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<th>ANTI-CANCER PHOSPHINE CONTAINING [AuIlIm(CNC)mL]n+ COMPLEXES AND DERIVATIVES THEREOF AND METHODS FOR TREATING CANCER USING SUCH COMPOSITIONS</th>
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<td><strong>Inventor(s)</strong></td>
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(54) ANTI-CANCER PHOSPHINE CONTAINING [AUHIM(CNC)MLN]⁺ COMPLEXES AND DERIVATIVES THEREOF AND METHODS FOR TREATING CANCER USING SUCH COMPOSITIONS

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ABSTRACT

Gold(III)phosphine complexes [Au₃(C₅N₇L)₆]⁺ (where HCNCH=2,6-diphenylpyridine) and their use as anti-tumor agents are disclosed. Notable results for the appearance of new potential anti-tumor application of these gold(III) complexes are reported. The described complexes show promising cytotoxic properties toward cancer cells in both in vitro and in vivo studies.
**FIG. 2A**

time = 0h

**FIG. 2B**

time = 72 h

8.0 7.5 7.0 6.5 6.0 1H (ppm)
ANTI-CANCER PHOSPHINE CONTAINING [AUHIMCN(C)MILJN]+ COMPLEXES AND DERIVATIVES THEREOF AND METHODS FOR TREATING CANCER USING SUCH COMPOSITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of and priority to U.S. Provisional Patent Application No. 60/870,509 filed on Dec. 20, 2006, and which is incorporated herein in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates to gold(III)phosphine complexes as anti-tumor and antiviral agents, pharmaceutical compositions including these complexes, and methods for treatment of cancer and viral diseases using such compositions.

BACKGROUND OF THE INVENTION

[0003] The success of cisplatin and its derivatives as anti-cancer agents has stimulated the development of metal-based compounds, including that of gold, for anticancer treatment [C. F. Shaw III, Chem. Rev. 1999, 99, 2589]. In this context, extensive investigations on the biological properties of gold(I) and gold(III) have been reported. The development of gold(III) compounds as potential anti-cancer agents has been hampered by their poor stability in solution. Very few cytotoxic gold(III) compounds such as [Au(bipy−H)(OH)][PF6] (bipy=4-deprotonated 6-(1,1-dimethylbenzyl)-2,2′-bipyridine), [Au(dmanp)Cl] (dmanp=2-(dMethylaminomethyl)phenyl), and gold(II)tetraarylporphyrins [C.-M. Che, R. W.-Y. Sun, W.-Y. Yu, C.-B. Ko, N. Zhu, H. Sun, Chem. Commun. 2003, 1718] have been reported to have significant stability.


[0005] The synthesis and study of a series of gold(III)porphyrins that exhibit potent in vitro and in vivo anti-cancer properties toward hepaticcellular carcinoma and nasopharyngeal carcinoma has been reported. As demonstrated by DNA microarray and proteome analyses, the gold(III)tetraarylporphyrins up-regulated the transcription and translation of a number of apoptosis-related gene and protein expressions. [Au(CNC)2L]⁺ compounds (where HNCN=−2,6-diphenylnitridine, and L−phosphate-containing ligand, Scheme 1), were first developed and reported in 1998 [K.-H. Wong, K.-K. Cheung, M. C.-W. Chan, C.-M. Che, Organometalics 1998, 17, 3505].

SUMMARY OF THE INVENTION

[0007] Methods of using gold(III) complexes as anti-tumor agents are described. In one embodiment, cancer cell death is achieved by administering to a patient in need thereof a composition comprising an effective amount of a gold(III) complex. The gold (III) complexes can be represented by structural formula I and II:

![Chemical structure](image)

or a pharmaceutically acceptable salt thereof, wherein:

[0008] R₁−R₁⁵ are each independently hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkyl cyanocarbonyl, substituted alkynyl, phenyl, substituted phenyl, halo, nitro, hydroxyl, alkoxy, substituted alkoxy, phenoxyl, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl,
carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfinyl, substituted sulfinyl, sulfonyl, substituted sulfonyl, sulfonic acid, substituted sulfonic acid, phosphonato, substituted phosphonato, phosphonamide, substituted phosphonamide, C$_r$-C$_{20}$ cyclic, substituted C$_r$-C$_{20}$ cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, or polypeptide group;

0009 ligand L is independently —P-donor ligand;
0010 each X is independently a pharmaceutically acceptable counter-ion;

0011 m is an integer ranging from 1 to 10;
0012 n is an integer ranging from −10 to 100;
0013 p is an integer ranging from −3 to 3;
0014 q is equal to the absolute value of n+p;
0015 qX is absent when n is 0; and
0016 a pharmaceutically acceptable carrier. Preferred values for m are 1 to 10, and for n are −10 to 100.

BRIEF DESCRIPTION OF THE DRAWINGS

0017 FIG. 1 shows ORTEP drawings with atom-numbering scheme of phosphine-containing [Au$^{III}$]$_{m}$[CNC]$_{n}$] complexes in accordance with the present invention. Hydrogen atoms and solvent molecules are omitted for clarity. Thermal ellipsoids are drawn at the 30% probability level.

0018 FIG. 2 shows the 'H NMR spectra of compound 3d in D$_2$O/[D6] DMSO (9:1) at time = (a) 0 and (b) 72 h.

0019 FIGS. 3A and 3B show the induction of cell-cycle arrest in the SUNE1 cells after treatment with cisplatin, and gold(III) compound 3d.

0020 FIG. 4 shows the survival curves of HCC-bearing rats in different treatment groups. * p<0.05, compared to the vehicle control group.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

0021 Gold(III) [or Au(III) or Au$^{III}$] phosphine complexes [Au$^{III}$]$_{m}$[CNC]$_{n}$] are used as anti-tumor and anti-viral agents and as pharmaceutical compositions for combating cancer. The pharmaceutical compositions contain different synthetic [Au$^{III}$]$_{m}$[CNC]$_{n}$] compounds in amounts effective to induce cancer cell death.

0022 The cationic [Au$_{m}$[CNC]$_{n}$]$^+$ (m = 1, n = 1, L = phosphine ligand) compounds were predicted to be structurally analogous to the classical metallointercalating [Pt (terpy)I$^+$] compounds [K. Becker, C. Herold-Mende, J. J. Park, G. Lowe, R. H. Schirmer, J. Med. Chem. 2001, 44, 2784]. This class of compounds should be highly robust in solution, since the dianionic nature of CNC ligand would stabilize the electrophilic gold(III), making its reduction to gold(II) and gold(I) at negative reduction potential. One should also be able to make structural modifications to the [Au(CNC)$_r$]$_r$ complexes through ligand substitution reactions of L (phosphine ligand), which would be highly useful for studying the relationship between structure and cytotoxicity. By using appropriate polydentate phosphine ligands, polyvalent gold(II) compounds comprising more than one [Au(CNC)$_r$]$^+$ moiety (m = 2 or 3) could be obtained. It is understood that the CNC molecule and the gold(III) center may not form a charge neutral complex. For instance, the net positive charge on the gold(III) may be greater than the absolute net negative charge of the CNC molecule; or the net positive charge on the gold(III) may be less than the absolute net negative charge of the CNC molecule. In view of this, there should be at least one anion or counter-ion coordinated to the gold(III) complex for charge neutralization. Accordingly, the phrase “pharmaceutically acceptable salt” as used herein, includes salts formed from charged gold(III) complex and the anion or counter-ion.

1. DEFINITIONS

0023 As used herein, the term “CNC” refers to a molecule of either one of the following chemical structures:

where R$_1$-R$_{15}$ are each independently hydrogen, alkyl, substituted alkyl, alkanyl, substituted alkanyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, halo, nitro, hydroxyl, alkoxyl, substituted alkoxyl, phenoxyl, substituted phenoxyl, aroxyl, substituted aroxyl, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carbonyl, substituted carbonyl, amino, substituted amino, amido, substituted amido, sulfinyl, substituted sulfinyl, sulfonyl, substituted sulfonyl, sulfonic acid, substituted sulfonic acid, phosphonato, substituted phosphonato, phosphonamide, substituted phosphonamide, C$_r$-C$_{20}$ cyclic, substituted C$_r$-C$_{20}$ cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, or polypeptide group
As used herein, the phrase “counter-ion” refers to an ion associated with a positively or negatively charged gold (III) complex. Non-limiting examples of counter-ions include fluoride, chloride, bromide, iodide, sulfate, phosphate, trifluoromethanesulfonate, acetate, nitrate, perchlorate, acetylacetonate, hexafluoroacetacetonate, sodium and potassium.

As used herein, the term “[AuIII(CN)₃]⁺ complexes or complexes” refers to complex of gold(III) metal bound to any CNC molecule to any phosphine molecule. The gold(III) ion can have one or more various neutral, positively or negatively charged CNC molecule(s). The structure of [AuIII(CN)₃]⁺ complexes can be either in monomeric, dimeric or polymeric form. Also, they can exist as a single molecule or aggregated molecules.

As used herein, the phrase of “pharmacologically acceptable carrier” means a carrier combination of carrier ingredients approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, mammals, and more particularly in humans. Non-limiting examples of pharmacologically acceptable carriers include liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin. Water is preferred vehicle when the compound of the invention is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid vehicles, particularly for injectable solutions.

As used herein, the term “patient” refers to any animal, preferably a human.

II. METHODS OF TREATMENT

The compositions are useful for induction of cancer cell death (including but not limited to apoptosis) of cancer cells by administering to a patient afflicted with a responsive form of cancer a composition comprising an effective amount of one or more [AuIII(CN)₃]⁺ complexes. The [AuIII(CN)₃]⁺ complexes can be represented by structural formulas I or II, or a pharmaceutically acceptable salt thereof:

As used herein, the term “L” in structural formulae I or II refers to an ion or molecule, including all P-donor ligands, that binds to the gold(III) ion. It includes one or more than one phosphino molecules.

As used herein, the term “P-donor ligand” refers to an ion or molecule using the electron rich phosphorus atom, that binds to the gold(III) ion of the invention. It includes but not limited to one or more than one coordinating compounds such as triphenylphosphine (TPP), 1,2-bis(diphenylphosphino)methane (dpmm), 1,2-bis(diphenylphosphino)ethane (dpe), 1,2-bis(diphenylphosphino)propane (dppp), 1,2-bis(diphenylphosphino)butane (dppb), 1,2-bis(diphenylphosphino)pentane (dpppe), 1,2-bis(diphenylphosphino)hexane (dpph), or bis(diphenylphosphinoethyl)diphenylphosphine (dpppe).

In structural formulas I and II, R¹-R¹⁵ are each independently hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, halo, nitro, hydroxyl, alkoxy, substituted alkoxy, phenoxyl, substituted phenoxyl, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfanyl, substituted sulfanyl, sulfonyl, substituted sulfonyl, sulfonic acid, substituted sulfonic acid, phosphono, substituted phosphonato, phosphoramide, substituted phosphoramide, C₁-C₂₀ cyclic, substituted C₁-C₂₀ cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, or polypeptide group.

In one embodiment, the method relates to the induction of cancer cell death by administering to a patient in need thereof a composition comprising an effective amount of an [AuIII(CN)₃]⁺ complex of formula I or a pharmaceutically acceptable salt thereof, wherein R¹-R¹⁵ are each —H; m is 1; n is 0; qXP is absent; and L is:

and a pharmaceutically acceptable carrier.
[0033] In yet another embodiment, the method for the induction of cancer cell death is by administering to a patient in need thereof a composition comprising an effective amount of a [AuII\textsubscript{m} (CNC)\textsubscript{n} L\textsuperscript{m+}] complex of formula I or a pharmaceutically acceptable salt thereof, wherein R\textsuperscript{1}-R\textsuperscript{11} are each —H; m is 1; n is 0; q X\textsuperscript{0} is absent; and L is:

\[ \text{I} \]

and a pharmaceutically acceptable carrier.

[0034] In a further embodiment, the method for the induction of cancer cell death is by administering to a patient in need thereof a composition comprising an effective amount of a [AuII\textsubscript{m} (CNC)\textsubscript{n} L\textsuperscript{m+}] complex of formula I or a pharmaceutically acceptable salt thereof, wherein R\textsuperscript{1}-R\textsuperscript{11} are each —H; m is 2; n is 2; q is 2; X\textsuperscript{0} is CF\textsubscript{3}SO\textsubscript{3}⁻; and L is:

\[ \text{II} \]

and a pharmaceutically acceptable carrier.

[0035] In another embodiment, the method for the induction of cancer cell death is by administering to a patient in need thereof a composition comprising an effective amount of a [AuII\textsubscript{m} (CNC)\textsubscript{n} L\textsuperscript{m+}] complex of formula I or a pharmaceutically acceptable salt thereof, wherein R\textsuperscript{1}-R\textsuperscript{11} are each —H; m is 2; n is 2; q is 2; X\textsuperscript{0} is CF\textsubscript{3}SO\textsubscript{3}⁻; and L is:

\[ \text{III} \]

and a pharmaceutically acceptable carrier.

[0036] In another embodiment, the method for the induction of cancer cell death is by administering to a patient in need thereof a composition comprising an effective amount of a [AuII\textsubscript{m} (CNC)\textsubscript{n} L\textsuperscript{m+}] complex of formula I or a pharmaceutically acceptable salt thereof, wherein R\textsuperscript{1}-R\textsuperscript{11} are each —H; m is 2; n is 2; q is 2; X\textsuperscript{0} is CF\textsubscript{3}SO\textsubscript{3}⁻; and L is:

\[ \text{IV} \]

and a pharmaceutically acceptable carrier.

[0037] In another embodiment, the method for the induction of cancer cell death is by administering to a patient in need thereof a composition comprising an effective amount of a [AuII\textsubscript{m} (CNC)\textsubscript{n} L\textsuperscript{m+}] complex of formula I or a pharmaceutically acceptable salt thereof, wherein R\textsuperscript{1}-R\textsuperscript{11} are each —H; m is 2; n is 2; q is 2; X\textsuperscript{0} is CF\textsubscript{3}SO\textsubscript{3}⁻; and L is:

\[ \text{V} \]

and a pharmaceutically acceptable carrier.

[0038] In another embodiment, the method for the induction of cancer cell death is by administering to a patient in need thereof a composition comprising an effective amount of a [AuII\textsubscript{m} (CNC)\textsubscript{n} L\textsuperscript{m+}] complex of formula I or a pharmaceutically acceptable salt thereof, wherein R\textsuperscript{1}-R\textsuperscript{11} are each —H; m is 2; n is 2; q is 2; X\textsuperscript{0} is CF\textsubscript{3}SO\textsubscript{3}⁻; and L is:

\[ \text{VI} \]

and a pharmaceutically acceptable carrier.
[0039] In another embodiment, the method for the induction of cancer cell death is by administering to a patient in need thereof a composition comprising an effective amount of a \([\text{Au}^{III}_n(\text{CNC})_m\text{L}_p\text{]}^{n+}\) complex of formula I or a pharmaceutically acceptable salt thereof, wherein \(R^1-R^{15}\) are each \(-\text{H}; m = 2; n = 2; q = 2; X^0 = \text{CF}_3\text{SO}_3\); and \(L\) is.

and a pharmaceutically acceptable carrier.

[0040] In another embodiment, the method for the induction of cancer cell death is by administering to a patient in need thereof a composition comprising an effective amount of a \([\text{Au}^{III}_n(\text{CNC})_m\text{L}_p\text{]}^{n+}\) complex of formula I or a pharmaceutically acceptable salt thereof, wherein \(R^1-R^{15}\) are each \(-\text{H}; m = 3; n = 3; q = 3; X^0 = \text{CF}_3\text{SO}_3\); and \(L\) is:

and a pharmaceutically acceptable carrier.

[0041] In another embodiment, the method for the induction of cancer cell death is by administering to a patient in need thereof a composition comprising an effective amount of a \([\text{Au}^{III}_n(\text{CNC})_m\text{L}_p\text{]}^{n+}\) complex of formula II or a pharmaceutically acceptable salt thereof, wherein \(R^1-R^{15}\) are each \(-\text{H}; m = 2; n = 2; q = 2; X^0 = \text{CF}_3\text{SO}_3\); and \(L\) is:

and a pharmaceutically acceptable carrier.

[0042] In another embodiment, the method for the induction of cancer cell death is by administering to a patient in need thereof a composition comprising an effective amount of a \([\text{Au}^{III}_n(\text{CNC})_m\text{L}_p\text{]}^{n+}\) complex of formula II or a pharmaceutically acceptable salt thereof, wherein \(R^1-R^{15}\) are each \(-\text{H}; m = 1; n = 0; qX^0\) is absent; and \(L\) is:

and a pharmaceutically acceptable carrier.

[0043] In another embodiment, the method for the induction of cancer cell death is by administering to a patient in need thereof a composition comprising an effective amount of a \([\text{Au}^{III}_n(\text{CNC})_m\text{L}_p\text{]}^{n+}\) complex of formula II or a pharmaceutically acceptable salt thereof, wherein \(R^1-R^{15}\) are each \(-\text{H}; m = 2; n = 2; q = 2; X^0 = \text{CF}_3\text{SO}_3\); and \(L\) is:

and a pharmaceutically acceptable carrier.

[0044] In another embodiment, the method for the induction of cancer cell death is by administering to a patient in need thereof a composition comprising an effective amount of a \([\text{Au}^{III}_n(\text{CNC})_m\text{L}_p\text{]}^{n+}\) complex of formula II or a pharmaceutically acceptable salt thereof, wherein \(R^1-R^{15}\) are each \(-\text{H}; m = 2; n = 2; q = 2; X^0 = \text{CF}_3\text{SO}_3\); and \(L\) is:

and a pharmaceutically acceptable carrier.
In another embodiment, the method for the induction of cancer cell death is by administering to a patient in need thereof a composition comprising an effective amount of a \([\text{Au}^{III}_{m}(\text{CNC})_{n}L]^+\) complex of formula II or a pharmaceutically acceptable salt thereof wherein \(R^1-R^{11}\) are each —H; \(m\) is 3; \(n\) is 3; \(q\) is 3; \(X\) is \(\text{CF}_3\text{SO}_3^-\); and \(L\) is:

and a pharmaceutically acceptable carrier.

In another embodiment, the method for the inhibition of tumor growth in an orthotopic rat hepatocellular carcinoma model using the rat hepatoma cells McA-RH7777, is by administering to a patient in need thereof an effective amount of the \([\text{Au}^{III}_{m}(\text{CNC})_{n}L]^+\) complexes of formula I and II or a pharmaceutically acceptable salt.

Compounds designated 3d, 3e, 3f, 3g, 3h, 5c, 5d, 5e and 5f have not previously been described in the literature: 2,6-diphenylpyridine-gold co-ordination complexes of formula I:

—wherein each of \(R^1-R^{11}\) is \(H\); \(m\) is 2; \(n\) is 0; \(q\) is absent or is a pharmaceutically acceptable counter-ion such as trifluoroacetate, and \(L\) is a 1,2-bis(diphenylphosphino) propane.

Of particular interest, and therefore constituting a specific preferred embodiment is the compound of the above formula in which the group \(L\) is 1,2-bis(diphenylphosphino) propane, compound 3d in the specific examples which follow. This compound has been found to have a potency against cancers which is about 200 times greater than that of the clinically used cis-platin. It is, in addition, highly active toward hepatocellular carcinoma, for which the mortality rate in human patients is high. It can be taken orally, and intestine adsorbed. Moreover, in vivo toxicity studies have revealed that this compound is non-toxic to nude mice even at concentrations 20-fold higher than the effective dose, and does not cause DNA mutation upon usage.

Scheme 1 shows illustrative examples of the \([\text{Au}^{III}_{m}(\text{CNC})_{n}L]^+\) complexes.
-continued

-1,2-bis(diphenylphosphino)ethane,
  \([\text{Au}_2(C^\text{N-N}C)_2(\mu\text{-dppm})]^2^- (3b)\)
-1,2-bis(diphenylphosphino)ethane,
  \([\text{Au}_2(C^\text{N-N}C)_2(\mu\text{-dppc})]^2^- (3c)\)
-1,2-bis(diphenylphosphino)propane,
  \([\text{Au}_2(C^\text{N-N}C)_2(\mu\text{-dppp})]^2^- (3d)\)
-1,2-bis(diphenylphosphino)butane,
  \([\text{Au}_2(C^\text{N-N}C)_2(\mu\text{-dppb})]^2^- (3e)\)
-1,2-bis(diphenylphosphino)pentane,
  \([\text{Au}_2(C^\text{N-N}C)_2(\mu\text{-dppp})]^2^- (3f)\)
-1,2-bis(diphenylphosphino)hexane,
  \([\text{Au}_2(C^\text{N-N}C)_2(\mu\text{-dpph})]^2^- (3g)\)

-\text{bis(diphenylphosphinoethyl)phenylphosphine},
  \([\text{Au}_{10}(C^\text{N-N}C)_3(\mu\text{-dpep})]^2^- (3h)\)

-\text{PPPh}_3, n = 1,
  \([\text{Au}(\text{Np}-C^\text{N-N}C)(\text{PPPh}_3)]^+ (3i)\)

-\text{R} = \text{PPPh}_3, n = 1,
  \([\text{Au}(\text{Np}-C^\text{N-N}C)(\text{PPPh}_3)]^+ (3i)\)

-\text{Au} = 1,
  \([\text{Au}_2(\text{Np}-C^\text{N-N}C)_2(\mu\text{-dppm})]^2^- (5d)\)
-\text{Au} = 2,
  \([\text{Au}_2(\text{Np}-C^\text{N-N}C)_2(\mu\text{-dppp})]^2^- (5e)\)
EXAMPLES

Example 1
Preparation and Characterization of the Gold(III) Complexes


[0051] The syntheses and characterization of 3a-6 had been reported previously [K.-H. Wong, K.-K. Cheung, M. C.-W. Chan, C.-M. Che, Organometallics 1998, 17, 3505].

[0052] Analytical data for the [Au(μ-CN(CN)2)L] complexes are shown below:

[0053] [Au2(CN)3(μ-dppp)][(CF3SO3)2] (3d (CF3SO3)2).

[0054] [Au2(CN)3(μ-dppp)][(CF3SO3)2] (Ce (CF3SO3)2).

[0055] [Au2(CN)3(μ-dppp)][(CF3SO3)2] (Se (CF3SO3)2).

[0056] [Au2(CN)3(μ-dpph)][(CF3SO3)2] (3g (CF3SO3)2).

[0057] [Au2(CN)3(μ-dppp)][(CF3SO3)2] (3h (CF3SO3)2).

[0058] [Au2(CN)3(μ-dppp)][(CF3SO3)2] (5c (CF3SO3)2).

The procedure was similar to that for 3b except bis(diphenylphosphino) methane (dpmm) was used. Yield: 48%; Caled for C25H18F4N2O4P5S5Au: C, 47.66; H, 2.94; N, 1.86. Found: C, 47.66; H, 2.94; N, 1.86. H NMR (400 MHz, [D4] DMSO): δ=8.21 (t, J=8.0 Hz, 2H), 7.94-7.88 (m, 12H), 7.61-7.46 (m, 16H), 6.91 (t, J=7.3 Hz, 4H), 6.50 (t, J=7.4 Hz, 4H), 6.10 (d, J=7.5 Hz, 4H), 3.78 (s, 4H), 3.47 ppm (s, 2H); UV/Vis (DMSO): λmax/nm (log e) 264 (4.68), 308 (4.23, sh), 389 (3.81), 403 (3.78); FAB-MS: m/z: 1414 [M+]..

[0059] [Au2(Np-CN(CN)2)3(μ-dppp)][(CF3SO3)2] (5d (CF3SO3)2).

The procedure was similar to that for 3b except bis(diphenylphosphino) methane (dpmm) was used. Yield: 80%; Caled for C27H18F4N2O4P5S5Au: C, 53.30; H, 3.02; N, 1.61. Found: C, 53.38; H, 3.05; N, 1.60. H NMR (400 MHz, CDCl3): δ=8.35 (t, J=7.2 Hz, 2H), 7.33 (d, J=8.0 Hz, 4H), 6.61 (s, 4H), 7.12 (t, J=8.0 Hz, 4H), 7.24 (t,
J=8.0 Hz, 4H), 7.34 (br, 12H), 7.38 (d, J=8.0 Hz, 4H), 7.43 (d, J=8.0 Hz, 4H), 7.58 (s, 4H), 7.80 (t, J=8.0 Hz, 1H), 8.26 ppm
(br, 8H); 31P NMR (162 MHz, CD3CN): δ=23.89 ppm; UV/Vis (DMSO): λmax/ε (nm (log ε)) 258 (5.01), 276 (5.01), 396
(4.41); FAB-MS: m/z 1431 [M+];

Example 3

Cytotoxicity Studies of the gold(III) Complexes
Towards Different Cancer and Normal Cell Lines

[0065] DNA is a major target for anticancer drugs [L. H. Harley, Nature Rev. Cancer 2002, 2, 188], and the binding of
gold(III) compounds to DNA has been studied extensively. The binding affinities of the phosphino-containing 3a and 3d
compounds to calf thymus DNA (ctDNA) were examined by means of UV/Vis absorption titration. Their binding constants
(Kb) toward ctDNA were determined from the plot of [ctDNA]/[Δε] vs [ctDNA] [C. V. Kumar, E. H. Asuncion, J.
Am. Chem. Soc. 1993, 115, 8547], with the Kb values of (2.1±0.7)×10^10 mol^-1 and (7.1±0.7)×10^10 mol^-1 for 3a and
3d respectively, which are comparable to that of the

Example 4

Cytotoxicity Studies of the gold(III) Complexes
Towards Different Cancer and Normal Cell Lines

[0066] Compound 3d does not interfere double-stranded DNA. A gel mobility shift assay was employed to determine
the intercalating property 3d [E. C. Long, J. K. Barton, Acc.
Chem. Res. 1990, 23, 271] to evaluate the effect 3d, had upon the
DNA binding affinities. A 100-base pair DNA ladder treated with 3d, or ethidium bromide (EB, DNA intercalator)
as well as a control were resolved by agarose gel electrophoresis. Briefly, gel electrophoresis of 100-bp DNA ladder
on a 1.5% (w/v) agarose gel was performed showing the mobility of DNA (50 μM bp-1) in the absence or presence of
ethidium bromide (EB), or compound 3d. Only EB exhibited a tailing effect, which is due to the intercalation of this
complex to the DNA, since the binding would cause elongation of
DNA. The bound DNA would have lower mobility compared
to that of the free DNA. In contrast, 3d did not cause this
tailing effect indicating that this compound does not bind to
DNA by intercalation.

Example 5

Cytotoxicity Studies of the gold(III) Complexes
Towards Different Cancer and Normal Cell Lines

[0067] Example 5 describes the results of in vitro cytotoxicity
studies of the gold(III)-phosphino complexes.

[0068] The cytotoxicities of the gold(III) compounds toward several human cancer cell lines including
nasopharyngeal carcinoma (SUNE1, and its cisplatin variant CNE1), hepatocellular carcinoma (HepG2), and cervical
epithelial carcinoma (HeLa.a) were determined using a well-established
MTT assay. The results are listed in Table 1.

[0069] Cisplatin chemotherapy is currently the last-line treatment for several types of cancer including nasopharyngeal
carcinoma (NPC). However, resistance to cisplatin is frequently encountered. Thus, new anti-cancer agents that are
active against cisplatin-resistant cell lines are in obvious
need. As shown in Table 1, the gold(III) compounds are
equally cytotoxic toward the cisplatin-sensitive and -resistant
NPC (SUNE1 and CNE1, respectively). The resistance factor,
Ic_{50} (CNE1/SUNE1), for cisplatin is 3.5, whereas the
corresponding values for the gold(III) compounds are close to
unity (Table 1). The lack of cross resistance suggests that the gold(III) compounds and cisplatin may induce cytotoxicity via different mechanisms, or the gold(III) compounds may bypass the cellular sequestration mechanism for cytotoxic agents (e.g., stable in elevated level of GSH).

Phosphine-containing compounds have long been known as potential anti-cancer agents. However, their instability under physiological conditions (e.g., formation of phosphine oxide) and non-specific binding affinities toward various biomolecules have hindered their clinical development as anticancer agents. In this work, we have found that the [Au(CNC)]^+ moiety may be used as carrier for phosphino compounds.

All the treated cells showed similar cellular absorption of Au which spanned over the range of 1.18-3.81 ng/cell. These cellular absorptions of Au, obviously, do not follow the trend of IC50 values the gold(III)-1,2-bis(diphenylphosphino)C6, thus revealing the presence of non-gold mediated cytotoxicity.

As mentioned earlier, ligation of the phosphine ligands to the [Au(CNC)]^+ moieties improved their stabilities and aqueous solubilities. Taking its inherent cytotoxicity into consideration, our results show that [Au(CNC)]^+ moiety can serve as a pendant carrier of phosphine ligands in biological systems.

| TABLE 1 |
| Cytotoxicities (IC50, 72 h) of the gold(III) compounds against selected human cancer cell lines. |

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>3a</td>
<td>n.d.</td>
<td>n.d.</td>
<td>17 ± 2</td>
<td>11 ± 2</td>
<td>0.65</td>
</tr>
<tr>
<td>3b</td>
<td>0.81 ± 0.08</td>
<td>n.d.</td>
<td>0.92 ± 0.12</td>
<td>1.2 ± 0.2</td>
<td>1.3</td>
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<tr>
<td>3c</td>
<td>0.14 ± 0.03</td>
<td>0.32 ± 0.08</td>
<td>0.25 ± 0.03</td>
<td>0.40 ± 0.06</td>
<td>1.6</td>
</tr>
<tr>
<td>3d</td>
<td>0.043 ± 0.02</td>
<td>0.21 ± 0.09</td>
<td>0.055 ± 0.01</td>
<td>0.99 ± 0.10</td>
<td>1.7</td>
</tr>
<tr>
<td>3e</td>
<td>3.4 ± 0.2</td>
<td>n.d.</td>
<td>1.5 ± 0.2</td>
<td>2.2 ± 0.3</td>
<td>1.5</td>
</tr>
<tr>
<td>3f</td>
<td>1.3 ± 0.3</td>
<td>n.d.</td>
<td>1.6 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>1.6</td>
</tr>
<tr>
<td>3g</td>
<td>3.2 ± 0.4</td>
<td>n.d.</td>
<td>4.3 ± 0.9</td>
<td>3.2 ± 0.5</td>
<td>0.74</td>
</tr>
<tr>
<td>3h</td>
<td>0.92 ± 0.09</td>
<td>3.8 ± 0.6</td>
<td>0.26 ± 0.03</td>
<td>0.40 ± 0.05</td>
<td>1.5</td>
</tr>
</tbody>
</table>


Example 5
Induction of Cell-Cycle Arrest by the Gold(III) Complexes

Example 5 describes the study of cell-cycle arrest by the gold(III) complexes.

Example 5: 1c:2q To find out whether cellular DNA is a major target of the gold(III) compounds, we studied the cell cycle profiles of 3d-treated cancer cells. Cell cycle analysis was performed by assessing the DNA content of cells stained with propidium iodide using flow cytometry. This enables quantification of the total cellular populations in the different phases of the cell cycle (G0/G1, S and G2/M). The flow cytometric data for SUNE1 cells treated with cisplatin or 3d is presented in Figs. 3A and 3B. Treatment with 3d did not significantly affect the cell cycle after 24- or 48-h incubation. Compound 3d appears to affect its cytotoxicity via some alternative mechanistic pathways.

Example 6
In Vivo Studies of Antitumor Activity of the Gold (III) Complexes in an Orthotopic Rat Hepatocellular Carcinoma (HCC) Model Using the Rat Hepatoma Cell McA-RH7777

Example 6 describes the in vivo anti-cancer activities of the gold(III) complexes.
The anti-tumor effect of complex 3d was examined in the rat HCC model. Male Buffalo rats, 8-12 weeks old and weighing 250-320 g, were purchased from Charles River Labs (Wilmington, Mass.). The animals were kept under standard condition with constant day-night rhythm and free access to water and standard laboratory food. Tumor model was induced by injection of 2×10^6 McA-RH7777 cells into the left lobe of the liver. Seven days after tumor induction, the rat livers were exposed to confirm the formation of tumor nodules. All the experimental rats had developed a single tumor nodule with size ranging from 1×2 to 2×3 mm^2. The animals were then divided into the following 4 groups: (1) sham operation (NT); (2) 3d, 0.25 mg/kg; (3) 3d, 0.5 mg/kg; and (4) 3d, 0.75 mg/kg. Complex 3d suspension was prepared by dissolving the complex in dimethyl sulfoxide (DMSO) and diluted with phosphate-buffered saline (pH 7.4, 1:1, v:v). Different doses of 3d (0.25, 0.5 or 0.75 mg/kg) were injected intratumorally at the first instance, followed by intraperitoneal injection twice weekly until they died.

What is claimed is:

1. A method for treating cancer in a patient in need of such treatment, comprising administering to the patient a composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a gold(III) complex having the structure I or structure II set out below:

\[ R^1-R^{15} \text{ are each independently hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, halo, nitro, hydroxyl, alkoxy, substituted alkoxy, phenoxyl, substituted phenoxyl, aroxyl, substituted aroxyl, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, cyano, isocyno, substituted isocyano, carboxyl, substituted carboxyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfanyl, substituted sulfanyl, sulfonyl, substituted sulfonyl, sulfonyl, acid, substituted sulfonic acid, phosphonato, substituted phosphonato, phosphoramidate, substituted phosphoramidate, } C_7-C_{20} \text{ cyclic, substituted } C_7-C_{20} \text{ cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, or polypeptide group;}

L is a P-donor ligand;

each X^p is independently a pharmaceutically acceptable counter-ion;

m is an integer ranging from 1 to 10;

n is an integer ranging from 1 to 100;

p is an integer ranging from 3 to 3;

q is equal to the absolute value of m or p; and

qX^p is absent when n is 0.

2. The method according to claim 1, wherein the gold (III) complexes induce apoptosis in cancer cells.

3. The method of claim 1, wherein R^1-R^{15} are each —H; m is 1; n is 0; qX^p is absent; and L is:
4. The method of claim 1, wherein $R^1$-$R^{15}$ are each $-H$; $m$ is 1; $n$ is 0; $qX^p$ is absent; and $L$ is:

5. The method of claim 1, wherein $R^1$-$R^{15}$ are each $-H$; $m$ is 2; $n$ is 2; $q$ is 2; $X^p$ is $CF_3SO_3^-$; and $L$ is:

and wherein each $P$ atom is separately connected to one $[Au(CNC)]^+$ unit.

6. The method of claim 1, wherein $R^1$-$R^{15}$ are each $-H$; $m$ is 2; $n$ is 2; $q$ is 2; $X^p$ is $CF_3SO_3^-$; and $L$ is:

7. The method of claim 1, wherein $R^1$-$R^{15}$ are each $-H$; $m$ is 2; $n$ is 2; $q$ is 2; $X^p$ is $CF_3SO_3^-$; and $L$ is:

and wherein each $P$ atom is separately connected to one $[Au(CNC)]^+$ unit.

8. The method of claim 1, wherein $R^1$-$R^{15}$ are each $-H$; $m$ is 2; $n$ is 2; $q$ is 2; $X^p$ is $CF_3SO_3^-$; and $L$ is:

and wherein each $P$ atom is separately connected to one $[Au(CNC)]^+$ unit.

9. The method of claim 1, wherein $R^1$-$R^{15}$ are each $-H$; $m$ is 2; $n$ is 2; $q$ is 2; $X^p$ is $CF_3SO_3^-$; and $L$ is:

and wherein each $P$ atom is separately connected to one $[Au(CNC)]^+$ unit.

10. The method of claim 1, wherein $R^1$-$R^{15}$ are each $-H$; $m$ is 2; $n$ is 2; $q$ is 2; $X^p$ is $CF_3SO_3^-$; and $L$ is:

and wherein each $P$ atom is separately connected to one $[Au(CNC)]^+$ unit.
11. The method of claim 1, wherein R₁⁻R₁⁵ are each —H; 
m is 3; n is 3; q is 3; X is CF₂SO₂⁻; and L is:

and wherein each P atom is separately connected to one 
[Au(CNC)]⁺ unit.

12. The method of claim 1, wherein R₁⁻R₁⁵ are each —H; 
m is 3; n is 3; q is 3; X is CF₂SO₂⁻; and L is:

and wherein each P atom is separately connected to one 
[Au(CNC)]⁺ unit.

13. A pharmaceutical composition for the treatment or 
prophylaxis of cancer in an animal patient, the composition 
comprising a pharmaceutically acceptable carrier and an 
effective amount of gold compound having the following 
structure I or structure II:

L is a P-donor ligand;
each XP is independently a pharmaceutically acceptable 
counter-ion;
m is an integer ranging from 1 to 10;
n is an integer ranging from —10 to 100;
p is an integer ranging from —3 to 3;
q is equal to the absolute value of n/p;
qX is absent when n is 0.

14. The composition of claim 13 wherein, in the gold 
compound, each R is hydrogen, m is 1 or 2, n is 0, 1 or 2 and 
L is a phenyl phosphine group.

15. The composition of claim 14 wherein the gold 
compound is of general formula I,
m is 2 and L is selected from the group consisting of

and m is 2.

17. 2,6-diphenylpyridine-gold co-ordination complexes of formula I:

wherein each of $R^4$-$R^{11}$ is H, m is 2, n is 2+, q is 2, $X^0$ is (CF$_3$SO$_2$)$_2$N and L is a 1,2-bis(diphenylphosphino) C$_6$H$_{10}$ alkane group.

18. A 2,6-diphenylpyridine-gold co-ordination complex according to claim 17 in which the group L is 1,2-bis(diphenylphosphino)propane.

* * * * *