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Platinum complexes and methods for inhibiting tumor cell proliferation

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(54) **PLATINUM COMPLEXES AND METHODS FOR INHIBITING TUMOR CELL PROLIFERATION**

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C07F 15/00 (2006.01)

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USPC **514/492**; 514/184; 544/225; 546/2; 548/101; 556/136; 556/137

(58) **Field of Classification Search**
USPC 514/184, 492; 544/225; 546/2; 548/101; 556/136, 137
See application file for complete search history.

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(57) **ABSTRACT**

The subject invention concerns platinum complexes that exhibit antitumor cell and/or antiparasitic activity. The subject invention also concerns the use of platinum complexes of the invention to treat oncological and inflammatory disorders. The platinum complexes of the invention can also be used to treat or prevent infection by a virus or a bacterial or parasitic organism in vivo or in vitro.

1 Claim, No Drawings

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PLATINUM COMPLEXES AND METHODS FOR INHIBITING TUMOR CELL PROLIFERATION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. application Ser. No. 11/703,497, filed Feb. 7, 2007, which is a continuation of U.S. application Ser. No. 11/030,567, filed Jan. 6, 2005, which claims the benefit of U.S. Provisional Application Ser. No. 60/534,575, filed Jan. 6, 2004, the disclosure of each of which is incorporated herein by reference in its entirety, including all tables.

BACKGROUND OF THE INVENTION

Platinum complexes, the prototype of cisplatin, have been widely used as active anticancer agents (Ardizzoni et al., 1999; Nitiss, 2002) in a variety of human tumors, including testicular, ovarian, bladder carcinoma, head and neck, and non-small cell lung cancers. The outcome of treatments with cisplatin and other platinum-containing compounds is

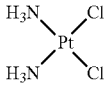
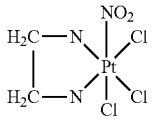
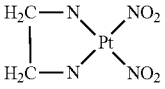
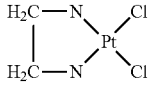
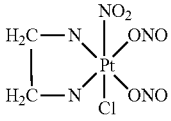
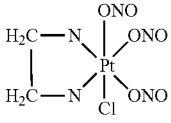
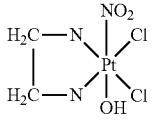
strongly linked to their alkylating effects on DNA. However, the potential impact of platinum-complex-based therapy on cellular signaling and the therapeutic importance of such interactions have yet to be explored. Reports show that cisplatin induces activation of members of the mitogen-activated protein kinase (MAPK) pathways (Persons et al., 1999; Sanchez-Perez et al., 1998), which may influence drug-induced apoptosis.

BRIEF SUMMARY OF THE INVENTION

The subject invention concerns platinum complexes and methods for treating disease conditions, such as cancer and tumors, using platinum complexes of the invention.

DETAILED DISCLOSURE OF THE INVENTION

The subject invention concerns platinum complexes and methods for inducing apoptosis and inhibiting tumor cell growth and for treating animals having tumors, cancers, and oncological disease conditions using the subject platinum complexes. Platinum complexes of the subject invention are shown in the Table below:

Designation	Structure	IC50
CisPt		A549: 9.9 ± 4.0 Calu-1: 8.6 ± 4.5 Panc-1: 4.5 ± 1.9 T-24: 1.3 ± 0.4
CPA-1		A549: 50 ± 23 Calu-1: 26 ± 11 Panc-1: 19 ± 8.1 T-24: 6.2 ± 1.1
CPA-2		A549: >1000 Calu-1: 497 Panc-1: 890 T-24: 560
CPA-3		A549: >250 Calu-1: >250 Panc-1: 94 ± 65 T-24: 23/152/>250
CPA-4 ("dirty mix")		A549: 50 ± 23 Calu-1: 26 ± 11 Panc-1: 19 ± 8.1 T-24: 6.2 ± 1.1
CPA-4 ("clean")	same as above	A549: >250 Calu-1: >250 Panc-1: >250 T-24: >250
CPA-5		A549: 213 Calu-1: 187 Panc-1: >250 T-24: 111
CPA-6		

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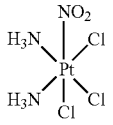
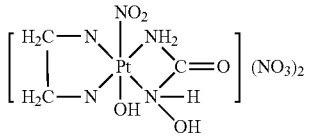
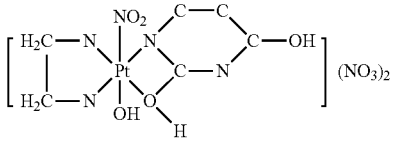
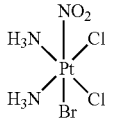
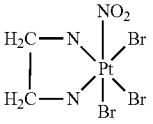
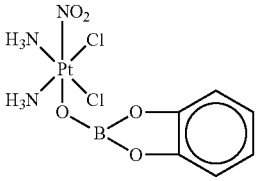
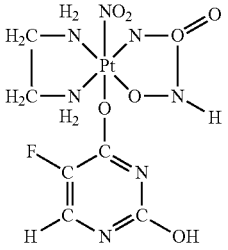
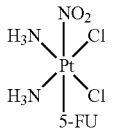
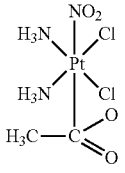
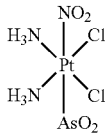
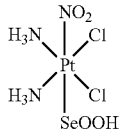
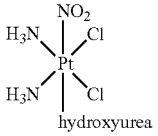
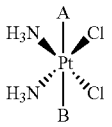
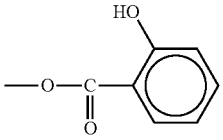
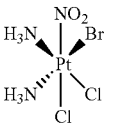
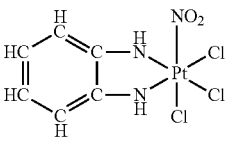
CPA-7			A549: 20 Calu-1: — Panc-1: 6.0 T-24: —
CPA-8			A549 : >250 Calu-1: — Panc-1: >250 T-24: —
CPA-9			A549 : >250 Calu-1: — Panc-1: 6.0 T-24: —
CPA-10			A549 : >250 Calu-1: — Panc-1: 6.0 T-24: —
CPA-11			A549: 138 Calu-1: — Panc-1: 48 T-24: —
CPA-12	JP1076B cis-[diaminodichloronitro-$C_6H_4O_2$—B—O-Pt^{IV}] FW 479.98 rec'd May 3, 1999		A549: > 250 μM 70 μM Calu-1: — Panc-1: >250 μM 28 μM T-24: —
CPA-13	JP1078B Pt^{IV}(en) (HOHN—CO—NH_2)(NO_2) 5-FU FW 508.39 rec'd May 3, 1999		Panc-1: <1.953 μM 2.3 μM 4.4 μM 2.3 μM A549: >250 μM 64 μM >250 μM Calu-1: 149 μM 112 μM
CPA-14	JP1079 cis-[$Pt^{IV}(NH_3)_2Cl_2NO_2$-5FU FW 476.2 rec'd Aug. 13, 1999		Panc-1: <1.953 μM 1.9 μM 2.4 μM 10 μM A549: 18 μM 4.8 μM 38 μM Calu-1: 13 μM
CPA-15	JP1080 cis-[$Pt^{IV}(NH_3)_2Cl_2NO_2$-acetate FW 482.2 rec'd Aug. 13, 1999		Panc-1: 3.3 μM 17 μM A549: 13.5 μM 42 μM

-continued

CPA-16	JP1082 cis-[$Pt^{IV}(NH_3)_2Cl_2NO_2$—AsO_2 FW 453.0 rec'd Aug. 13, 1999		Panc-1: 4.8 μM 20 μM A549: 15 μM 39 μM
CPA-17	JP1085A cis-[$Pt^{IV}(NH_3)_2Cl_2NO_2$—SeOOH FW 474.1 rec'd Aug. 13, 1999		Panc-1: 5.8 μM 20 μM A549: 14 μM 40 μM
CPA-18	JP1087A cis-[$Pt^{IV}(NH_3)_2Cl_2NO_2$-hydroxyurea FW 422.1 rec'd Aug. 13, 1999		Panc-1: 5.6 μM 3.0 μM 4.7 μM A549: 13 μM 12 μM Calu-1: 4.2 μM



	Designation	A	B	IC50
CPA-19	JP1081 cis-[$Pt^{IV}(NH_3)_2Cl_2NO_2$-salicylate] FW 483 rec'd Aug. 30, 1999	—NO_2		Panc-1: 20 μM 20 μM A549: 37 μM
CPA-20	JP1092A cis-[$Pt^{IV}(NH_3)_2Cl_2NO_2$(ONO)] FW 392 rec'd Aug. 30, 1999	—NO_2	—ONO	Panc-1: 15 μM A549: 28 μM
CPA-21	JP1093B cis-[$Pt^{IV}(NH_3)_2Cl_2NO_2Cl$ FW 382 rec'd Aug. 30, 1999	—NO_2	—Cl	Panc-1: 9.2 μM A549: 4.2 μM
CPA-22	JP1091A cis-[$Pt^{IV}(NH_3)_2Cl_2NO_2(ONO_2)$ FW 408 rec'd Aug. 30, 1999	—NO_2	—ONO_2	Panc-1: 5.4 μM A549: 4.6 μM
CPA-23	JP1089A cis-[$Pt^{IV}(NH_3)_2Cl_2NO_2(H_2PO_4")$ FW 443 rec'd Aug. 30, 1999	—NO_2	—OPO_3H_2	Panc-1: 3.3 μM 5.7 μM 1.9 μM A549: 4.2 μM
CPA-24	JP1090A cis-[$Pt^{IV}(NH_3)_2Cl_2NO_2(HSO_4")$] FW 443 rec'd Aug. 30, 1999	—NO_2	—OSO_3H	Panc-1: 5.2 μM 9.2 μM 5.9 μM A549: 3.8 μM

	Designation	Structure	IC50
CPA-25	JP0097B cis-[$Pt^{IV}(NH_3)_2BrCl_2NO_2$ FW 426 rec'd Aug. 30, 1999		Panc-1: 5.6 μM 13 μM A549: 8.0 μM
CPA-26	1094C		Panc-1: 228 μM

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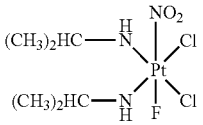
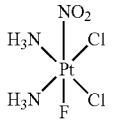
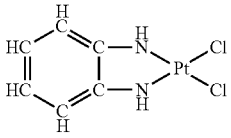
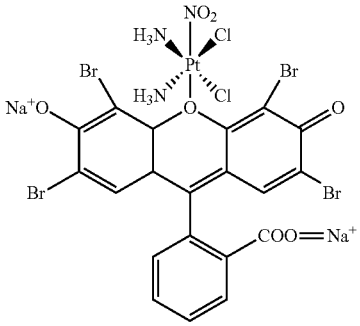
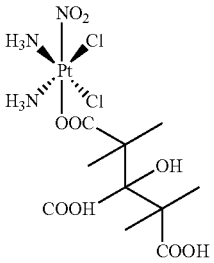
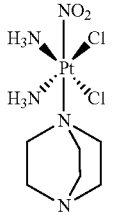
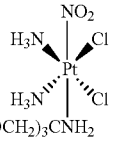
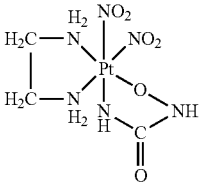
CPA-27	1084A		Panc-1: 31 μM 35 μM
CPA-28	1083A		Panc-1: 80 μM
CPA-29	1094B		Panc-1: 5.7 μM 2.1 μM
CPA-30	Eosin		Panc-1: 5.7 μM 4.5 μM
CPA-31	Citrate		Panc-1: 31 μM
CPA-32	Dabco		Panc-1: 15 μM
CPA-33	Tris		Panc-1: 10 μM
CPA-34	1084C		

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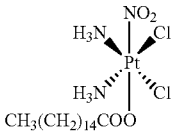
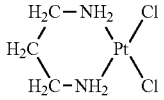
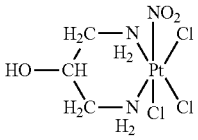
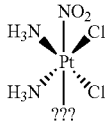
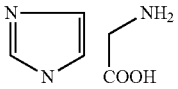
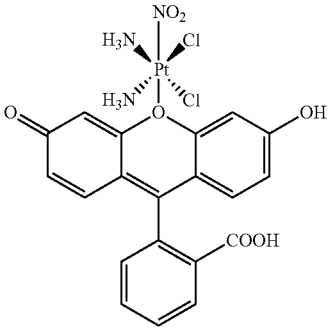
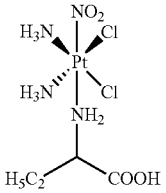
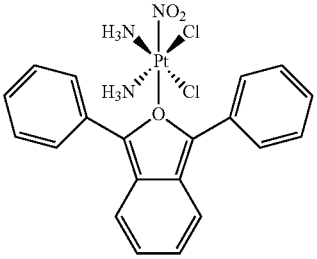
CPA-35	Palmitic		Panc-1: 7.7 μM 21 μM
CPA-36	1,3-diaminopropane		Panc-1: 20 μM
CPA-37	Hydroxydiaminopropane		Panc-1: 27 μM
CPA-38	Histidine	 	Panc-1: 5.6 μM
CPA-39	Fluorescein		Panc-1: 17 μM 12 μM
CPA-40	2-aminobutyric acid		Panc-1: 3.1μM
CPA-41	IBF		Panc-1: 1.0 μM

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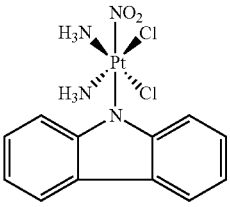
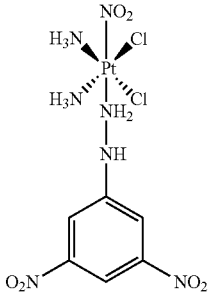
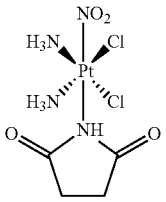
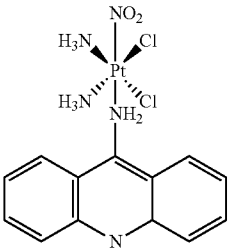
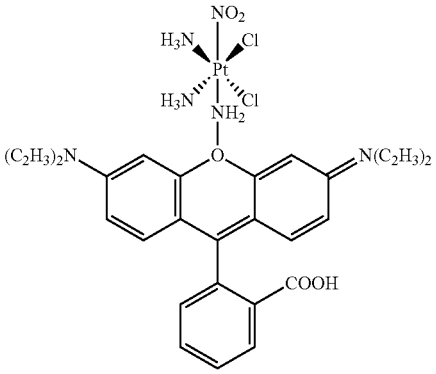
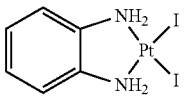
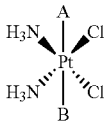
CPA-42	CZ		Panc-1: 20 μM
CPA-43	DNP		Panc-1: 3.3 μM 4.5 μM
CPA-44	Succinamide		Panc-1: 6.0 μM
CPA-45	Acridine		Panc-1: 3.0 μM
CPA-46	Rhodamine		Panc-1: 3.6 μM 4.8 μM
CPA-47	1094A		Panc-1: 3.3 μM
CPA-48	Methyl thymol blue		Panc-1: 8.6 μM



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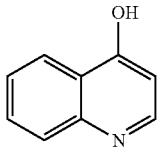
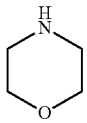
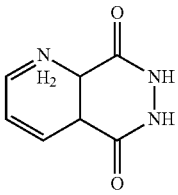
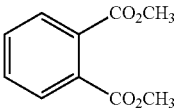
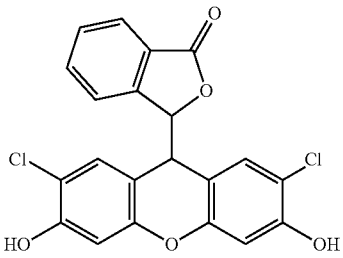
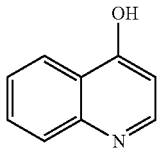
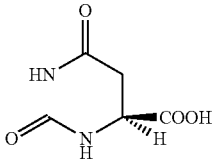
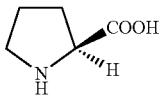
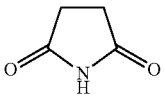
	Designation	A	B	IC50
CPA-49	D-+-maltose	—NO_2		Panc-1: 49 μM
CPA-50	Morpholine	—NO_2		
CPA-51	3-aminophthalhydrazide	—NO_2		
CPA-52	Dimethylphthalate	—NO_2		
CPA-53	2,7-dichlorofluorescein	—NO_2		
CPA-54	8-hydroxyquinoline	—NO_2		
CPA-55	Hydroorotic acid	—NO_2		
CPA-56	Proline	—NO_2		
CPA-57	Stearic acid	—NO_2	$CH_3(CH_2)_{16}COOH$	
CPA-58	succinimide	—NO_2		

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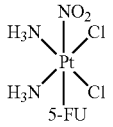
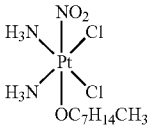
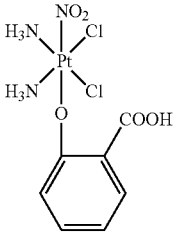
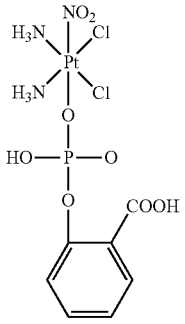
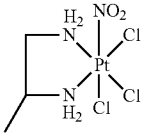
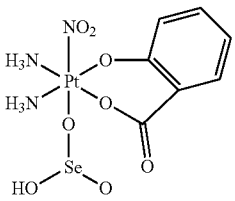
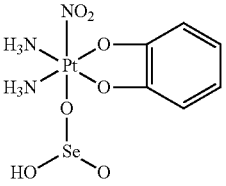
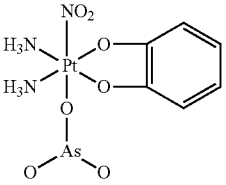
	Designation	Structure	IC50
JP3	[Cis-Pt IV$(NH_3)_2(Cl)_2(NO_2)$ (5-FU)]		Panc-1: 2.6 μM
JP4	[Cis-Pt IV$(NH_3)_2(Cl)_2(NO_2)$ (n-octanol)]		Panc-1: 18.5 μM
JP5	[Cis-Pt IV$(NH_3)_2(Cl)_2(NO_2)$ (salicylate)]		Panc-1: 6.2 μM
JP6	[Cis-Pt IV$(NH_3)_2(Cl)_2(NO_2)$ (OP(-salicylate)(OH)O)]		Panc-1: 5.5 μM
GD6	[Pt IV (1,2-diaminopropane) $(Cl)_3(NO_2)$]		Panc-1: 9.5 μM
GD2	[cis-diaminonitrosalicylatohydroselenito-Pt IV]		Panc-1: 60 μM
GD3	[cis-diaminonitrocaticholatohydroselenito-Pt IV]		Panc-1: 49 μM
GD4	[cis-diaminonitrocaticholatoarsenito-Pt IV]		Panc-1: 73 μM

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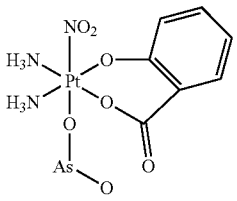
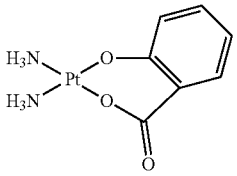
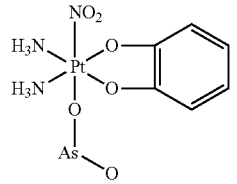
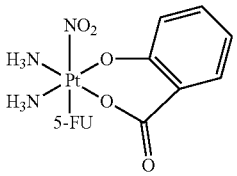
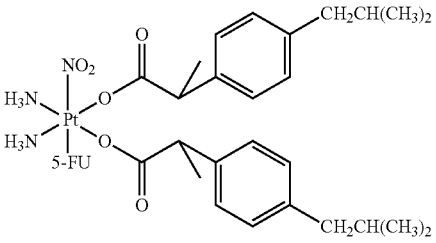
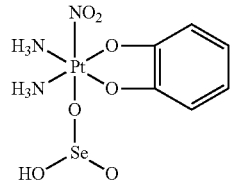
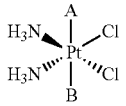
JP13A	[cis-diaminonitrosalicylatoarsenito-Pt IV]		
JP14B	[cis-diaminosalicylato-Pt II]		
JP14C	[cis-diaminonitrosalicylato-(5-FU) Pt IV]		
JP14D	[Cis-diaminonitrosalycilatoarenito-Pt IV]		
JP15	[Cis-diaminonitro-bis(ibuprofen)-Pt IV]		
	5-FU		Panc-1: 5.0 μM Calu: 250 μM
	hydroxyurea		Panc-1: 1.3 μM Calu-1: >250 μM
	Cisplatin		Panc-1: 1.3 μM Calu: 2.0 μM

Those platinum complexes designated as CPA-19 through CPA-24 and CPA-49 through CPA-58 have the structure:



wherein the A and B substituents are as identified in the Table for the particular complex. The substituent can attach to the platinum atom through any suitable atom therein, e.g., a nitrogen, sulfur, or oxygen atom. As used herein, "5FU" means 5-fluorouracil.

The Table shows the results of the various platinum complexes in MTT assays using cell lines A549, Calu-1, Pane-1, and T-24. The IC50 for a particular platinum complex is shown in the far right column of the Table.

Compounds of the subject invention also include pharmaceutically-acceptable salts of the subject platinum complexes.

The term pharmaceutically-acceptable salts means salts of the platinum complexes of the invention which are prepared with acids or bases, depending on the particular substituents found on the subject complexes described herein. Examples of a pharmaceutically-acceptable base addition salts include sodium, potassium, calcium, ammonium, or magnesium salt. Examples of pharmaceutically-acceptable acid addition salts include hydrochloric, hydrobromic, nitric, phosphoric, carbonic, sulphuric, and organic acids like acetic, propionic, benzoic, succinic, fumaric, mandelic, oxalic, citric, tartaric, maleic, and the like. Pharmaceutically-acceptable salts of platinum complexes of the invention can be prepared using conventional techniques.

It will be appreciated by those skilled in the art that certain of the platinum complexes of the invention may contain one or more asymmetrically substituted carbon atoms which can give rise to stereoisomers. It is understood that the invention extends to all such stereoisomers, including enantiomers, and diastereoisomers and mixtures, including racemic mixtures thereof. In one embodiment, methods of the invention comprise inhibiting proliferation of cancerous or tumorigenic cells using the platinum complexes of the present invention. Platinum complexes of the invention can be delivered to a cell either through direct contact with the cell or via a carrier means. Carrier means for delivering compositions to cells are known in the art and include encapsulating the composition in a liposome moiety, and attaching the platinum complexes to a protein or nucleic acid that is targeted for delivery to the target cell. Published U.S. Patent Application Nos. 20030032594 and 20020120100 disclose amino acid sequences that can be coupled to another composition and that allows the composition to be translocated across biological membranes. Published U.S. Patent Application No. 20020035243 also describes compositions for transporting biological moieties across cell membranes for intracellular delivery.

The subject invention also concerns methods for treating oncological or inflammatory disorders in a patient. In one embodiment, an effective amount of a platinum complex of the present invention is administered to a patient having an oncological or inflammatory disorder and who is in need of treatment thereof. Methods of the invention can optionally include identifying a patient who is or may be in need of treatment of an oncological or inflammatory disorder. The patient can be a human or other mammal, such as a primate (monkey, chimpanzee, ape, etc.), dog, cat, cow, pig, or horse, or other animals having an oncological disorder. Means for administering and formulating platinum complexes for administration to a patient are known in the art, examples of which are described herein. Oncological disorders include cancer and/or tumors of the bone, breast, kidney, mouth, larynx, esophagus, stomach, testis, cervix, head, neck, colon, ovary, lung, bladder, skin, liver, muscle, pancreas, prostate, blood cells (including lymphocytes), and brain. Inflammatory disorders include arthritis, multiple sclerosis, lupus, Crohn's disease, and related neurological and inflammatory connective tissue diseases (e.g., Sjögren's syndrome).

For the treatment of oncological disorders, the platinum complexes of this invention can be administered to a patient in need of treatment in combination with other antitumor or anticancer substances or with radiation therapy or with surgical treatment to remove a tumor. These other substances or radiation treatments may be given at the same as or at different times from the platinum complexes of this invention. For example, the platinum complexes of the present invention can be used in combination with mitotic inhibitors such as taxol or vinblastine, alkylating agents such as cyclophosamide or ifosfamide, antimetabolites such as 5-fluorouracil or hydroxyurea, DNA intercalators such as adriamycin or bleomycin, topoisomerase inhibitors such as etoposide or camptothecin, antiangiogenic agents such as angiostatin, antiestrogens such as tamoxifen, and/or other anti-cancer drugs or antibodies, such as, for example, GLEEVEC (imatinib) (Novartis Pharmaceuticals Corporation) and HERCEPTIN (trastuzumab) (Genentech, Inc.), respectively.

Many tumors and cancers have viral genome present in the tumor or cancer cells. For example, Epstein-Barr Virus (EBV) is associated with a number of mammalian malignancies. The platinum complexes of the subject invention can be used alone or in combination with anticancer or antiviral agents, such as ganciclovir, azidothymidine (AZT), lamivudine (3TC), etc., to treat patients infected with a virus that can cause cellular transformation and/or to treat patients having a tumor or cancer that is associated with the presence of viral genome in the cells. The platinum complexes of the subject invention can also be used in combination with viral based treatments of oncologic disease. For example, platinum complexes of the invention can be used with mutant herpes simplex virus in the treatment of non-small cell lung cancer (Toyoizumi et al., 1999).

The subject invention also concerns methods for treating bacterial and viral infections of a patient using a platinum complex of the invention. In one embodiment, an effective amount of a platinum complex of the invention is administered to a patient having a bacterial or viral infection. Methods of the invention can optionally include identifying a patient who is or may be in need of treatment of a bacterial or viral infection. The patient can be a human or other mammal, such as a primate (monkey, chimpanzee, ape, etc.), dog, cat, cow, pig, or horse, or other animal infected with a bacteria or virus. Bacterial infections that can be treated according to the present invention include those from *Staphylococcus, Streptococcus, Salmonella, Bacillus, Clostridium, Pseudomonas, Neisseria, Mycobacterium*, and *Yersinia*. Viral infections that can be treated according to the present invention include, but are not limited to, those associated with human immunodeficiency virus (HIV), human T cell leukemia virus (HTLV), Papillomavirus (e.g, human papilloma virus), Polyomavirus (e.g., SV40, BK virus, DAR virus), orthopoxvirus (e.g., variola major virus (smallpox virus)), EBV, herpes simplex virus (HSV), hepatitis virus, Rhabdovirus (e.g., Ebola virus) and cytomegalovirus (CMV). Platinum compositions of the present invention can also be used to treat viral diseases in the presence of photodynamic therapy (Cuny et al., 1999).

Platinum complexes of the subject invention can also be used to treat patients infected with a parasitic organism. In one embodiment, the patient is administered a therapeutically effective amount of a platinum complex of the present invention. Methods of the invention can optionally include identifying a patient who is or may be in need of treatment of a parasitic infection. The patient can be a human or other mammal, such as a primate (monkey, chimpanzee, ape, etc.), dog, cat, cow, pig, or horse, or other animal infected with a parasitic organism. Disease conditions that can be treated according to the present invention include, but are not limited to, *leishmania*, toxoplasmosis, schistosomiasis, trypanosomiasis, pneumocystis, malaria, and trichinosis. Parasitic organisms that can cause disease conditions treatable according to the present invention include, but are not limited to, *Leishmania, Toxoplasma, Schistosoma, Plasmodium*, and *Trypanosoma*. The subject invention can also be used to treat gastro-intestinal disorders caused by parasitic organisms such as, *Entamoeba, Giardia, Trichomonas*, and nematodes such as *Ascaris, Trichuris, Enterobius, Necator, Ancylostoma, Strongyloides*, and *Trichinella*. In another embodiment, a

platinum complex of the present invention can be administered to patients prophylactically, wherein an uninfected patient is traveling to or will be present in an area where parasitic disease is prevalent or poses a risk to the patient. Accordingly, the patient can be treated with a composition of the present invention prior to the patient's exposure to or presence in the area where parasitic disease is prevalent or poses a risk and/or prior to infection with the parasitic organism.

Platinum complexes of the present invention can also be used to treat biological products in vitro that are contaminated with or suspected of being contaminated with a virus on a bacterial or parasitic organism. Biological products which can be treated with a platinum complexes of the present invention include, but are not limited to, whole blood, fractionated blood, plasma, serum, whole organs, or parts of organs, and cells, including blood cells, muscle cells, skin cells, and neural cells, and products derived from cells. Products derived from cells which can be treated with a platinum complex of the present invention include, but are not limited to, interferons, interleukins, blood clotting factors such as factor VIII, IX, X, and the like, insulin, polyclonal and monoclonal antibodies, growth factors, cytokines, and other products. Treatment of biological products comprises contacting the product for an effective amount of time and with an effective amount of a platinum complex of the present invention. If necessary, the biological product can be subsequently washed, preferably with a suitable sterile wash solution such as phosphate buffered saline, to remove the platinum complex that was used to treat the product.

Therapeutic application of the subject platinum complexes, and compositions containing them, can be accomplished by any suitable therapeutic method and technique presently or prospectively known to those skilled in the art. The subject platinum complexes can be administered by any suitable route known in the art including, for example, oral, nasal, rectal, and parenteral routes of administration. As used herein, the term parenteral includes subcutaneous, intravenous, intramuscular, and intrasternal administration, such as by injection. Administration of the subject platinum complexes of the invention can be continuous or at distinct intervals as can be readily determined by a person skilled in the art.

Compounds useful in the methods of the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in detail in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* by E. W. Martin describes formulations which can be used in connection with the subject invention. In general, the compositions of the subject invention will be formulated such that an effective amount of the bioactive platinum complex is combined with a suitable carrier in order to facilitate effective administration of the composition. The compositions used in the present methods can also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspension, suppositories, injectable and infusible solutions, and sprays. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional pharmaceutically acceptable carriers and diluents which are known to those skilled in the art. Examples of carriers or diluents for use with the subject platinum complexes include ethanol, dimethyl sulfoxide, glycerol, alumina, starch, and equivalent carriers and diluents. To provide for the administration of such dosages for the desired therapeutic treatment, new pharmaceutical compositions of the invention will advantageously comprise between about 0.1% and 99%, and especially, 1 and 15% by weight of the total of one or more of the subject platinum complexes based on the weight of the total composition including carrier or diluent.

The compounds of the subject invention can also be administered utilizing liposome technology, slow release capsules, implantable pumps, and biodegradable containers. These delivery methods can, advantageously, provide a uniform dosage over an extended period of time. The platinum complexes of the present invention can also be administered in their salt derivative forms or crystalline forms known to those of ordinary skill in the art.

The subject invention also concerns a packaged dosage formulation comprising in one or more containers at least one platinum compound of the subject invention formulated in a pharmaceutically acceptable dosage.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Materials and Methods

Synthesis of NitroPlatinum (IV) Complexes.

Cis-diammineoplatinum(II) dichloride (cisplatin) can be purchased at 99.9% purity from Sigma-Aldrich (#P4394). Using 0.300 grams of Cisplatin (0.00100 moles, FW=300.1), 150 mL of ultra deionized water and 50 mL of dichloroethane are added to a 250-mL Erlenmeyer flask. However, hexane or any organic solvents can be substituted in place of the dichlorethane used here. The choice of a sixth ligand includes the availability of a nitrogen, sulfur or oxygen atom in the chemical structure providing a Lewis base for bonding to the oxidized Pt. Other bondings are possible with metals, halides (such as HCl) or through chelation or interaction with pi molecular orbitals. One mole of the chosen ligand per mole of cisplatin should be weighed and added to the mixture. Organic solvents, such as dichloroethane, provide solubility for organic ligands of hydrophobic nature. A magnetic stir bar is placed in the mixture and the flask placed on a magnetic stir plate in a chemical fume hood. A lecture bottle of dinitrogen tetroxide is fitted with a regulator and Teflon hose, with a glass pipet attached to the hose outlet. The pipet tip is inserted into the lower solvent (e.g., dichloroethane) and the lecture bottle warmed slightly with a warm water bath. Nitrogen dioxide gas is released at a rate of approximately one bubble per second into the stirring mixture. The gas should be added until all the yellow cisplatin is consumed; the disappearance of yellow solids and yellow solution will indicate consumption of the available cisplatin. A blue color is noted to indicate formation of the nitrosyl intermediate; variations in hue and duration of this color have been observed. Gas addition is then terminated (remove the pipet to prevent vacuum suction into the lecture bottle) and the flask covered in aluminum foil to prevent light exposure. The flask should be left to stir overnight, uncovered.

Additional nitrogen dioxide may be added the next day to check for completeness of reaction. A blue color would indicate incomplete oxidation of platinum (II). Normally, this blue fades within ten minutes. For a colorless ligand, the solution has become yellow overnight. If blue color remains, allow it to continue stirring. The mixture requires air for complete oxidation, so should not be tightly covered. Continued oxidation with air can be accelerated using air blown through a trap into the Erlenmeyer, over the liquids. The solvents will evaporate in about two days, leaving a yellow precipitate, which is the product.

The precipitate can be purified via recrystallization in methanol, DMSO, or other suitable solvent. Alternatively, the product can be purified on silica columns or using HPLC.

MTT Assay.

Inhibition of the growth of human tumor cells was carried out in 96-well plates using the MTT assay Cells were plated and treated with various concentrations of the platinum complex for 4 days. Cell viability then was determined by adding to the cells 1 mg/ml media of 3-(4-5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and incubating the cells for 3 hrs at 37° C. The dye-containing media was replaced by DMSO. After a 5 min incubation with DMSO, absorbance of the control compared to inhibitor treated columns of the 96-well plate was then read at 540 nM with a microtiter plate reader and IC50s determined.

XTT Assay.

A 96-well plate was used for the assays. Approximately 2.5×10^4 cells in log phase were added to each well. A platinum complex of the invention was dispensed into each well (dissolved in 20% DMSO and 80% media), with additional media added as needed to account for uniform volumes. Control wells contained only cells and media. Each concentration assay was performed in triplicate. Plates were incubated for 48 hours at 37° C. with 7.5% CO2. XTT from MD Biosciences, Quebec, was then added according to the provided protocol concentrations and allowed to react for 3 hours. Plates were agitated 5 minutes before reading absorbance at 475 nm on a Varian Cary 50 spectrophotometer with a fibre-optic probe. Percent survival as compared to control wells was plotted against platinum complex concentration.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

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Published U.S. Patent Application No. 20020035243

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Nitiss, J. L. (2002) "A copper connection to the uptake of platinum anticancer drugs" *Proc. Natl. Acad. Sci. USA* 99:13963-13965.

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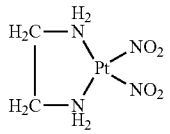
We claim:

1. A platinum complex having the structure:

CPA-2



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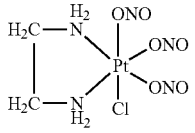

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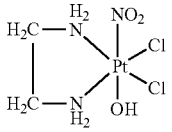

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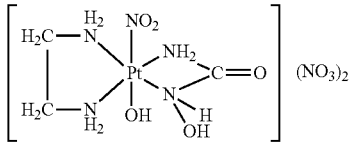

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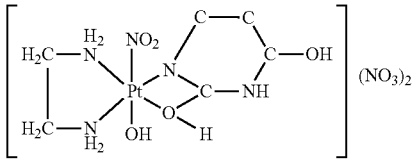

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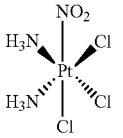

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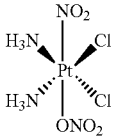

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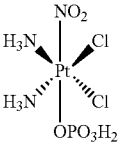

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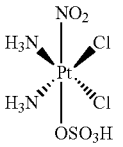

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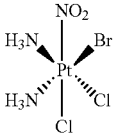

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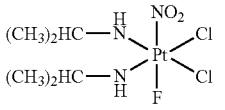

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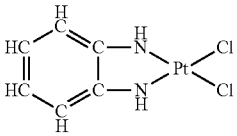

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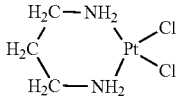

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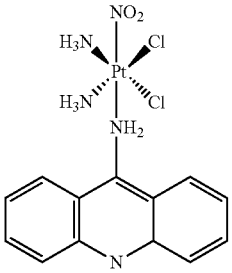

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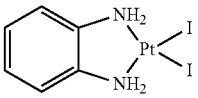

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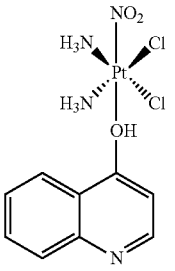

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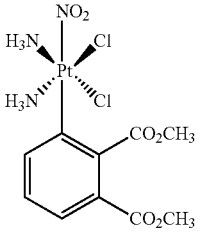

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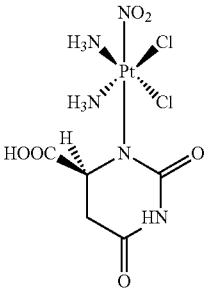

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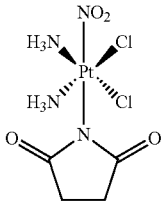

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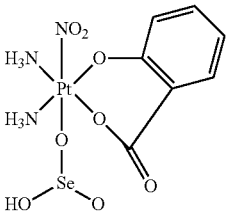
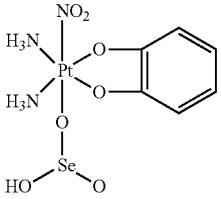
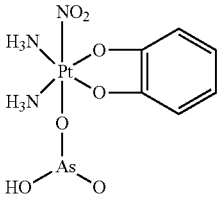


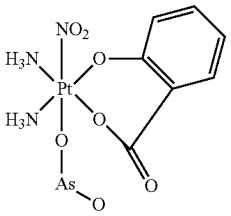
GD3



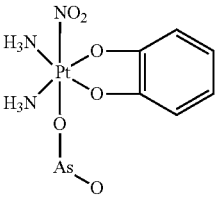
GD4



JP13A



JP14C



or a pharmaceutically acceptable salt thereof.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

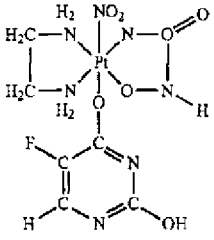
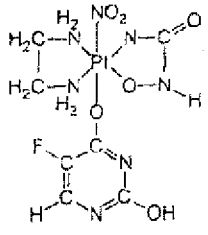
CERTIFICATE OF CORRECTION

PATENT NO. : 8,455,543 B2
APPLICATION NO. : 13/158060
DATED : June 4, 2013
INVENTOR(S) : Heidi Kay et al.

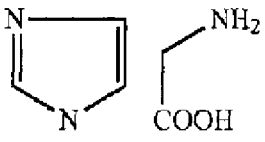
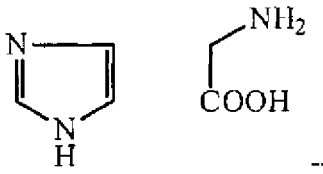
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

Column 4,
Lines 39-44 (CPA-13)

“  ” should read --  --.

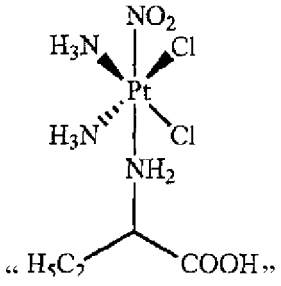
Column 10,
Lines 28-31 (CPA-38)

“  ” should read --  --.

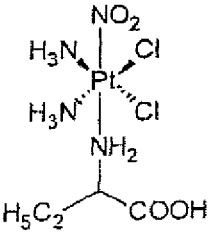
Signed and Sealed this
Eighth Day of October, 2013

Teresa Stanek Rea
Deputy Director of the United States Patent and Trademark Office

Column 10,
Lines 46-49 (CPA-40)



" should read --



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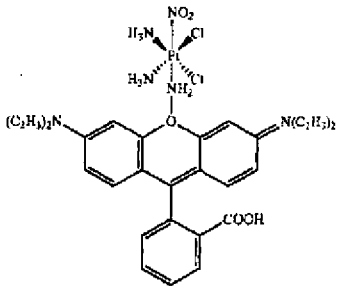
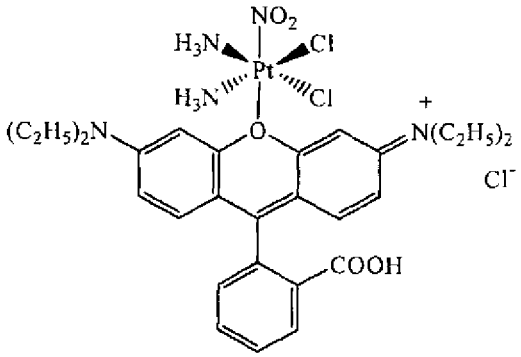
Column 12,
Lines 46-49 (CPA-46)



" should read --



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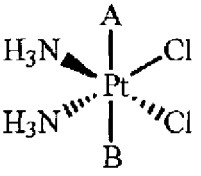
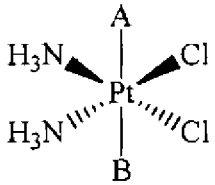
Column 17,
Lines 62-65



" should read --



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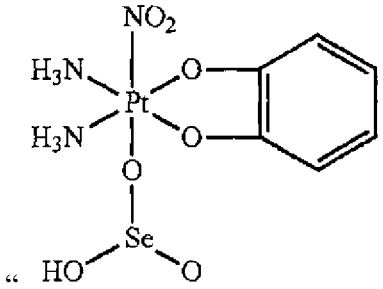
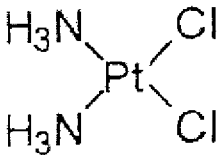
Column 18,
Lines 47-54 (Cisplatin)



“ ” should read --



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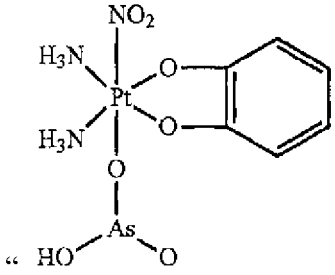
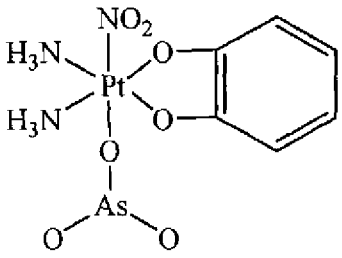
In the Claims

Column 26,
Lines 33-40 (GD4)



“ ” should read --



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