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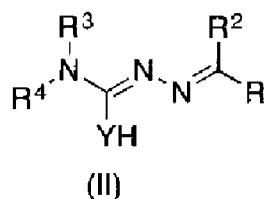
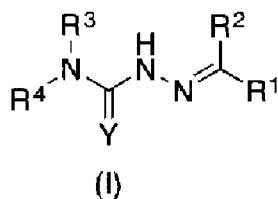
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(54) Title: (THIO)SEMICARBAZONE DERIVATIVES AND THEIR USE FOR TREATING CANCER



(57) Abstract: The invention provides compounds of formula I and II and salts thereof, wherein R¹, R², Y, R³, and R⁴ have any of the meanings described in the specification, as well as compositions comprising such compounds and salts, and methods for treating cancer using such compounds and salts.

CROSS-REFERENCE TO RELATED APPLICATIONS

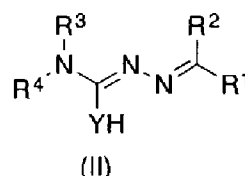
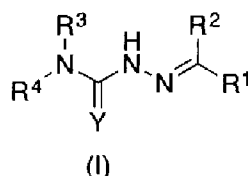
This patent application claims the benefit of priority of U.S. application serial No. 62/108,415, filed January 27, 2015, and of U.S. application serial No. 62/258,236, filed November 20, 2015, which applications are herein incorporated by reference.

BACKGROUND OF THE INVENTION

TP53 is the most commonly mutated gene in human cancer for which no effective targeted anti-cancer drug exists. The majority of TP53 mutations (>70%) are mis-sense mutations that generate a defective protein that is generally found at high levels in cancer cells due to loss of MDM2 negative feedback. Restoring the function of p53 in mouse models of cancer is highly therapeutic. Reactivating mutant p53 using small molecules has been highly sought after, yet remains an elusive goal in the development of cancer therapeutics.

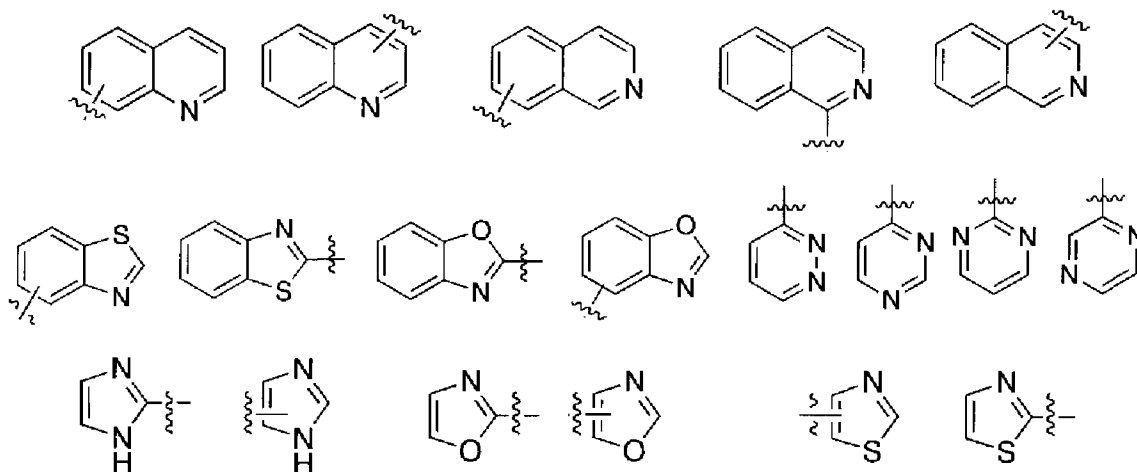
SUMMARY OF THE INVENTION

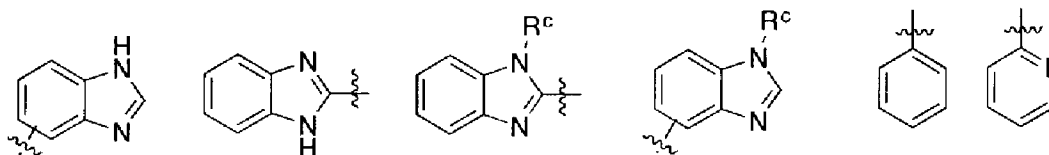
The invention provides novel compounds, compositions, and methods for treating cancer. More specifically, one aspect of the present invention provides a compound of formula (I) or (II):



wherein:

R¹ is selected from the group consisting of:





wherein R^1 is optionally substituted with one or more groups independently selected from halo, cyano, hydroxy, nitro, $-N(R^a)_2$, carboxy, phenyl, (C_1-C_6) alkyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, (C_3-C_6) cycloalkyl, (C_1-C_6) alkoxy, (C_1-C_6) alkanoyl, (C_1-C_6) alkoxycarbonyl, (C_2-C_6) alkanoyloxy, (C_4-C_6) heterocycloalkyl, (C_2-C_6) alkylaminocarbonyl and (C_2-C_6) alkanoylamino wherein any phenyl, (C_1-C_6) alkyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, (C_1-C_6) alkoxy, and (C_3-C_6) cycloalkyl, is optionally substituted with one or more groups independently selected from halo, cyano, hydroxy, nitro, $-N(R^a)_2$, carboxy, (C_3-C_6) cycloalkyl, (C_1-C_6) alkoxy, (C_1-C_6) alkanoyl, (C_1-C_6) alkoxycarbonyl, (C_2-C_6) alkanoyloxy, (C_4-C_6) heterocycloalkyl, (C_2-C_6) alkylaminocarbonyl and (C_2-C_6) alkanoylamino;

R^2 is selected from the group consisting of H, phenyl, heteroaryl, (C_1-C_6) alkyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, and (C_3-C_6) cycloalkyl, wherein any phenyl, heteroaryl, (C_1-C_6) alkyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, and (C_3-C_6) cycloalkyl is optionally substituted with one or more groups independently selected from halo, $-N(R^b)_2$, (C_3-C_6) cycloalkyl, (C_1-C_6) alkoxy, (C_2-C_6) alkanoyloxy, (C_2-C_6) alkoxycarbonyl, (C_2-C_6) alkylaminocarbonyl, and (C_2-C_6) alkanoylamino;

Y is S, O, Se;

R^3 and R^4 are each independently selected from H, (C_1-C_6) alkyl, piperidinyl, or piperazinyl, which piperidinyl or piperazinyl is optionally substituted with pyridyl; or R^3 and each R^4 taken together with the nitrogen to which they are attached form a 3, 4, 5, 6, 7, 8, or 9 membered ring that is optionally substituted with one or more groups independently selected from the group consisting of halo;

each R^a is independently selected from the group consisting of H, (C_1-C_6) alkyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, (C_3-C_6) cycloalkyl, (C_1-C_6) alkanoyl, and (C_1-C_6) alkoxycarbonyl, wherein any (C_1-C_6) alkyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, (C_3-C_6) cycloalkyl, (C_1-C_6) alkanoyl, and (C_1-C_6) alkoxycarbonyl, is optionally substituted with one or more groups independently selected from halo, (C_3-C_6) cycloalkyl, and (C_1-C_6) alkoxy; or two R^a taken together with the nitrogen to which they are attached form a azetidino, pyrrolidino, piperidino, or morpholino ring;

each R^b is independently selected from the group consisting of H, (C_1-C_6) alkyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, (C_3-C_6) cycloalkyl, (C_1-C_6) alkanoyl, and (C_1-C_6) alkoxycarbonyl,

wherein any (C₁-C₆)alkyl, (C₂-C₆)alkenyl, (C₂-C₆)alkynyl, (C₃-C₆)cycloalkyl, (C₁-C₆)alkanoyl, and (C₁-C₆)alkoxycarbonyl, is optionally substituted with one or more groups independently selected from halo, (C₃-C₆)cycloalkyl, and (C₁-C₆)alkoxy; or two R^b taken together with the nitrogen to which they are attached form a azetidino, pyrrolidino, piperidino, or morpholino ring; and

each R^c is independently selected from the group consisting of H and (C₁-C₆)alkyl that is optionally substituted with one or more groups independently selected from halo, (C₃-C₆)cycloalkyl, and (C₁-C₆)alkoxy;

or a salt thereof;

provided that if R¹ is 2-pyridinyl, then R² is not H or (C₁-C₆)alkyl.

Another aspect of the present invention provides a pharmaceutical composition, comprising, a compound of formula I or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

Another aspect of the present invention provides a method of inhibiting cancer cell growth, comprising contacting the cancer cell with an effective amount of a compound of formula I or a salt thereof.

Another aspect of the present invention provides a method of treating cancer in an animal (e.g. a human), comprising administering to the animal a compound of formula I or a pharmaceutically acceptable salt thereof.

The invention further includes methods of preparing, methods of separating, and methods of purifying the compounds described herein.

Additional advantages and novel features of this invention shall be set forth in part in the description that follows, and in part will become apparent to those skilled in the art upon examination of the following specification, or may be learned by the practice of the invention. The advantages of the invention may be realized and attained by means of the instrumentalities, combinations, compositions, and methods particularly pointed out in the appended claims.

DESCRIPTION OF THE INVENTION

The following definitions are used, unless otherwise described: halo is fluoro, chloro, bromo, or iodo. Alkyl, alkoxy, alkenyl, alkynyl, etc. denote both straight and branched groups; but reference to an individual radical such as propyl embraces only the straight chain radical, a branched chain isomer such as isopropyl being specifically referred to.

The term allyl as used herein refers to a substituent, molecular fragment, or radical

having the chemical formula $-\text{CH}_2-\text{CH}=\text{CH}_2$.

The term "butyl" as used herein refers to a four-carbon alkyl radical, substituent, or molecular fragment having the chemical formula $-\text{C}_4\text{H}_9$.

The term "cyclopropyl" as used herein refers to a radical, substituent, or molecular fragment having a chemical structure derived from cyclopropane and having the chemical formula C_3H_5 .

The term "ethyl" as used herein refers to an alkyl substituent, radical, or molecular fragment having the chemical formula $-\text{C}_2\text{H}_5$.

The term "isopropyl" as used herein refers to a propyl with a group attached to the secondary carbon.

The term "methyl" as used herein refers to an alkyl derived from methane and containing one carbon atom bonded to three hydrogen atoms and having the chemical formula $-\text{CH}_3$.

The term "propyl" as used herein refers to a linear three-carbon alkyl substituent, molecular fragment, or radical having the chemical formula $-\text{C}_3\text{H}_7$.

The term "phenyl" refers to a cyclic group of atoms, radical, substituent, or molecular fragment having the chemical formula $-\text{C}_6\text{H}_5$.

It will be appreciated by those skilled in the art that compounds of the invention having a chiral center may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein, it being well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase).

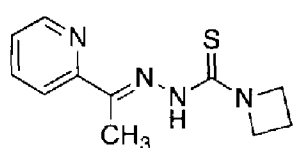
When a bond in a compound formula herein is drawn in a non-stereochemical manner (e.g. flat), the atom to which the bond is attached includes all stereochemical possibilities. When a bond in a compound formula herein is drawn in a defined stereochemical manner (e.g. bold, bold-wedge, dashed or dashed-wedge), it is to be understood that the atom to which the stereochemical bond is attached is enriched in the absolute stereoisomer depicted unless otherwise noted. In one embodiment, the compound may be at least 51% the absolute stereoisomer depicted. In another embodiment, the compound may be at least 60% the

absolute stereoisomer depicted. In another embodiment, the compound may be at least 80% the absolute stereoisomer depicted. In another embodiment, the compound may be at least 90% the absolute stereoisomer depicted. In another embodiment, the compound may be at least 95 the absolute stereoisomer depicted. In another embodiment, the compound may be at least 99% the absolute stereoisomer depicted.

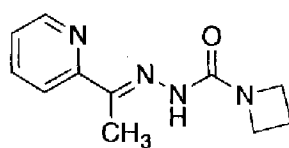
Specific values listed below for radicals, substituents, and ranges, are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents.

Specifically, (C₁-C₆)alkyl can be methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, sec-butyl, pentyl, 3-pentyl, or hexyl; (C₃-C₆)cycloalkyl can be cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl; (C₁-C₆)alkoxy can be methoxy, ethoxy, propoxy, isopropoxy, butoxy, iso-butoxy, sec-butoxy, pentoxy, 3-pentoxy, or hexyloxy; (C₂-C₆)alkenyl can be vinyl, allyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, or 5-hexenyl; (C₂-C₆)alkynyl can be ethynyl, 1-propynyl, 2-propynyl, 1-butylnyl, 2-butylnyl, 3-butylnyl, 1-pentylnyl, 2-pentylnyl, 3-pentylnyl, 4-pentylnyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, or 5-hexynyl; (C₁-C₆)alkanoyl can be acetyl, propanoyl or butanoyl; (C₁-C₆)alkoxycarbonyl can be methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, pentoxy carbonyl, or hexyloxycarbonyl; and (C₂-C₆)alkanoyloxy can be acetoxyl, propanoyloxy, butanoyloxy, isobutanoyloxy, pentanoyloxy, or hexanoyloxy.

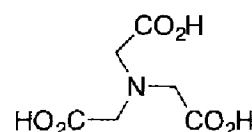
The ability of ZMC1, NTA (Zn²⁺-binding homolog), and A6 (structural homolog) to increase intracellular [Zn²⁺]_{free} was evaluated by treating cells with the fluorescent Zn²⁺ indicator FluoZin-3-AM (FZ3-AM) in complete media and imaging them using confocal microscopy. In both HEK293 (non-cancer, p53-WT) and TOV112D (ovarian cancer, p53-R175H) cells, ZMC1 increased intracellular [Zn²⁺]_{free} as indicated by increased fluorescence, but NTA and A6 did not. This result is consistent with the metallochaperone (MC) model for ZMC1 function and explains the inability of NTA and A6 to reactivate p53-R175H at micromolar concentrations. Of the two control compounds, A6 shuttled Zn²⁺ into the liposomes, but NTA did not.



ZMC1



A6



NTA

NTA binds Zn^{2+} with an affinity similar to that of ZMC1, but it cannot cross either liposomal or cellular membranes, likely because it possesses negative charges. A6, on the other hand, lacks charges and is similar in structure to ZMC1, but binds Zn^{2+} weakly ($K_d = 1.1 \mu\text{M}$). It can function as an ionophore in conditions of the liposome experiments where external $[\text{Zn}^{2+}]_{\text{free}}$ was $10 \mu\text{M}$. However, in complete media containing 10% fetal bovine serum (FBS), Zn^{2+} -binding proteins from the serum (e.g. albumin) necessarily compete for Zn^{2+} with any putative MC, making the effective $[\text{Zn}^{2+}]_{\text{free}}$ much lower than $[\text{Zn}^{2+}]_{\text{total}}$. A6 therefore likely does not increase intracellular $[\text{Zn}^{2+}]_{\text{free}}$ in culture because $K_{d,A6}$ is greater than extracellular $[\text{Zn}^{2+}]_{\text{free}}$. Thus, both an appropriate Zn^{2+} K_d and ionophore activity influence ZMC1 activity. To determine whether ZMC1 can traverse lipid bilayers as a free compound, the $[\text{Zn}^{2+}]_{\text{free}}$ gradient was reversed by adding a large excess of metal ion chelator EDTA to the solution outside of the liposomes; fluorescence was monitored in the presence and absence of ZMC1. EDTA alone did not cause a significant decrease in RZ-3 fluorescence as the liposomal membranes are impermeable to EDTA. After subsequent addition of ZMC1, there was a time dependent decrease in RZ-3 fluorescence. This result indicates that free ZMC1 crossed the liposomal membranes, bound internal Zn^{2+} , and transported it back outside the liposome where the metal was then bound by the much stronger chelator EDTA. Thus, ZMC1 can cross biological membranes both as free drug and drug- Zn^{2+} complex, and therefore can transport Zn^{2+} into cells without becoming trapped as either species.

To ensure that the fluorescence results were due to Zn^{2+} transport and not to non-specific disruption of liposomal membranes, a liposomal leakage assay was performed using the self-quenching fluorophore calcein. When calcein is encapsulated at concentrations above 4 mM its fluorescence is decreased via self-quenching. Leakage is detected by a fluorescence increase as the dye dilutes and its fluorescence dequenches. At the highest concentrations of ZMC1 and ZnCl_2 a significant fluorescence increase was not detected. Disruption of liposomes can also be detected by alteration of their size distribution. The size distribution of liposomes treated with the highest concentrations of ZnCl_2 and ZMC1 was identical to that of untreated liposomes. Together, these data indicate the liposomal membranes remained intact upon ZMC1 treatment, and therefore the RZ-3 fluorescence changes are attributable only to specific Zn^{2+} transport.

Characterization of ZMC1-mediated Zn^{2+} transport in live cells

To extend the investigation of ZMC1 as an ionophore to living systems, ZMC1-mediated Zn^{2+} transport was quantified in cells. The kinetics of intracellular $[\text{Zn}^{2+}]_{\text{free}}$ increase was measured by loading HEK293 and TOV112D cells with FZ3-AM, treating the cells with ZMC1 and ZnCl_2 , and monitoring fluorescence by time-lapse microscopy. To minimize the potential for Zn^{2+} contamination and contributions from poorly defined elements in complete media (e.g. FBS), cells were treated and imaged in Ca^{2+} and Mg^{2+} -free Earle's Balanced Salt Solution supplemented with 10 mM HEPES pH 7.4 (EBSS/H (-)Ca/Mg). Excess ZnCl_2 with the Zn^{2+} ionophore pyrithione (PYR) was used as a positive control. Excess membrane-permeable Zn^{2+} chelator N,N,N',N'-tetrakis(2-pyridylmethyl)ethane-1,2-diamine (TPEN) was used as a negative control. When treated with ZnCl_2 alone or ZMC1 alone, neither cell type showed an increase in intracellular $[\text{Zn}^{2+}]_{\text{free}}$. When treated with both ZMC1 and ZnCl_2 , both cell lines showed a time dependent increase at two different ZnCl_2 concentrations, demonstrating that both ZMC1 and extracellular Zn^{2+} are required. When the fluorescence increases were fit to first-order exponentials, both concentrations of ZnCl_2 yielded identical half-lives in their respective cell types, which we combine to report $t_{1/2}$ (HEK293) = 124 ± 20 s and $t_{1/2}$ (TOV112D) = 156 ± 50 s (mean $\pm$ SD, n=4).

The steady-state intracellular $[\text{Zn}^{2+}]_{\text{free}}$ of both cell types was then quantified after treatment with the 2:1 ratio of ZMC1: ZnCl_2 . Cells were again loaded with FZ3-AM, treated with 1 μM ZMC1 and 0.5 μM ZnCl_2 in EBSS/H (-)Ca/Mg, and imaged as above. To normalize for differential dye loading, cells were then sequentially treated with excess PYR/ ZnCl_2 , imaged, treated with TPEN, and imaged again. PYR/ ZnCl_2 and TPEN served to saturate and apoize the intracellular FZ3, respectively. In the absence of drug an intracellular $[\text{Zn}^{2+}]_{\text{free}}$ of 0.69 ± 0.25 nM was measured for HEK293 cells and 0.71 ± 0.19 nM was measured for TOV112D cells. These values reflect the lower limit of detection by FZ3-AM and are likely overestimates. Upon treatment with ZMC1 and ZnCl_2 intracellular $[\text{Zn}^{2+}]_{\text{free}}$ rose to 18.1 ± 4.7 nM for HEK293 cells and 15.8 ± 2.5 nM for TOV112D cells. These concentrations are theoretically sufficient to reactivate ~90 % of p53-R175H based on the K_{d1} value of 2.1 nM measured for DBD-R175H.

MATERIALS AND METHODS

Reagents

FZ3-AM, RZ-3 (K^+ salt), and cell culture media were purchased from Life Technologies. DOPC was purchased from Avanti Polar Lipids. ZMC1 and A6 were similarly obtained. Zn^{2+} (ZMC1)₂ was synthesized and crystallized. HEK293 and TOV112D cells were purchased from ATCC and maintained in DMEM + GlutaMAX with 10% FBS and 1 mg/mL penicillin-streptomycin under a 5% CO₂ atmosphere at 37 °C. All non-cell based experiments were conducted in 50 mM Tris pH 7.2, 0.1 M NaCl at 25 °C.

Liposome Import Assay

DOPC-liposomes were prepared by film rehydration and extrusion followed by gel filtration and diluted to an OD₆₀₀ = 0.06 in buffer. The size distribution of the liposomes was determined by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS. Fluorescence measurements were taken on a Horiba Fluoromax-4 spectrofluorimeter in a 5 x 5 mm quartz cuvette with $\lambda_{ex}/\lambda_{em}$ = 550/572 nm for RZ-3 and 490/515 nm for calcein. Initial Zn^{2+} import/export was quantified by fitting the first 10-30 s of data after each treatment to a line and converted to units of flux using the following Eqn 1:

$$Eqn\ 1: \quad J_i = \frac{\Delta F}{\Delta t} \cdot \left(\frac{F_{max} - F_{min}}{[RZ3]} \right) \cdot \left(\frac{SA}{Vol} \right)$$

where J_i is the initial flux, $\Delta F/\Delta t$ is the slope of the fit line, F_{max} is RZ-3 fluorescence in the presence of saturating Zn^{2+} and 1% TritonX-100, F_{min} is RZ-3 fluorescence in the presence of excess EDTA and 1% TritonX-100, [RZ3] is the concentration of encapsulated RZ-3, and SA/Vol is the surface area to volume ratio calculated assuming hollow spheres of the mean diameter determined by DLS.

Intracellular $[Zn^{2+}]_{free}$ Imaging

TOV112D or HEK293 cells (40,000 cells/well) were plated on either 8-well BD Falcon chambered culture slides (Corning Life Sciences) or 8-chambered #1.5 Nunc Lab-Tek II chambered coverglasses (Thermo Scientific) treated with poly-L-lysine. After 48 h, cells were washed 2 x 5 m in serum-free media and incubated with 1 μ M FZ3-AM for 40 m at 37 °C. Cells were then washed 2 x 5 m in either EBSS/H (-)Ca/Mg or phenol-red free DMEM + 10% FBS containing the indicated treatments for 20 m before imaging. For nuclear colocalization, 1 μ g/mL Hoechst 33342 was also included. Cells were imaged using a Zeiss

LSM510 META NLO confocal microscope equipped with 37 °C environmental control chamber. FZ3 and Hoechst 33342 were excited at 488 nm (argon laser) and 790 nm (Chameleon Ti:sapphire laser), respectively. To determine the kinetics of fluorescence change, each background-subtracted image in the time-lapse series was integrated in ImageJ and normalized to the integrated fluorescence of the first frame after treatment. For quantification of intracellular $[Zn^{2+}]_{free}$, each cell was analyzed in the treated, 50 μ M PYR/ $ZnCl_2$ (1:1), and 100 μ M TPEN images by taking the mean fluorescence of an ROI inside the cell subtracted by an ROI immediately outside the cell measured in ImageJ. The $[Zn^{2+}]_{free}$ for each cell was then calculated by Eqn 2:

$$Eqn. 2: \quad [Zn^{2+}]_{free} = \frac{F - F_{min}}{F_{max} - F} \cdot K_d$$

Where F, F_{max} , and F_{min} are fluorescence in the treatment, PYR/ $ZnCl_2$, and TPEN images, respectively, and K_d is that of FZ3 for Zn^{2+} (15 nM) (31). To minimize the effects of outliers the lowest and highest 5% of cells in each series were rejected, and the remaining values averaged to give the value from that experiment. The number of cells analyzed in each trial ranged from 54-163. For nuclear colocalization, treated, PYR/ $ZnCl_2$, and TPEN treated images costained with Hoechst 33342 were aligned and each pixel subjected to Eqn. 2 in MATLAB (MathWorks). The resultant images were Gaussian mean filtered and false-colored by calculated $[Zn^{2+}]_{free}$.

p53-R175H Immunofluorescence

DMEM + 10% FBS was treated with 5 g Chelex 100 resin per 100 mL media for 1 hour with gentle shaking. The media was then decanted and filtered through 0.2 μ m sterile filter. TOV112D cells were then incubated with 1 μ M ZMC1 in untreated media, Chelex-treated media, or media + 10 μ M TPEN at 37 °C for 2 h, fixed, and stained with PAB240 and PAB1640.

Assays:

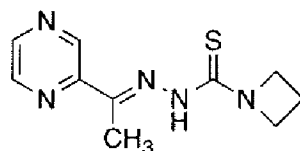
Cell growth inhibition assay using human tumor cell lines with different p53 status (wildtype, null, p53-R175H) were employed to determine if wildtype structure is restored to mutant p53 after treatment with a zinc metallochaperone.

An immunofluorescence assay using conformation specific antibodies was used to determine if a test compound could induce a wildtype conformation of mutant p53.

The invention will now be illustrated by the following non-limiting Examples.

Examples

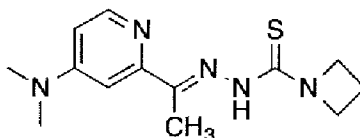
Example 1



(*E*)-*N'*-(1-(Pyrazin-2-yl)ethylidene)azetidine-1-carbothiohydrazide (1) General Method A:

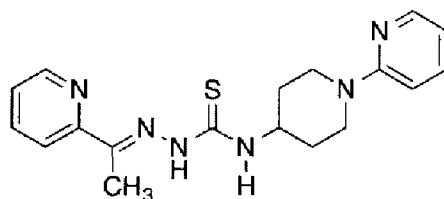
To a solution of azetidine-1-carbothiohydrazide (156 mg, 1.19 mmol, 1.0 eq) and 1-(pyrazin-2-yl)ethan-1-one (152 mg, 1.25 mmol, 1.05 eq) in DCM (6 ml) was added AcOH (4 drops). After stirring overnight at room temperature, the reaction was concentrated under reduced pressure and recrystallized from MeOH to afford **1** as a crystalline white solid (132 mg, 0.56 mmol, 47%). ¹H-NMR (400 MHz, CDCl₃) δ 2.38 (t, J = 7.72 Hz, 1H), 2.42 (t, J = 7.88 Hz, 1H), 4.36 (br. t, J = 7.52 Hz, 1H), 4.73 (br. t, J = 7.40 Hz, 1H), 8.50 (d, J = 2.56 Hz, 1H), 8.53 (m, 1H), 8.78 (s, 1H, NH), 9.13 (m, 1H). MS: 236.1 [M + H]⁺.

Example 2

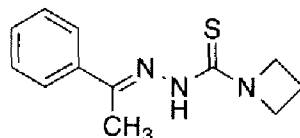


(*E*)-*N'*-(1-(4-(dimethylamino)pyridin-2-yl)ethylidene)azetidine-1-carbothiohydrazide (2):

Following **General Method A** for the condensation of azetidine-1-carbothiohydrazide and 1-(4-(dimethylamino)pyridin-2-yl)ethan-1-one the title compound **2** was isolated as a white solid after recrystallization from MeOH. ¹H-NMR (400 MHz, CDCl₃) δ 2.34 (m, 5H), 3.02 (s, 6H), 4.34 (m, 2H), 4.70 (m, 2H), 6.49 and 6.54 (E/Z dd, J = 6.04 Hz, 2.64 Hz, 1H), 6.62 and 7.08 (E/Z d, J = 2.44 Hz, 1H), 8.26 (m, 1H), 8.71 (br. s, 1H, NH). MS: 278.0 [M + H]⁺.

Example 3**(E)-2-(1-(pyridin-2-yl)ethylidene)-N-(1-(pyridin-2-yl)piperidin-4-yl)hydrazine-1-carbothioamide**

(3): Following **General Method A** for the condensation of *N*-(1-(pyridin-2-yl)piperidin-4-yl)hydrazinecarbothioamide and 1-(pyridin-2-yl)ethan-1-one the title compound **3** was isolated as a white solid after recrystallization from MeOH. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 1.76 (ddd, $J = 15.33$ Hz, 11.72 Hz, 3.92 Hz, 2H), 2.30 (m, 2H), 2.42 (s, 3H), 3.04 (dt, $J = 13.73$ Hz, 2.40 Hz, 2H), 3.72 (m, 2H), 4.57 (m, 1H), 7.16 and 7.19 (E/Z d, 1.56 Hz, 1H), 7.21 and 7.24 (E/Z m, 1H), 7.30 (ddd, $J = 5.87$ Hz, 4.92 Hz, 1.04 Hz), 7.52 (br. d, $J = 8.16$ Hz, 1H, NH), 7.72 (dt, $J = 7.76$ Hz, 1.72 Hz, 1H), 7.90 (d, $J = 8.04$ Hz, 1H), 8.11 (dd, $J = 4.44$ Hz, 1.32 Hz, 1H), 8.35 (d, $J = 2.68$ Hz, 1H), 8.61 (d, $J = 4.12$ Hz, 1H), 8.68 (br. s, 1H, NH). MS: 287.0 $[\text{M} + \text{H}]^+$.

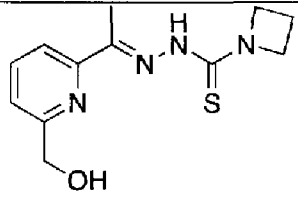
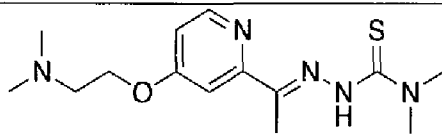
Example 4

(E)-N'-(1-phenylethylidene)azetidine-1-carbothiohydrazide (4): Following **General Method A** for the condensation of azetidine-1-carbothiohydrazide and acetophenone, the title compound **4** was isolated as a white solid after recrystallization from MeOH. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 2.33 (t, $J = 7.80$ Hz, 1H), 2.37 (t, $J = 7.88$ Hz, 1H), 4.33 (m, 2H), 4.69 (m, 2H), 7.37 (m, 3H), 7.65 (m, 2H), 8.63 (br. s, 1H, NH). MS: 234.1 $[\text{M} + \text{H}]^+$.

Examples 5 and 6

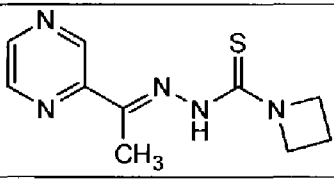
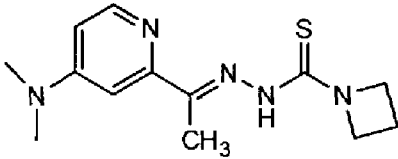
The compounds of Examples 4 and 5 were prepared using **General Method A**. The structures, names, NMR data and mass spectral data for the compounds of Examples 5 and 6 are shown in Table 1.

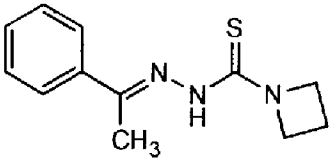
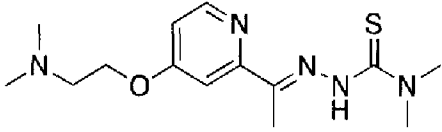
Table 1

Ex	Structure and Name	¹ H NMR MS
5	 (E)-N'-(1-(6-(hydroxyl-methyl)pyridin-2-yl)ethylidene)azetidine-1-carbothiohydrazide	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ 10.16 (s, 1H), 7.78 (dt, <i>J</i> = 15.7, 8.0 Hz, 2H), 7.43 (d, <i>J</i> = 6.9 Hz, 1H), 5.41 (t, <i>J</i> = 5.9 Hz, 1H), 4.56 (d, <i>J</i> = 5.8 Hz, 4H), 4.10 (s, 2H), 2.31 (s, 3H), 2.27 – 2.18 (m, 2H). (MS+H) ⁺ 265
6	 mixture of diastereomers (E)-2-(1-(4-(2-(dimethylamino)ethoxy)-pyridin-2-yl)ethylidene)-N,N-dimethylhydrazine-1-carbothioamide	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ 9.61 (bs, 1H), 8.43 (d, <i>J</i> = 5.7 Hz, 1H), 7.57 (s, 1H), 7.07 - 6.98 (m, 1H), 4.32 - 4.22 (m, 2H), 3.32 (s, 6H), 2.94 (bs, 2H), 2.62 (s, 3H), 2.42 (s, 6H). (MS+H) ⁺ 310.05

Cell-based TOV112D activity for representative compounds is shown in Table 2.

Table 2.

Example	Structure	TOV112D IC ₅₀ [*]
1		++
2		+++

4		+
6	 mixture of diastereomers	+

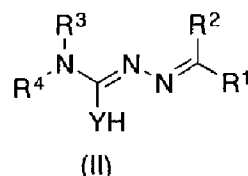
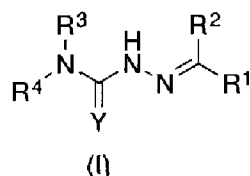
* +++, most active; ++, moderately active; +, less active, - no measurable activity

All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

Claims

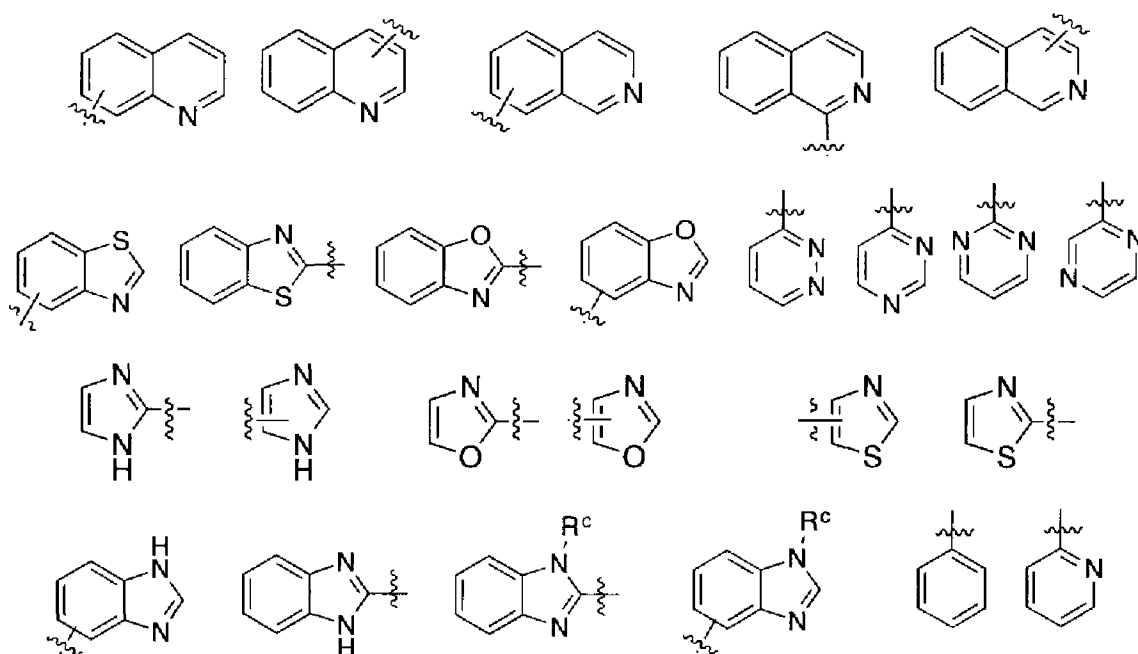
What is claimed is:

1. A compound selected from compounds of formulas (I) or (II):



and salts thereof, wherein:

R^1 is selected from the group consisting of:



wherein R^1 is optionally substituted with one or more groups independently selected from halo, cyano, hydroxy, nitro, $-N(R^a)_2$, carboxy, phenyl, (C_1-C_6) alkyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, (C_3-C_6) cycloalkyl, (C_1-C_6) alkoxy, (C_1-C_6) alkanoyl, (C_1-C_6) alkoxycarbonyl, (C_2-C_6) alkanoyloxy, (C_4-C_6) heterocycloalkyl, (C_2-C_6) alkylaminocarbonyl and (C_2-C_6) alkanoylamino wherein any phenyl, (C_1-C_6) alkyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, (C_1-C_6) alkoxy, and (C_3-C_6) cycloalkyl, is optionally substituted with one or more groups independently selected from halo, cyano, hydroxy, nitro, $-N(R^a)_2$, carboxy, (C_3-C_6) cycloalkyl, (C_1-C_6) alkoxy, (C_1-C_6) alkanoyl, (C_1-C_6) alkoxycarbonyl, (C_2-C_6) alkanoyloxy, (C_4-C_6) heterocycloalkyl, (C_2-C_6) alkylaminocarbonyl and (C_2-C_6) alkanoylamino;

R^2 is selected from the group consisting of H, phenyl, heteroaryl, (C_1-C_6) alkyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, and (C_3-C_6) cycloalkyl, wherein any phenyl, heteroaryl, $(C_1-$

C₆)alkyl, (C₂-C₆)alkenyl, (C₂-C₆)alkynyl, and (C₃-C₆)cycloalkyl is optionally substituted with one or more groups independently selected from halo, -N(R^b)₂, (C₃-C₆)cycloalkyl, (C₁-C₆)alkoxy, (C₂-C₆)alkanoyloxy, (C₂-C₆)alkoxycarbonyl, (C₂-C₆) alkylaminocarbonyl, and (C₂-C₆) alkanoylamino;

provided that if R¹ is 2-pyridinyl, then R² is not H or (C₁-C₆)alkyl;

Y is S, O, Se;

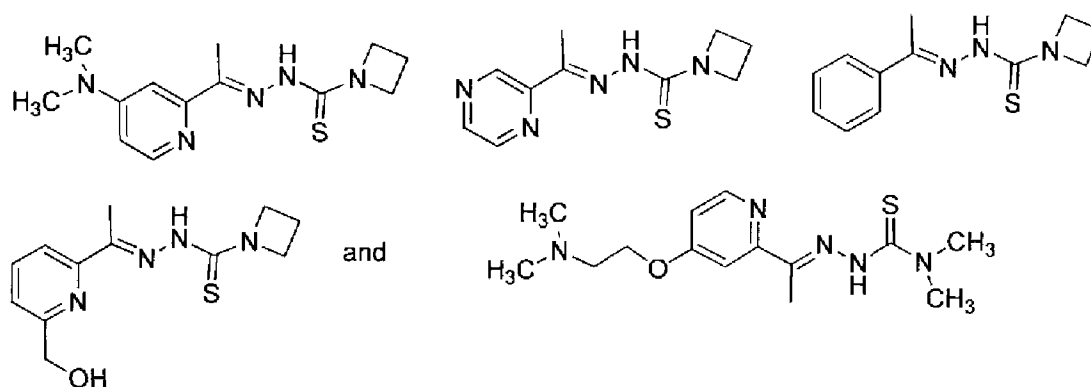
R³ and R⁴ are each independently selected from H, (C₁-C₆)alkyl, piperidinyl, or piperazinyl, which piperidinyl or piperazinyl is optionally substituted with pyridyl; or R³ and each R⁴ taken together with the nitrogen to which they are attached form a 3, 4, 5, 6, 7, 8, or 9 membered ring that is optionally substituted with one or more groups independently selected from the group consisting of halo;

each R^a is independently selected from the group consisting of H, (C₁-C₆)alkyl, (C₂-C₆)alkenyl, (C₂-C₆)alkynyl, (C₃-C₆)cycloalkyl, (C₁-C₆)alkanoyl, and (C₁-C₆)alkoxycarbonyl, wherein any (C₁-C₆)alkyl, (C₂-C₆)alkenyl, (C₂-C₆)alkynyl, (C₃-C₆)cycloalkyl, (C₁-C₆)alkanoyl, and (C₁-C₆)alkoxycarbonyl, is optionally substituted with one or more groups independently selected from halo, (C₃-C₆)cycloalkyl, and (C₁-C₆)alkoxy; or two R^a taken together with the nitrogen to which they are attached form a azetidino, pyrrolidino, piperidino, or morpholino ring;

each R^b is independently selected from the group consisting of H, (C₁-C₆)alkyl, (C₂-C₆)alkenyl, (C₂-C₆)alkynyl, (C₃-C₆)cycloalkyl, (C₁-C₆)alkanoyl, and (C₁-C₆)alkoxycarbonyl, wherein any (C₁-C₆)alkyl, (C₂-C₆)alkenyl, (C₂-C₆)alkynyl, (C₃-C₆)cycloalkyl, (C₁-C₆)alkanoyl, and (C₁-C₆)alkoxycarbonyl, is optionally substituted with one or more groups independently selected from halo, (C₃-C₆)cycloalkyl, and (C₁-C₆)alkoxy; or two R^b taken together with the nitrogen to which they are attached form a azetidino, pyrrolidino, piperidino, or morpholino ring; and

each R^c is independently selected from the group consisting of H and (C₁-C₆)alkyl that is optionally substituted with one or more groups independently selected from halo, (C₃-C₆)cycloalkyl, and (C₁-C₆)alkoxy.

2. A compound selected from the group consisting of:



and salts thereof.

3. A pharmaceutical composition, comprising, a compound of claim 1 or 2 or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.
4. An injectable pharmaceutical formulation comprising, a compound of claim 1 or 2 or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.
5. A method of inhibiting cancer cell growth *in vivo* or *in vitro*, comprising contacting a cancer cell with a compound of any one of claims 1-2 or a pharmaceutically acceptable salt thereof.
6. A method of treating cancer in an animal comprising administering a compound of any one of claims 1-2 or a pharmaceutically acceptable salt thereof to the animal.
7. The method of claim 6, further comprising administering zinc to the animal.
8. The method of any one of claims 5-7, wherein the cancer is caused by mutations affecting zinc binding proteins.
9. The method of any one of claims 5-8, wherein the cancer is associated with a zinc binding p53 mutation.
10. The method of any one of claims 5-9, wherein the cancer is associated with a zinc binding p53 mutation selected from R175, C176, H179, C238, C242, and G245.

11. A compound of any one of claims 1-2 or a pharmaceutically acceptable salt thereof, for use in medical treatment.
12. A compound of any one of claims 1-2 or a pharmaceutically acceptable salt thereof, for the prophylactic or therapeutic treatment of cancer.
13. The compound or pharmaceutically acceptable salt of claim 12 wherein the cancer is caused by mutations affecting zinc binding proteins.
14. The compound or pharmaceutically acceptable salt of claim 13, wherein the cancer is associated with a zinc binding p53 mutation.
15. The compound or pharmaceutically acceptable salt of claim 12, wherein the cancer is associated with a zinc binding p53 mutation selected from R175, C176, H179, C238, C242, and G245.
16. The use of a compound of any one of claims 1-2 or a pharmaceutically acceptable salt thereof, to prepare a medicament for treating cancer in an animal.
17. The use of claim 16 wherein the cancer is caused by mutations affecting zinc binding proteins.
18. The use of claim 16, wherein the cancer is associated with a zinc binding p53 mutation.
19. The use of claim 16, wherein the cancer is associated with a zinc binding p53 mutation selected from R175, C176, H179, C238, C242, and G245.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/015177

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D403/12 C07D401/14 C07D401/12 C07D205/04 C07D213/68
A61K31/4427 A61K31/44 A61K31/397 A61K31/50 A61P35/00

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D A61K A61P

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

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

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KRISHNA C. AGRAWAL ET AL: "Potential antitumor agents. 13. 4-Methyl-5-amino-1-formylisoquinoline thiosemicarbazone", JOURNAL OF MEDICINAL CHEMISTRY, vol. 19, no. 7, 1 July 1976 (1976-07-01), pages 970-972, XP055258301, US ISSN: 0022-2623, DOI: 10.1021/jm00229a023 abstract page 971; compound 8 page 971; table 1 ----- -/--	1-19



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search

16 March 2016

Date of mailing of the international search report

24/03/2016

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Marzi, Elena

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/015177

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IPPOLITO ANTONINI ET AL: "Elucidation of the structure of the antineoplastic agents, 2-formylpyridine and 1-formylisoquinoline thiosemicarbazones", JOURNAL OF MEDICINAL CHEMISTRY, vol. 20, no. 3, 1 March 1977 (1977-03-01), pages 447-449, XP055258307, US ISSN: 0022-2623, DOI: 10.1021/jm00213a024 abstract page 448; compounds (E)-IQ-1, (Z)-IQ-1 -----	1-19
X	DES R. RICHARDSON ET AL: "Dipyridyl Thiosemicarbazone Chelators with Potent and Selective Antitumor Activity Form Iron Complexes with Redox Activity", JOURNAL OF MEDICINAL CHEMISTRY, vol. 49, no. 22, 1 November 2006 (2006-11-01), pages 6510-6521, XP055258311, US ISSN: 0022-2623, DOI: 10.1021/jm0606342 abstract page 6511; figure 1; compounds HDpT, HDp4mT, HDp4eT, HDp44mT page 6513; table 3 Biological studies; page 6519 -----	1-19
X	HE HUANG ET AL: "A Series of [alpha]-Heterocyclic Carboxaldehyde Thiosemicarbazones Inhibit Topoisomerase II[alpha] Catalytic Activity", JOURNAL OF MEDICINAL CHEMISTRY, vol. 53, no. 8, 22 April 2010 (2010-04-22), pages 3048-3064, XP055258314, US ISSN: 0022-2623, DOI: 10.1021/jm9014394 abstract compound HDp44mT page 3049, paragraph 2.3 - page 3050, paragraph 2.10 page 3051; table 1; compounds TSC23, TSC24, TSC30, TSC31 page 3053, paragraph 3.3 - page 3060, paragraph 4.3 -----	1,3-6, 11-19
X	WO 2012/175962 A1 (MEDICAL RES COUNCIL [GB]; BOECKLER FRANK [DE]; WILCKEN RAINER [DE]; FE) 27 December 2012 (2012-12-27) page 136; compound 878 claims 1, 59-66 page 66, line 34 - page 78, line 6 ----- -/--	1-19

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/015177

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANNA MROZEK-WILCZKIEWICZ ET AL: "Iron Chelators in Photodynamic Therapy Revisited: Synergistic Effect by Novel Highly Active Thiosemicarbazones", ACS MEDICINAL CHEMISTRY LETTERS, vol. 5, no. 4, 10 April 2014 (2014-04-10), pages 336-339, XP055258567, United States ISSN: 1948-5875, DOI: 10.1021/ml400422a abstract page 337; figure 1; compounds 1-4 page 338; table 1 -----	1-19
X	DANUTA S KALINOWSKI ET AL: "Design, Synthesis, and Characterization of Novel Iron Chelators: Structure-Activity Relationships of the 2-Benzoylpyridine Thiosemicarbazone Series and Their 3-Nitrobenzoyl Analogues as Potent Antitumor Agents", JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 50, no. 15, 26 July 2007 (2007-07-26), pages 3716-3729, XP002667595, ISSN: 0022-2623, DOI: 10.1021/JM070445Z [retrieved on 2007-06-30] abstract DpT, Dp4mT, Dp44mT, Dp4eT, BpT, Bp4mT, B44mT, Bp4eT, NBpT, NB4mT, NB44mT, NB4eT; page 3717; table 1 page 3719; table 2 -----	1-19
X	WO 2006/019955 A2 (HARVARD COLLEGE [US]; COEN DONALD M [US]; LOREGIAN ARIANNA [US]) 23 February 2006 (2006-02-23) page 31, lines 31-33 page 32, lines 9-11 figure 2; compounds A114, A120 tables 1-2 page 42, line 12 - page 43, line 24 -----	1-4
X	US 4 665 173 A (KLAYMAN DANIEL L [US] ET AL) 12 May 1987 (1987-05-12) claims 1-70 -----	1-19

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2016/015177

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012175962	A1	27-12-2012	NONE

WO 2006019955	A2	23-02-2006	US 2007225310 A1 27-09-2007
			WO 2006019955 A2 23-02-2006

US 4665173	A	12-05-1987	NONE
