Title: METAL CONJUGATES OF INDOLE 3-ALDEHYDE AZINE AND ITS DERIVATIVES AND USES THEREOF

Abstract: Provided are compounds which are metal complexes of indole 3-aldehyde azine and its derivatives, as well as pharmaceutical compositions containing the compounds. Also provided are methods of use of these compounds for preventing or treating a disease such as cancer, caused by one or more conditions such as enhanced angiogenesis, uncontrolled cell proliferation and lack of apoptosis in an animal including human. The method comprises administering to the animal an effective amount of a metal complex of indole 3-aldehyde azine or a metal complex of any of its derivatives.
Metal Conjugates of Indole 3-Aldehyde Azine and its Derivatives and Uses Thereof

Field of Invention

The invention relates to the metal conjugates of the indole 3-aldehyde azine and its derivatives and their use in the treatment of diseases or conditions including cancers, as well as diseases associated with conditions including enhanced angiogenesis, uncontrolled cell proliferation and lack of apoptosis.

Background of Invention

Cancer is a disease involving multiple mechanisms. The progression of cancer involves factors such as cell proliferation, apoptosis and angiogenesis. Several biochemical pathways control these factors, and many of these pathways include kinases. The modulation of kinase activity is associated with several types of cancers, and kinase inhibitors have been developed as anticancer agents.

There is a great need to find effective drugs for variety of high prevalence cancers, including breast cancer, prostrate cancer, colon cancer as well as complicated cancers such as the pancreatic cancers. In the United States, the prevalence of these cancer types is: breast-2,369,036; prostate- 1,937,798; and pancreas- 27,688. The incidences reported in 2008 in the United States for these cancers are: breast- 184,450; prostate- 186,320; Colon- 108,070; pancreas- 37,680 (American Cancer Society. Cancer Facts & Figures 2008. Atlanta: American Cancer Society; 2008).

Among the aforementioned cancer types, patients with pancreatic cancer have a high mortality rate. Treatment of pancreatic cancer is rarely successful because this disease has usually metastasized widely by the time it is diagnosed. Therapy consists of surgery and possibly radiation and chemotherapy. The drugs that are currently used for pancreatic cancer patients include gemcitabine as monotherapy, and in combination with erlotinib. There are several clinical trials underway for pancreatic cancer using various drug combinations.

The mechanisms involved in the progression of cancer are mainly cell proliferation, lack of cell apoptosis, and enhanced angiogenesis. Among the key pathways are those controlling cell proliferation, which coordinate a response to the cellular environment, with the mTOR kinase as a critical node, tumor development is influenced by infections and inflammation, and the complex role of the nuclear factor-κB transcription factors has been
reported (M. Karin and F. R. Greten, Nature Reviews Immunology, 5, 749-759 (2005)). Expansion of tumor cells depends on nutrient supply and vascularization, which is orchestrated by the transcription factor known as hypoxia-inducible factor (HIF). The metastatic spread of primary tumors to other organs is facilitated by many signalling pathways. On the other hand, apoptosis (programmed cell death) is triggered by a variety of stimuli, including cell surface receptors like FAS, mitochondrial response to stress, and cytotoxic T cells. There are several pathways through which cell apoptosis can happen. Some of the signaling pathways that are known to involve in apoptosis are: Protein Kinase B (PKB/AKT) Signaling Pathway, Apoptotic DNA fragmentation and tissue homeostasis, Apoptotic Signaling in Response to DNA Damage, Ataxia Telangiectasia-Mutated gene (ATM) Signaling Pathway, Caspase Cascade in Apoptosis, D4-GDI Signaling Pathway, p53 Signaling Pathway. Angiogenesis is a very complex, tightly regulated, multi-step process. Vascular endothelial growth factor (VEGF) plays a key role in physiological blood vessel formation and pathological angiogenesis such as tumor growth and ischemic diseases. Hypoxia is a potent inducer of VEGF in vitro. The secreted VEGF is a major angiogenic factor that regulates multiple endothelial cell functions, including mitogenesis. Cellular and circulating levels of VEGF are elevated in hematologic malignancies and are adversely associated with prognosis. VEGF inhibitors and antibodies have shown anticancer activities indicating that controlling angiogenesis can be a critical therapeutic option.

One of the most relevant pathway involved in most of the cancer progression mechanisms is EGFR/PI3K/Akt/NF-κB pathway. In most of the aforementioned cancers it has been shown that there is an over-expression of epidermal growth factor receptor (EGFR) leading to activation of the Akt and NF-κB signalling pathways, suggesting that these pathways are important targets. It has been shown that Akt can inhibit death by apoptosis induced by various stimuli in a certain number of cell types and in tumor cells. In accordance with these findings, it has been shown that Akt can, via phosphorylation of given serine residues, inactivate BAD, GSK3β-caspase-9 and Forkhead transcription factor, and activate IKKalpha and e-NOS. Various experimental data suggest that the activation of EGFR leads to the activation of Akt which in turn activates NF-κB and, hence, strategies to disrupt this pathway or down regulate EGFR/NF-κB may be useful for achieving maximal therapeutic response in these cancers (M.M. Hill, B.A. Hemmings, Pharmacology & Therapeutics 93, 243-251 (2002)).
Epidemiological surveys have provided evidence that consumption of certain phytochemicals through diets/specific foods is associated with reduced risk of several types of cancers (Ghaneh et al., J. Hepatobiliary Pancreat Surg., 9: 1-11, 2002; Lee et al., Cancer Epidemiol Biomarkers Prent., 12: 665-668, 2003; Mukhtar et al., Toxicol, ScL, 52: 111-117, 1999). These phytochemicals generally act as competitive inhibitors of ATP and/or non-competitive inhibitors with substrate molecules. However, they are of little use in themselves, as they are broad range inhibitors and are effective only when used at high concentrations. However, they can prove to be valuable as models in designing synthetic molecules that can disrupt the phosphorylation reactions as well as signal transduction processes. Thus, synthetic manipulations of certain phytochemicals may be beneficial for evolving highly efficient and selective therapeutic agents, particularly those targeting specific proteins in signal transduction processes.

Indole 3-carbaldehyde (I3C) is an important phyto-chemical found in many vegetables such as broccoli and cabbage (B.B. Aggarwal, S. Shishodia, Biochemical Pharmacology 71 1397-1421, 2006). I3C is known to induce a G(I) cell-cycle arrest in the cells of human lymph node carcinoma of prostate (LNCaP). I3C represses AR expression and responsiveness in LNCaP cells as a part of its antiproliferative mechanism (M.M.R. Bhuiyan, et al. Cancer Res 66, 10064-10072, 2006). Translocation of Bax followed by mitochondrial depolarization and cytochrome c release is responsible for anti breast cancer activity of I3C. I3C also functions as an inhibitor of Akt and nuclear factor kB (NF-kB), which play important roles in cell survival (L.M. Howells, et. al. Molecular Cancer Therapeutics, 1, 1161-1172, 2002). Several synthetic anticancer compounds have been derived from I3C and its active metabolite 3,3'-diindolylmethane (DIM). The cyclization of methylene part of DIM leading to indolo [3,2b] carbazole and corresponding derivatives are reported for antitumor activity (US 6800655). More compounds have been reported where methylene part of DIM converted to ketone and corresponding derivatives have shown anticancer activity (US 2006/021 1759).

Some compounds are reported that have metal-complexes of substituted bis-indole derivatives with indoles being linked by alkylenic group, as MRI contrasting agents (WO 2002/038546). Metal complexes of formula ML2X2 are reported as antibacterial agents with M being Cu(II), Co(II) and Ni(II), L being indole 2-carbaldehyde and X being nitrate and acetate (Chohan, Z.H. and Sherazi, S. K.A., Metal Based Drugs, 4(6), 327-332 , 1997). However, the ligand used in this work is not in a bis form.
Synthesis and spectral analysis of azines is reported in several articles (Bulatova, N.N. and Suvorov, N.N., Khimiya Geterotsiklicheskikh Soedinenii, 5, 813-817 (1969) and Journal Chemistry of Heterocyclic Compounds, 5, 602-604 (1969); El-Rayyes et al., Journal of Chemical and Engineering Data, 28, 132-4 (1983); M.R. Rizal, H.M. Ali and S.W. Ng Acta Cryst. E64, o555, (2008)). Some azines have shown antipsychotic activity by inhibition of monoamine oxidase (Anales de Quimica (1968-1979) (1970), 66(7-8), 681-8 ), whereas anti-tubercular testing of 30 azines showed no activity (Fujikawa, F. et al., Yakugaku Zasshi, 90(1), 78-82 (1970)). Further, some azines like 4,4′-diacetylaminobenzalazine have demonstrated moderate anti-tumor activity (Hirayama et al., Yakugaku Zasshi, 100(12), 1225-34 (Japanese) 1980) in animal models of Ehrlich carcinoma, however azine of 3-indolyl does not show any anti-tumor activity.

Summary of Invention

In one embodiment, metal conjugates of compounds corresponding to the following structure, and derivatives thereof, are provided.

![Chemical structure](image)

wherein Ri is selected from hydrogen, (C₁-Ce) alkyl, (C₃-C₇) cycloalkyl, or phenyl, -CO-R¹ with R' being (C₁-C₆) alkyl, (C₃-C₇) cycloalkyl, phenyl or substituted phenyl group, halogens like F, Cl, Br, I; acyl, acyloxy, C₂-C₈ alkoxycarbonyl, halocarbonyl, C₂-C₈ alkylcarbonato, carboxy, carboxylato;
wherein R₂ is selected from hydrogen, (C₁-C₆) alkyl; (C₃-C₇) cycloalkyl; or phenyl, -CO-R¹ with R' being (C₁-C₆) alkyl, (C₃-C₇) cycloalkyl, or phenyl group; -OH, -OR' (alkoxy), -NH₂, -NR₁'R₂' with R¹ being (C₁-C₆) alkyl groups;

wherein R₃, R₄, R₅ and R₆ are each independently selected from hydrogen, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₆ alkynyl, phenyl, substituted phenyl, halo, hydroxyl, sulfhydryl, haloalkyl, C₁-C₈ alkoxy, C₂-C₈ alkenyloxy, C₂-C₈ alkynloxy, C₂-C₈ acyloxy, C₂-C₈ alkoxycarbonyl, halocarbonyl, C₂-C₈ alkylcarbonato, carboxy, carboxylato, carbamoyl, mono-(C₁-C₈ alkyl)-substituted carbamoyl, di-(C₁-C₈ alkyl)-substituted carbamoyl, thiocarbamoyl, carbamido, cyano, isocyano, cyanato, isocyanato, isothiocyanato, azido, formyl, thioformyl, amino, mono- and di-(C₁-C₈ alkyl)-substituted amino, C₂-C₈ alkylamido, imino, alkylimino, arylimino, nitro, nitroso, sulfo, sulfonato, C₁-C₈ alkylsulfanyl, C₁-C₈ alkylsulfinyl, C₁-C₈ alkylsulfonyl, phosphono, phosphonato, phosphinito, phospho, phosphino, and combinations thereof.

In another embodiment, a composition is provided comprising a pharmaceutically acceptable diluent, carrier or excipient and a compound as described above.

In another embodiment, a method is provided for preventing or treating a disease, or a condition that predisposes to a disease, wherein the disease or condition is associated with any of the conditions like excess cell proliferation, reduced apoptosis and induction of angiogenesis in an animal. The method comprises administering to the animal a preventive or treatment effective amount of a compound as described above.

In yet another embodiment, a method is provided for increasing apoptosis of an animal cell comprising contacting the cell with a compound as described above.

Other methods, features and advantages of the present invention will be or become apparent to one with skill in the art upon examination of the following detailed descriptions. It is intended that all such additional methods, features and advantages be included within this description, be within the scope of the present invention, and be protected by the accompanying claims.

**Detailed Description of Invention**

Before the present compositions and methods are described, it is to be understood that the invention is not limited to the particular methodologies, protocols, assays, and reagents described, as these may vary. It is also to be understood that the terminology used herein is intended to describe particular embodiments of the present invention, and is in no way intended to limit the scope of the present invention as set forth in the appended claims.
It must be noted that as used herein and in the appended claims, the singular forms
"a," "an," and "the" include plural references unless the context clearly dictates otherwise.

Unless defined otherwise, all technical and scientific terms used herein have the same
meanings as commonly understood by one of ordinary skill in the art to which this invention
belongs. All publications cited herein are incorporated herein by reference in their entirety
for the purpose of describing and disclosing the methodologies, reagents, and tools reported
in the publications that might be used in connection with the invention. Nothing herein is to
be construed as an admission that the invention is not entitled to antedate such disclosure by
virtue of prior invention.

The terms "disorder," "disease," and "condition" are used inclusively and refer to any
status deviating from normal.

The terms "treating", and "treatment", and the like are used herein to generally mean
obtaining a desired pharmacological and physiological effect. More specifically, the
compounds and compositions described herein may have an effect on conditions associated
with the diseases states discussed herein including cell proliferation, apoptosis and
angiogenesis. The term "treatment", as used herein, covers any treatment of a disease in a
mammal, particularly a human.

The term "efficacy" and "effective amount" as used herein refers to the effectiveness
of a particular treatment regime. For example, with regard to cancer, efficacy can be
measured based on such characteristics (but not limited to these) as inhibition of tumor
growth, reduction of tumor mass, reduction of metastatic lesions as assessed, for example, by
radiologic imaging, slowed tumor growth, lack of detectable tumor associated antigens, and
the like. Additional methods of assessing tumor progression are discussed herein and would
be known to the treating and diagnosing physicians.

The term "cancer" refers to any disease characterized by uncontrolled, abnormal
growth of cells. The term "cancer" may include both benign and malignant growths and can
include both tumors and cells. Preferably, the tumor is malignant. The tumor can be a solid
tissue tumor such as a melanoma, or a soft tissue tumor such as a lymphoma, a leukemia, or a
bone cancer.

The term "alkyl" refers to saturated, monovalent hydrocarbon radicals having straight
or branched chains containing 1 to 8 carbon atoms.
The term "cycloalkyl" refers to cyclic ring-containing hydrocarbon moieties containing 3 to 7 carbon atoms.

The term "alkoxy" refers to O-alkyl groups.

The term "substituted phenyl" refers to phenyl group substituted at 2, 3 or 4 positions with groups such as hydrogen, C1-C8 alkyl, C2-C8 alkenyl, C2-C6 alkynyl, halo, hydroxyl, sulfhydryl, haloalkyl, C1-C8 alkoxy, C2-C8 alkenyloxy, C2-C8 alkynyloxy, acyl, acyloxy, C2-C8 alkoxy carbonyl, halocarbonyl, C2-C8 alkylcarbonato, carboxy, carboxylato, carbamoyl, mono-(Cl-C8 alkyl)-substituted carbamoyl, di-(Cl-C8 alkyl)-substituted carbamoyl, thiocarbamoyl, carbamido, cyano, isocyano, cyanato, isocyanatō, isothiocyanato, azido, formyl, thioformyl, amino, mono- and di-(Cl-C8 alkyl)-substituted amino, C2-C8 alkylamido, imino, alkylimino, arylmino, nitro, nitroso, sulfo, sulfonato, C1-C8 alkylsulfanyJ, C1-C8 alkylsulfinyl, C1-C8 alkylsulfonyl, phosphono, phosphonato, phosphinato, phospho, phosphino, or combinations thereof.

The term "aryloxy" refers to O-aryl groups, where aryl corresponds to phenyl and substituted phenyl groups.

The term "haloalkyl" refers to alkyl groups further bearing one or more halogen substituents, e.g., -CH₂Cl, -CH₂CH₂Cl.

The term "amino" refers to the group -NRR', wherein R and R¹ may be H or (C1-C6) alkyl group.

Compounds of the present invention are metal conjugates of indole 3-aldehyde azine compounds (also known as bis indole 3-aldehyde hydrazone) and derivatives thereof.
corresponding to Formula (I):

wherein $R_i$ is selected from hydrogen, (C$_1$-C$_6$) alkyl, (C$_3$-C$_7$) cycloalkyl, or phenyl, -CO-$R'$ with $R'$ being (C$_1$-C$_6$) alkyl, (C$_3$-C$_7$) cycloalkyl, phenyl or substituted phenyl group, halogens selected from F, Cl, Br, I; acyl, acyloxy, C$_2$-C$_8$ alkoxy carbonyl, halocarbonyl, C$_2$-C$_8$ alkyl carbonato, carboxy, carboxylato;

wherein $R_2$ is selected from hydrogen, (C)-C$_6$ alkyl; (C$_3$-$C_7$) cycloalkyl; or phenyl, -CO-$R^1$ with $R^1$ being (C$_1$-C$_6$) alkyl, (C$_3$-$C_7$) cycloalkyl, or phenyl group; -OH, -OR' (alkoxy), -NH$_2$, -NR$_1$ $R_2'$ with $R_1'$ and $R_2'$ being (C$_1$-$C_6$) alkyl groups;

wherein $R_3$, $R_4$, $R_5$ and $R_6$ are each independently selected from hydrogen, C$_1$-C$_8$ alkyl, C$_2$-C$_8$ alkenyl, C$_2$-C$_6$ alkynyl, phenyl, substituted phenyl, halo, hydroxyl, sulfhydryl, haloalkyl, C$_1$-C$_8$ alkoxy, C$_2$-C$_8$ alkenyloxy, C$_2$-C$_8$ alkynlyoxy, acyl, acyloxy, C$_2$-C$_8$ alkoxy carbonyl, halocarbonyl, C$_2$-C$_8$ alkyl carbonato, carboxy, carboxylato, carbamoyl, mono-(C$_1$-C$_8$ alkyl)-substituted carbamoyl, di-(C$_1$-C$_8$ alkyl)-substituted carbamoyl, thiocarbamoyl, carbamido, cyano, isocyano, cyanato, isocyanato, isothiocyanato, azido, formyl, thioformyl, amino, mono- and di-(C$_1$-C$_8$ alkyl)-substituted amino, C$_2$-C$_8$ alkylamido, imino, alkylimino, arylimino, nitro, nitroso, sulfo, sulfonato, C$_1$-C$_8$ alkylsulfanyl, C$_1$-C$_8$ alkylsulfynyl, C$_1$-C$_8$ alkylsulfonyl, phosphonato, phosphonato, phosphinato, phospho, phosphino, or combinations thereof.

The metal conjugates of these compounds are delivered in pharmaceutically acceptable salts. Metals which can be used in the metal conjugates include, for example, copper, iron, palladium, nickel, gold, zinc, vanadium or platinum.

Exemplary compounds of the present invention include the Cu(II) complex of indole-3-aldehyde azine also known as bis-indole 3-aldehyde hydrazone.

The compounds of the present invention may contain one or more stereocenters. The invention includes all possible diastereomers and all enantiomeric forms as well as all combinations of diastereomers and enantiomers, including racemic mixtures. In addition, compounds of the present invention exist as tautomeric forms and all tautomeric forms are encompassed by the present invention. The compounds can be separated into substantially optically pure compounds.

The compounds of the present invention are useful in inhibiting cell proliferation, inducing apoptosis and/or inhibiting angiogenesis. Thus, in one embodiment, the present invention provides a method for preventing or treating a disease, or a condition that predisposes to a disease, wherein the disease or condition is associated with excess cell
proliferation, reduced apoptosis and enhanced angiogenesis in an animal. The method comprises administering to the animal a preventive or treatment effective amount of a metal conjugate of compound of Formula (I).

Any disease, or condition that predisposes to a disease, which is associated with one or more conditions such as excess cell proliferation, reduced apoptosis and enhanced angiogenesis may be treated according to the methods of the present invention. Exemplary diseases and conditions that predispose to a disease include but are not limited to cancer and precancerous lesions (including breast cancer, lung cancer, ovarian cancer, uterine cancer, brain cancer, sarcoma, melanoma, leukemia, lymphoma, head and neck cancer, colorectal cancer, prostate cancer, pancreatic cancer, and liver cancer); a rheumatologic disease such as rheumatoid arthritis or osteoarthritis; a pulmonary disease such as chronic obstructive pulmonary disease (COPD); an ophthalmic disease such as retinopathy; a cardiovascular disease; a dermatologic disease; a gynecological disease; a vascular disease; a neurologic disease; and/or an infectious disease including a bacterial, viral, retroviral or parasitic disease.

When using a metal conjugate of compound of Formula (I) for preventing or treating a disease, or a condition that predisposes to a disease, an additional compound effective for treating such a disease or condition may be administered with the metal conjugate of compound of Formula (I). The additional compound may be administered before, after, or simultaneously with the compound of Formula (I). For example, in the treatment of cancer, the compound of Formula (I) may be administered before, after, or simultaneously with gemcitabine or a pharmaceutically acceptable salt thereof, 5-fluorouracil, capecitabine, taxotere, erlotinib, gefitinib or cisplatin.

In another embodiment, the present invention relates to a method for increasing apoptosis of an animal cell comprising contacting the cell with a metal conjugate of compound of Formula (I). For example, exposure to a metal conjugate compound of Formula (I) can induce apoptosis in a cancer cell and thereby result in treatment of cancer in a patient in need of such treatment.

The animals and cells in need of the treatment and prevention according to the methods of the present invention are preferably mammals and mammalian cells. The methods can be used in any mammalian species, including human, monkey, cow, sheep, pig, goat, horse, mouse, rat, dog, cat, rabbit, guinea pig, hamster and horse. Preferably, the mammal is human.
The compounds of the present invention can be delivered directly, or in pharmaceutical compositions along with suitable diluents, carriers or excipients, as is well known in the art. For example, a pharmaceutical composition of the invention may include a conventional additive, such as a stabilizer, buffer, salt, preservative, filler, flavor enhancer and the like, as known to those skilled in the art. Exemplary buffers include phosphates, carbonates, citrates and the like. Exemplary preservatives include EDTA, EGTA, BHA, BHT and the like.

An effective amount of such agents can readily be determined by routine experimentation, as can the most effective and convenient route of administration and the most appropriate formulation. Various formulations and drug delivery systems are available in the art. See, e.g., Gennaro, A. R., ed. (1995) Remington's Pharmaceutical Sciences.

Suitable routes of administration may include intavenous infusion, intravenous injection, oral, rectal, transmucosal, transdermal, topical, nasal, or intestinal administration and parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. In addition, the agent or composition thereof may be administered sublingually or via a spray, including a sublingual tablet or a sublingual spray. The agent or composition thereof may be administered in a local or a systemic manner. For example, a suitable agent can be delivered via injection or in a targeted drug delivery system, such as a depot or sustained release formulation. Preferably, the route of administration is intravenous, for example, by infusion or bolus.

The pharmaceutical compositions of the present invention may be manufactured by any of the methods well-known in the art, such as by conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. As noted above, the compositions of the present invention can include one or more physiologically acceptable carriers such as excipients and auxiliaries that facilitate processing of active molecules into preparations for pharmaceutical use.

Proper formulation is dependent upon the route of administration chosen. For example, for injection the composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks’ solution, Ringer’s solution, or physiological saline buffer. For transmucosal or nasal administration, penetrants appropriate to the barrier to be permeated may be used in the formulation. Such penetrants are generally known in the art. For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the
art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject. The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

Compositions formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion can be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with added preservatives as appropriate. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Formulations for parenteral administration may include aqueous solutions or other compositions in water-soluble form.

Suspensions of the active compounds may also be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil and synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

As mentioned above, the compositions of the present invention may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the present compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Suitable carriers for the hydrophobic molecules of the invention are well known in the art, and may include co-solvent systems comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The co-solvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system is effective in dissolving hydrophobic
compounds and produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied. For example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80, the fraction size of polyethylene glycol may be varied, other biocompatible polymers may replace polyethylene glycol, e.g., polyvinyl pyrrolidone, and other sugars or polysaccharides may substitute for dextrose.

Preferably, the present compounds are prepared in a formulation intended for intravenous administration via infusion or bolus injection. For intravenous administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers include non ionic surfactants such as Tween 20, Tween 40 or Tween 80 but not limited to, in the range of 1 to 40 % of volume; co-solvents such as ethanol and propylene glycol in the range of 1 to 20% of volume; vehicles such as Dextrose solution in the range of 1 to 99% and Phosphate buffered saline solution for injection.(B&D) in the range of 1 to 99%.

Alternatively, other delivery systems for hydrophobic molecules may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Liposomal delivery systems are discussed above in the context of gene-delivery systems. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity.

Pharmaceutical preparations’ for oral use can be obtained as solid excipients, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients may include, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; and/or cellulose preparations such as maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Wetting agents such as sodium dodecyl sulfate may be included.

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

Pharmaceutical preparations for oral administration include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in an admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate. Stabilizers may also be included. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

In one embodiment, the compounds of the present invention may be administered transdermally, such as through a skin patch or topically. In one aspect, the transdermal or topical formulations of the present invention can additionally comprise one or multiple penetration enhancers or other effectors, including agents that enhance migration of the delivered compound. Transdermal or topical administration may be preferred in situations in which location specific delivery is desired.

For administration by inhalation, the compounds for use according to the present invention may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide, or any other suitable gas. In the case of a pressurized aerosol, the appropriate dosage unit may be determined by providing a valve to deliver a metered amount. For example, capsules and cartridges of gelatin, for use in an inhaler or insufflator, may be formulated. These typically contain a powder mix of the compound as well as a suitable powder base such as lactose or starch.

Another system of delivery may be simple and rapid method for preparation of protein-drug nano particles using sol-oil chemistry. The protein nano particles of size 20-40 nm, with encapsulation of compound/drug particles attain a dimension of 60-80nm. The protein in nano particles is localized in the cell surface membrane, whereas compound will be released into the cells. The protein in the nano particles is recycled through exocytosis, while compound remain in the cells. This formulation may be used for IV bolus injection. The protein used for such system may be enzyme transferase.
Additionally, the compounds may be delivered using sustained-release systems, such as semi-permeable matrices of solid hydrophobic polymers containing the effective amount of the composition to be administered. Various sustained-release materials are established and available to those of skill in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for stabilization may be employed.

For any composition used in the present methods of treatment, a therapeutically effective dose can be estimated initially using a variety of techniques well known in the art. For example, in a cell culture assay, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC50 as determined in cell culture. Dosage ranges appropriate for human subjects can be determined, for example, using data obtained from cell culture assays and other animal studies.

A therapeutically effective dose of an agent refers to that amount of the agent that results in amelioration of symptoms or a prolongation of survival in a subject. Toxicity and therapeutic efficacy of such molecules can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the ratio LD50/ED50. Agents that exhibit high therapeutic indices are preferred.

Dosages preferably fall within a range of circulating concentrations that includes the ED50 with little or no toxicity. Dosages may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration, and dosage should be chosen, according to methods known in the art, in view of the specifics of a subject's condition.

The amount of agent or composition administered will, of course, be dependent on a variety of factors, including the sex, age, and weight of the subject being treated, the severity of the affliction, the manner of administration, and the judgment of the prescribing physician.

The present compositions may, if desired, be presented in a pack or dispenser device containing one or more unit dosage forms containing the active ingredient. Such a pack or device may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier
may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein, and are specifically contemplated.

Examples

The invention is further understood by reference to the following examples, which are intended to be purely exemplary of the invention. The present invention is not limited in scope by the exemplified embodiments, which are intended as illustrations of single aspects of the invention only. Any methods that are functionally equivalent are within the scope of the invention. Various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications fall within the scope of the appended claims.

Example 1

**Compound 1: Synthesis of Cu(II) complex of indole 3-aldehyde azine**

**General scheme of synthesis**

![Chemical structures]

**Synthesis of 3-beta-Nitropropenyl indole (B)**: A mixture of 7.5 g of indole-3-aldehyde, 0.9 g of ammonium acetate, and 15 ml of nitromethane were boiled with stirring for 10 minutes. The mixture was then diluted with 35 ml of hot ethanol, ImI of water and the mixture was allowed to crystallize.
Synthesis of Indole-3-aldehyde azine (C) Compound 2: 3-beta-nitro propenyl indole was dissolved in minimum quantity of ethanol and added to hydrazine hydrate (25% aqueous solution) in a 2:1 molar ratio. The mixture was magnetically stirred at room temperature for half an hour. The product obtained was filtered, washed and dried in vacuo.

Synthesis of Cu(II) complex of Indole 3-aldehyde azine (Compound 1):
The ligand (2g) was taken in 1-4 dioxane (850 ml) and CuC12.2H2O (2.507 g) in 20 ml methanol at 75°C was added and stirred at same temperature for 1 hr. After cooling to room temperature, greenish yellow complex precipitates out, which was filtered and washed with dioxane and dried in vacuo.

Ligand, indole 3-aldehyde azine (Compound 2) was characterized by IH NMR, 13C NMR, Mass and elemental analysis.

IH NMR (DMSO, D6) δ(ppm): 7.20 (2H, m, H5, H6); 7.47 (1H, d, H7); 7.90 (IH, s, H2); 8.34 (IH, d, H4); 8.90 (IH, s, H8); 11.69 (IH, s, H1).

13C NMR (DMSO, D6): 112.2 (C4, Quaternary); 112.5 (C5); 120.9 (C6); 122.5 (C7); 123.0 (C8); 125.1 (C3, Quaternary); 132.1 (C2); 137.5 (C9, Quaternary); 155.4 (ClO)

Elemental Analysis:
Calculated: C = 75.50%; H = 4.92%; N = 19.57%
Found: C = 74.96%; H = 4.28%; N = 19.71%

IR Spectrum:
(KBr, in cm⁻¹) 3191 (N-H stretching from indole); 1614 (C=N stretching); 1576 (C=N)
The Cu(II) complex of Indole 3-aldehyde azine was characterized by UV, ER, NMR, Mass and elemental analysis.

Cu(II) complex of Indole 3-aldehyde azine (Compound 1) was characterized by

IR spectrum:
(KBr, in cm⁻¹) 3213 (N-H stretching from indole); 1614 (C=N stretching); 1576 (C=N)
IHNM (DMSO, D6): δ (ppm): 7.18 (2H, m, H5, H6); 7.47 (1H, d, H7); 7.91 (IH, s, H2); 8.34 (IH, d, H4); 8.90 (IH, s, H8); 11.72 (IH, s, H1).

Mass Spectrum shows peaks at: 287 (corresponding to MH+ of ligand); 143 (corresponding to fragment after breakage of N-N bond from bis compound) and 115 (product ion peak corresponding to indole).

Elemental Analysis:
Calculated: C = 51.38%; H = 3.35%; N = 13.31%
Found: C = 51.81%; H = 3.90%; N = 15.50%

Example 2

a. Cell growth inhibition by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

COLO 357 cells (K-ras mutated pancreatic cancer) were seeded at a density of 3x10³ cells per well in 96-well microtiter culture plates. After overnight incubation, medium was removed and replaced with fresh medium containing different concentrations of compounds (0-100 µmol/L) diluted from a 10 mmol/L stock. On completion of 48 hours of incubation, 20 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-di phenyltetrazolium bromide (MTT) solution (5 mg/mL in PBS) were added to each well and incubated further for 2 hours. Upon termination, the supernatant was aspirated and the MTT formazan formed by metabolically viable cells was dissolved in 100 µL of isopropanol. The plates were mixed for 30 minutes on a gyratory shaker, and absorbance was measured at 595 nm using a plate reader (TECAN, Durham, NC). A similar protocol was used for other pancreatic cancer cell lines, viz. BxPC3, MiaPaCa2 and PANCl as well as PC3 (prostate cancer) and HCT (colon cancer) cell lines.

b. Cell growth inhibition by cytotoxic agents

Cells were plated as described above and allowed to attach overnight. Various cells were cultured with Compound 1 for 48 hours. The effect of Compound 1 pretreatment on cell viability was examined by the MTT assay method as described above. All the experiments were performed in triplicate and results are expressed as the IC50 value. Similarly, MTT assay of compound 2 which is ligand of Cu-Complex (Compound 1) was determined. The
ligand (Compound 2) shows no anti-tumor activity. This is in accordance with the earlier report (Hirayama et al., Yakugaku Zasshi, 100(12), 1225-34 (Japanese) 1980).

Table 1: MTT assay on pancreatic, prostate and colon cancer cell lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>MiaPaCa2</th>
<th>PANC1</th>
<th>BxPC3</th>
<th>COLO357</th>
<th>HCT116</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 1</td>
<td>12.7 ± 0.61</td>
<td>15.4 ± 0.37</td>
<td>17.4 ± 0.24</td>
<td>17.6 ± 1.32</td>
<td>18.6 ± 0.84</td>
<td>15.9 ± 0.93</td>
</tr>
<tr>
<td>Compound 2</td>
<td>ND</td>
<td>ND</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

ND: Not determined

Example 3
Quantification of apoptosis by ELISA

The Cell Apoptosis ELISA Detection Kit (Roche, Palo Alto, CA) was used to detect apoptosis in COLO357 cells, with different treatments according to the manufacturer’s protocol. Briefly, COLO357 cells were treated with 25 µmol/L of Compound 1 for 72 hours. After treatment, the cytoplasmic histone DNA fragments from COLO 357 cells with different treatments were extracted and bound to immobilized anti-histone antibody.

Subsequently, the peroxidase-conjugated anti-DNA antibody was used for the detection of immobilized histone DNA fragments. After addition of substrate for peroxidase, the spectrophotometric absorbance of the samples was determined using ULTRA Multifunctional Microplate Reader (TECAN) at 405 nm. A similar protocol was used for BxPC3, PC3 and HCT116 cell lines.

Table 2: Apoptosis measures for compounds against control

<table>
<thead>
<tr>
<th>Compound</th>
<th>BxPC3</th>
<th>COLO357</th>
<th>HCT116</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.50</td>
<td>0.75</td>
<td>0.35</td>
<td>0.50</td>
</tr>
<tr>
<td>Compound 1</td>
<td>2.20</td>
<td>1.90</td>
<td>1.60</td>
<td>0.60</td>
</tr>
</tbody>
</table>


Example 4
Colony Formation assay studies

For clonogenic assays, cells were plated in 6-well plates at a density of 250 cells/well, and after 24 h, Compound 1 was added for 72 h. Compound 1 was used at the concentrations corresponding to IC25 values for each drug, derived from the clonogenic assays. After addition of fresh media, cells were cultivated for 7-10 days; colonies (>50 cells) were then fixed in 75% ethanol, stained with Coomasste Blue (Sigma), and counted manually. The combination index for the clonogenic assay was determined as the ratio of the cumulative percentage of cells killed by each drug or signaling inhibitor alone to the percentage of cells killed by the combination. All experiments were performed in duplicate at least three times. The results of colony formation assay are shown in the Table 3.

Table 3: Clonogenic assay on pancreatic, prostate and colon cancer cell lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>Clonogenic Assay IC50 after 48h (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MiaPaCa2</td>
</tr>
<tr>
<td>Compound 1</td>
<td>12.9 ± 4.7</td>
</tr>
</tbody>
</table>

Example 5
Akt assay

Akt activity kit is an ELISA based activity kit that utilizes biotinylated peptide substrate (GRPRTSSFAEG) that is phosphorylated on second serine by Akt1, Akt2, Akt3, SGK and MSK1. Biotinylated Akt substrate and sample containing Akt are incubated in presence of ATP in the wells of a streptavidin coated 96-well plate, which allows for phosphorylation and substrate capture in a single step. The phosphorylated substrate is detected using phosphoserine detection antibody followed by HRP-antibody conjugate and color development with TMB substrate. Sensitivity is increased by addition of ELISA stop solution and relative activity is determined by reading dual absorbance at 450/540 nm or 450/595 nm. Inhibition profiles can be generated based on Akt activity in the presence and absence of test inhibitors.

Compound 1 shows Akt inhibitory activity in the micromolar range.
Example 6

Anti-angiogenic Activity

The anti-angiogenic activity of Compound 1 was tested on in vitro model of angiogenesis, namely, proliferation, migration and tube formation of human umbilical vein endothelial (HUVEC) cells. The inhibitory effects of Compound 1 on HUVEC cells proliferation were measured by cell counting, migration into the scratch wounded area in HUVEC cell monolayers and microvessel tube-like formation on collagen gel. The results showed that Compound 1 significantly inhibited angiogenesis in a dose-dependent form in range of 7.5-25 µM. The results indicated that inhibition of angiogenesis was achieved in a reasonable manner. The data showed that the inhibition of HUVECs growth occurred at higher concentration than the concentrations needed to inhibit cell migration and tube formation.

Example 7

Formulation for Intravenous Infusion

The formulation for intravenous infusion was obtained by dissolving Compound 1 in 5% tween 20 + 5% ethanol + 90% phosphate buffered saline for injection (B&D). The mixture was vortexed vigorously until compound was completely dissolved. Another formulation that was prepared by dissolving Compound 1 in 2% Tween 80 + 4% ethanol + 94% phosphate buffered saline for injection (B&D).

Example 8

Formulation for Intravenous Injection

The formulation for intravenous injection was obtained by dissolving 100 mg of Compound 1 in 2 gm of Tween 80 and 4.0 ml of Normal Saline (phosphate buffered saline) for injection (B&D). The mixture was vortexed vigorously until the compound was completely dissolved.

Example 9

*In-vivo* activity of Compound 1 in pancreatic cancer Xenograft model

The xenograft pancreatic cancer model of mice was created using BxPC3 cell line. 5x10^6 BxPC3 cells were injected subcutaneously, and tumors were allowed to grow up to 50 mm³, which was achieved after 6 days. Treatment was started on seventh day. Three doses of Compound 1 treatment were given on day 7, day 9 and day 11 to the respective groups. The
animals in a particular group were sacrificed when tumor size became approximately 1700 mm³. The tumor volumes were measured using vernier caliper on every alternate day after tumor induction. The weights of animals were monitored every alternate day.

The xenograft studies for pancreatic cancer were conducted using BxPC3 cell lines. Overall six groups were considered for the study with 10 animals in each group. The treatments were given via intravenous infusion for 2 hours using Harvard pump. The vehicle used for infusion was the 5% twin 20 + 5% ethanol + 90% phosphate buffered saline for injection (B&D) in which the Compound 1 was formulated. The study was carried out using three control groups, viz. Control animals with without any treatment, saline control animals having treated only with saline and vehicle control animals treated only with vehicle used for Compound 1 administration. Three groups were used for Compound 1 treatment: 50 mg/kg, 80 mg/kg and 120 mg/kg. The results show significant survival benefit as well as progression free survival for higher doses of Compound 1. As shown in Figures 1 highly reduced tumor volume was seen in animals receiving compound 1 as compared to a vehicle treated control, which did not receive Compound 1. The tumor volume that received compound 1 was always less that half of the control group. The body weights of animals remain unaffected indicating that compound 1 has no adverse effects.

It will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without departing from the spirit and scope of the invention. All such modifications and variations are intended to be included herein within the scope of this disclosure and the present invention and protected by the following claims.
Claims

What is claimed is:

1. A metal conjugate comprising indole 3-aldehyde azine and derivatives thereof represented by Formula (I):

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[D]
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wherein $R_1$ is selected from hydrogen, (C$_1$-C$_6$) alkyl, (C$_3$-C$_7$) cycloalkyl, or phenyl, -CO-$R^1$ or phenyl, -CO-$R^1$ with $R'$ being (C$_1$-C$_6$) alkyl, (C$_3$-C$_7$) cycloalkyl, phenyl or substituted phenyl group, halogens selected from F, Cl, Br, I; acyl, acyloxy, C2-C8 alkoxy carbonyl, halocarbonyl, C2-C8 alkyl carbonato, carboxy, carboxylato;

wherein $R_2$ is selected from hydrogen, (C$_1$-C$_6$) alkyl; (C$_3$-C$_7$) cycloalkyl; or phenyl, -CO-$R^1$ with $R^1$ being (C$_1$-C$_6$) alkyl, (C$_3$-C$_7$) cycloalkyl, or phenyl group; -OH, -OR ($^1$alkoxy), -NH$_2$, -NR$_1$R$_2$ with $R^1$ and $R^2$ being (C$_1$-C$_6$) alkyl groups;

wherein $R_3$, $R_4$, $R_5$ and $R_6$ are each independently selected from hydrogen, C1-C8 alkyl, C2-C8 alkenyl, C2-C6 alkynyl, phenyl, substituted phenyl, halo, hydroxyl, sulphydryl, haloalkyl, C1-C8 alkoxy, C2-C8 alkenyloxy, C2-C8 alkynlyoxy, acyl, acyloxy, C2-C8 alkoxy carbonyl, halocarbonyl, C2-C8 alkyl carbonato, carboxy, carboxylato, carbamoyl, mono-(C2-C8 alkyl)-substituted carbamoyl, di-(C1-C8 alkyl)-substituted carbamoyl, thiocarbamoyl, carbamido, cyano, isocyano, cyanato, isocyanato, isothiocyanato, azido, formyl, thioformyl, amino,
mono- and di-(Cl-C8 alkyl)-substituted amino, C2-C8 alkylamido, imino, alkylimino, arylimino, nitro, nitroso, sulfo, sulfonato, C1-C8 alkylsulfanyl, C1-C8 alkylsulfinyl, C1-C8 alkylsulfonyl, phosphono, phosphonato, phosphinato, phospho, phosphino, or combinations thereof.

2. The metal conjugate of claim 1, wherein the metal is a transition metal having an atomic number selected from the group consisting of 21 to 30; 39 to 48 and 72 to 80.

3. The metal conjugate of claims 1 and 2, wherein the metal is copper, cobalt, iron, palladium, molybdenum, tungsten, manganese, nickel, gold, zinc, vanadium or platinum.

4. The metal conjugate of claim 3, wherein the metal is Copper.

5. The metal conjugate according to claim 1, wherein the metal conjugate is a Cu(II) complex of indole 3-aldehyde azine.

6. A composition comprising the metal conjugate of claim 1 and a pharmaceutically acceptable diluent, carrier or excipient.

7. A composition comprising the metal conjugate of claim 5 and a pharmaceutically acceptable diluent, carrier or excipient.

8. A method of preventing or treating a disease, wherein the disease is associated with a condition selected from the group consisting of enhanced angiogenesis, uncontrolled cell proliferation, lack of apoptosis in an animal, and combinations thereof, wherein the method comprising administering to the animal in a suitable route of administration a preventive or treatment effective amount of a metal conjugate comprising indole 3-aldehyde azine and derivatives thereof represented by Formula (I):
wherein R₁ is selected from hydrogen, (C₁-C₆) alkyl, (C₃-C₇) cycloalkyl, or phenyl, -CO-R' with R' being (C₁-C₆) alkyl, (C₃-C₇) cycloalkyl, phenyl or substituted phenyl group, halogens selected from F, Cl, Br, I; acyl, acyloxy, C₂-C₈ alkoxy carbonyl, halocarbonyl, C₂-C₈ alkyl carbonato, carboxy, carboxylato;

wherein R₂ is selected from hydrogen, (C₁-C₆) alkyl; (C₃-C₇) cycloalkyl; or phenyl, -CO-R' with R¹ being (C₁-C₆) alkyl, (C₃-C₇) cycloalkyl, or phenyl group; -OH, -OR' (alkoxy), -NH₂, -NR₁R₂ with R¹ and R² being (C₁-C₆) alkyl groups;

wherein R₃, R₄, R₅ and R₆ are each independently selected from hydrogen, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₆ alkynyl, phenyl, substituted phenyl, halo, hydroxyl, sulfhydryl, haloalkyl, C₁-C₈ alkoxy, C₂-C₈ alkenyloxy, C₂-C₈ alkynyloxy, acyl, acyloxy, C₂-C₈ alkoxy carbonyl, halocarbonyl, C₂-C₈ alkyl carbonato, carboxy, carboxylato, carbamoyl, mono-(C₁-C₈ alkyl)-substituted carbamoyl, di-(C₁-C₈ alkyl)-substituted carbamoyl, thiocarbamoyl, thiocarbamoyl, carboxamido, cyanamido, isocyanamido, isothiocyanamido, azido, formyl, thiocarbonyl, amino, mono- and di-(C₁-C₈ alkyl)-substituted amino, C₂-C₈ alkylamido, imino, alkylimino, arylimino, nitro, nitroso, sulfo, sulfonamido, C₁-C₈ alkyl sulfonylamido, C₁-C₈ alkyl sulfinyl, C₁-C₈ alkyl sulfonyl, phosphono, phosphonamido, phosphinamido, phospho, phosphino, or combinations thereof.

9. The method of claim 8 wherein the animal is a mammal.
10. The method of claim 9 wherein the mammal is a human.

11. The method of claim 8, wherein the disease or condition is a proliferative disease selected from the group consisting of cancers or precancerous lesions, rheumatologic diseases, pulmonary diseases, ophthalmic diseases, cardiovascular diseases, dermatologic diseases, gynecological diseases, vascular diseases, neurologic diseases, and infectious diseases.

12. The method of claim 11, wherein the disease is a cancer or a precancerous lesion.


14. The method of claim 11, wherein the rheumatologic disease is rheumatoid arthritis, osteoarthritis, osteonecrosis, fibromyalgia, lupus osteoporosis, Lyme disease, polymyositis, polyarthritis, Carpal tunnel syndrome, tendonitis, vasculitis, sarcoidosis, scleroderma, psoriatic arthritis, or Paget's disease.

15. The method of claim 11, wherein the pulmonary disease is chronic obstructive pulmonary disease, acute respiratory distress syndrome (ARDS), chronic bronchitis, asthma, emphysema, influenza, histoplasmosis, cystic fibrosis, pneumonia, or pulmonary fibrosis.

16. The method of claim 11, wherein the ophthalmic diseases including retinal disorders such as retinopathy, diabetic retinopathy, age related macular degeneration, or retinitis pigmentosa and keratopathies.

17. The method of claim 11, wherein the infectious disease is a bacterial, viral, retroviral, or parasitic disease.

18. The method of claim 8, wherein the route of administration is selected from the group consisting of intravenous infusion, intravenous bolus injection, oral, rectal, transmucosal,
transdermal, topical, nasal, intestinal, parenteral, sublingual, intracameral, intraocular or via a spray.

19. The method of claim 18, wherein the route of administration is intravenous injection in the form of an infusion or bolus injection.

20. The method of claim 19, wherein the intravenous infusion is for a required time period as per severity of disease and condition of patient.

21. The method of claim 19, wherein the formulation of intravenous administration comprises one or more components from Tween 20 in the range 1 to 40% of volume; Tween 80 in the range of 1 to 40% of volume; ethanol in the range of 1 to 20% of volume; Dextrose solution in the range of 1 to 99%; Phosphate buffered saline solution for Injection (B&G) in the range of 1 to 99%.

22. The method of claim 19, wherein the composition for intravenous administration comprises 1 to 5% Tween 20; 1 to 5% ethanol and 90-99% of phosphate buffered saline solution for injection (B&G).

23. The method of claim 19, wherein the composition for intravenous administration comprises 1 to 5% Tween 80; 1 to 5% ethanol and 90-99% of phosphate buffered saline solution for injection (B&G).

24. A composition comprising an effective amount of metal conjugate according to claim 1 and further comprising an effective amount of alkylating agents, antimetabolites, DNA cutters, DNA binders, spindle poisons, antibodies such as gemcitabine, capecitabine, erlotinib, gefitinib, irinotecan, topotecan, paclitaxel, docetaxel, 5-fluoro uracil, nilutamide, flutamide, avastin, oxaliplatin or cisplatin, or a pharmaceutically acceptable salt thereof.

25. A composition comprising an effective amount of compound according to claim 1 for the treatment of cancer in an animal.

26. The method of claim 25, wherein the animal is a mammal.
27. The method of claim 26, wherein the mammal is a human.

28. A method for the treatment of a cancer in an animal comprising administering to said animal an effective amount of a metal conjugate according to claim 1 before, after or simultaneously with an effective amount of gemcitabine.

29. The method of claim 28, wherein the animal is a mammal.

30. The method of claim 29, wherein the mammal is a human.

31. A method for the treatment of a cancer in an animal comprising administering to said animal an effective amount of a metal conjugate according to claim 1 before, after or simultaneously with an effective amount of 5-Fluorouracil (5-FU).

32. The method of claim 31, wherein the animal is a human.

33. The method of claim 32, wherein the mammal is a human.

34. A method for the treatment of a cancer in an animal comprising administering to said animal a pharmaceutically effective amount of a metal conjugate according to claim 1 before, after or simultaneously with a pharmaceutically effective amount of Docetaxel or Cisplatin.

35. The method of claim 34, wherein the animal is a human.

36. The method of claim 35, wherein the mammal is a human.

37. A dispenser device comprising one or more unit dosage forms containing the composition of claims 6 and 7, and wherein the device comprises a blister pack.

38. The dispenser device of claim 37, further comprising instructions for administration.
Drawing 1 shows tumor volumes of animals in vehicle control group compared to animal groups receiving Compound 1 treatment. The C1-50 mg/kg corresponds to IV dose of Compound 1 of 50 mg/kg given by infusion of 2 hours. Similar data is provided for doses of 80 mg/kg and 120 mg/kg of Compound 1.