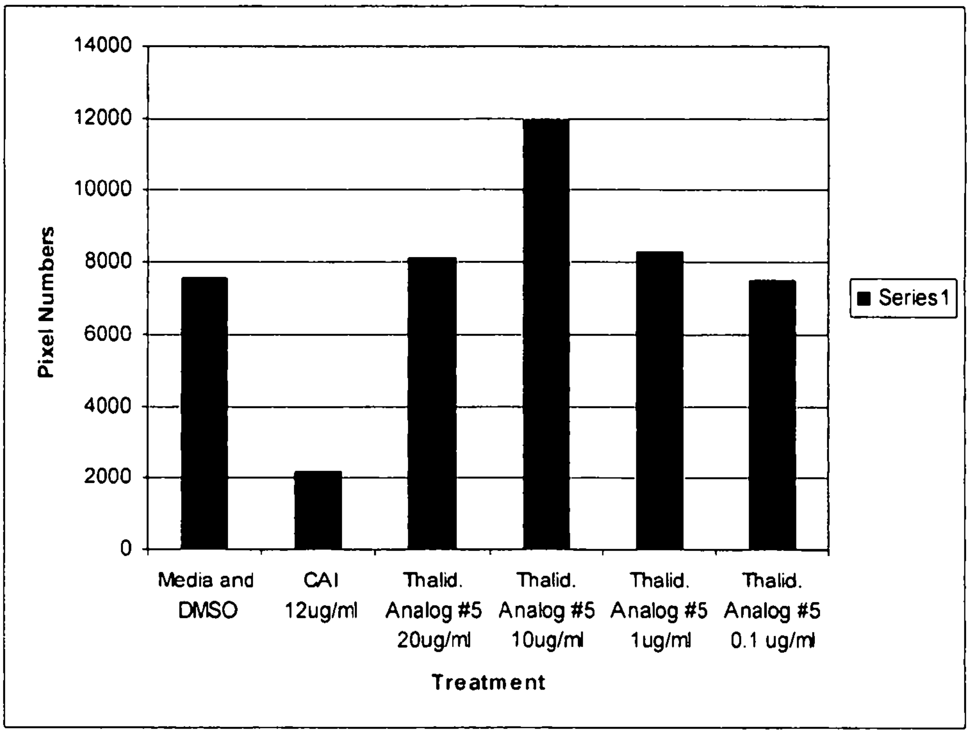
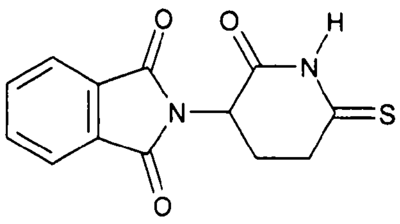

14000
12000
10000
8000
6000
4000
2000
0
Pixel Numbers
Series1
Media and DMSO
CAI 12ug/ml
Thalid. Analog #5 20ug/ml
Thalid. Analog #5 10ug/ml
Thalid. Analog #5 1ug/ml
Thalid. Analog #5 0.1 ug/ml
Treatment

FIG. 7



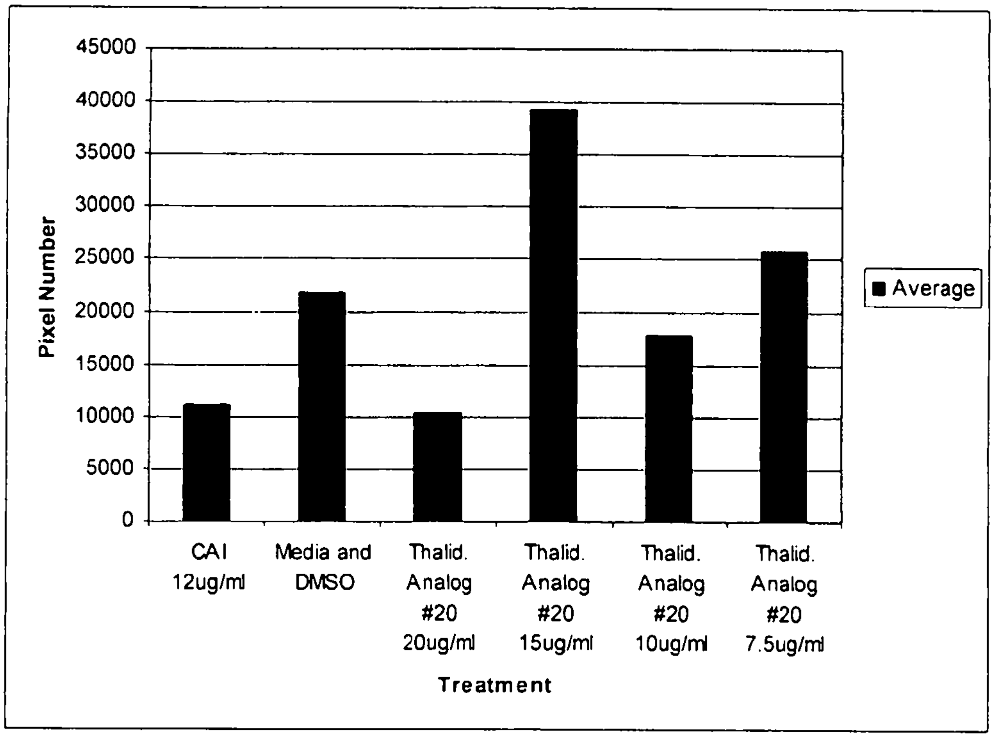

45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Pixel Number
Average
CAI 12ug/ml
Media and DMSO
Thalid. Analog #20 20ug/ml
Thalid. Analog #20 15ug/ml
Thalid. Analog #20 10ug/ml
Thalid. Analog #20 7.5ug/ml
Treatment

FIG. 8

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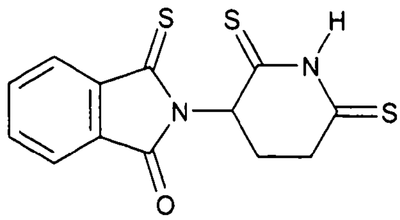


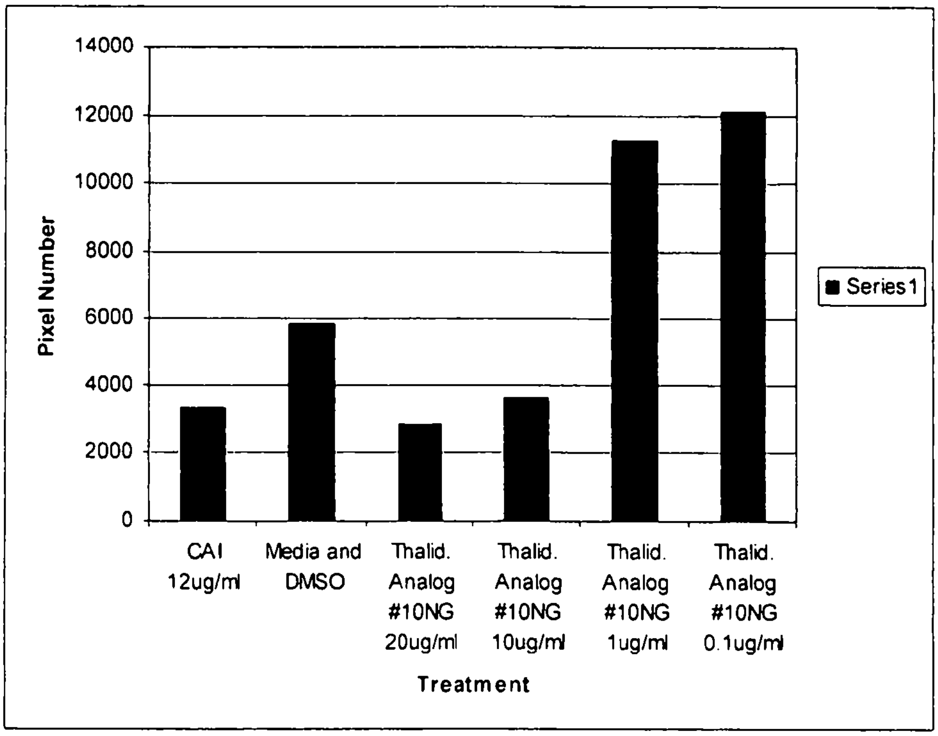
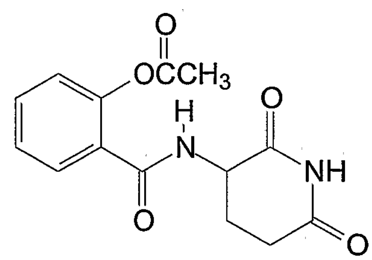


FIG. 9



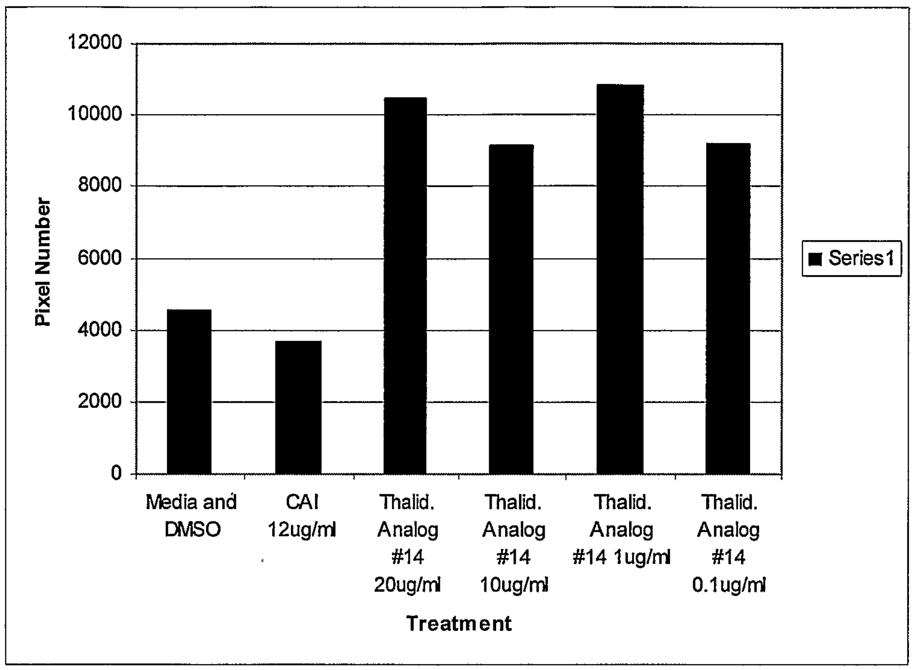
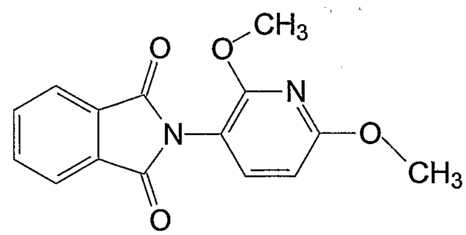

12000
10000
8000
6000
4000
2000
0
Pixel Number
Series1
Media and DMSO
CAI 12ug/ml
Thalid. Analog #14 20ug/ml
Thalid. Analog #14 10ug/ml
Thalid. Analog #14 1ug/ml
Thalid. Analog #14 0.1ug/ml
Treatment

FIG. 10



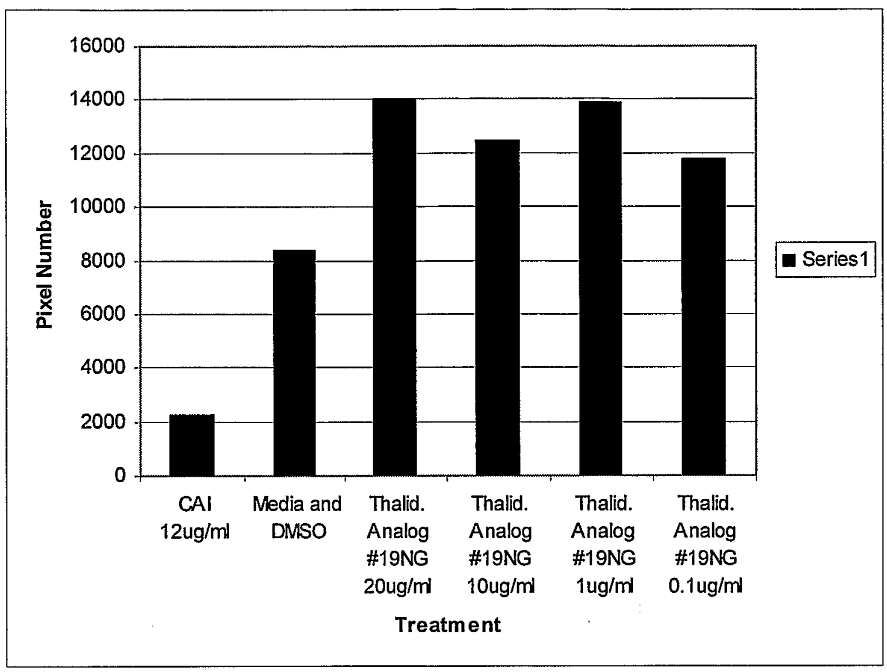

16000
14000
12000
10000
8000
6000
4000
2000
0
Pixel Number
Series1
CAI 12ug/ml
Media and DMSO
Thalid. Analog #19NG 20ug/ml
Thalid. Analog #19NG 10ug/ml
Thalid. Analog #19NG 1ug/ml
Thalid. Analog #19NG 0.1ug/ml
Treatment

FIG. 11