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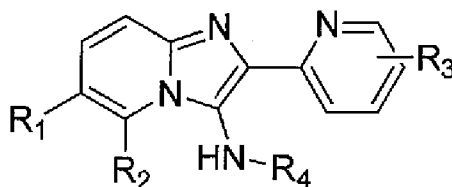
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(54) Title: IMIDAZO[1,2-A]PYRIDINE COMPLEXES WITH ANTICANCER ACTIVITY



Formula (A)

(57) Abstract: This invention relates to a metal complex of an imidazo[1,2-a]pyridine ligand and/or a metal complex of an imidazo[1,2-a] pyridine ligand derivative and/or an analogue thereof, wherein said metal complexes are for use in treating cancer. Typically, the cancer is breast cancer, colorectal cancer, colon cancer, and/or leukemia.

IMIDAZO[1,2-A]PYRIDINE COMPLEXES WITH ANTICANCER ACTIVITY

FIELD OF INVENTION

This invention relates to novel metal complexes of imidazo[1,2-a]pyridine derivatives and/or analogues thereof, and extends to their manufacture. This invention further extends to copper complexes of imidazo[1,2-a]pyridine derivatives and/or analogues thereof for use in the treatment of cancer, particularly breast cancer, colorectal cancer, and leukemia.

BACKGROUND

Cancer is considered as a leading cause of death in developed countries and consequently new and innovative anti-cancer pharmaceuticals are required to be developed. Many standard therapies are toxic to healthy non-cancerous cells resulting in unwanted side effects and/or serious life threatening adverse effects. Further, cancer often becomes resistant to known therapies resulting in its further spread and/or a need for increased and more toxic dosage regimes being administered to the cancer sufferer.

One such standard therapy includes the administration of cisplatin, a very toxic and expensive chemotherapeutic.

Imidazo[1,2-a]pyridines are a class of chemical compounds known to display a wide range of biological activities. For example, imidazo[1,2-a]pyridines are known to treat insomnia and anxiety and have been investigated in the treatment of HIV, possibly impacting several independent biochemical pathways. The Applicant has previously shown that certain derivatives and/or analogues of imidazo[1,2-a]pyridines show biological activity against colon cancer (see PCT/IB2010/051427).

There remains a need to provide new and inventive anti-cancer agents.

SUMMARY

In accordance with a **first aspect** of this invention there is provided a metal complex of an imidazo[1,2-a]pyridine ligand and/or a metal complex of an imidazo[1,2-a]pyridine ligand derivative and/or an analogue thereof.

The metal complex may further include at least one second ligand interacting with the metal. The interaction between the imidazo[1,2-a]pyridine ligand and the metal, and the interaction between the second ligand and the metal, may each be a molecular interaction, and may be at least one of the following group of molecular interactions: ionic, covalent, dative, coordinative, and van der Waals.

The at least one second ligand may be an oxygen containing ligand and/or a halogen interacting with the metal. Preferably, the at least one second ligand is an oxygen containing ligand.

The oxygen containing second ligand may include at least one of the following groups: nitrates, acetates, sulphates and phosphates. Typically, the oxygen containing second ligand is nitrate and/or acetate.

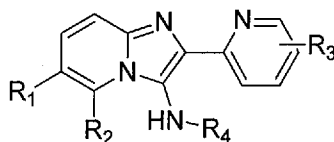
The metal may be a metal ion. The metal ion may be copper. The copper may be Cu^{1+} and/or Cu^{2+} . Preferably, the metal is Cu^{2+} .

The metal complex may be a copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue. Said copper (II) complex may provide a distorted square planar geometry.

There is provided for the copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue to provide a distorted square planar geometry, wherein the oxygen containing second ligand provides at least one oxygen atom being in a position out of plane relative to the remainder of the complex.

The copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue may form dimers.

The imidazo[1,2-a]pyridine ligand derivative and/or analogue may be a compound of Formula A:



Formula (A)

wherein,

R₁, R₂ and R₃ may each be a hydrogen or a halogen; and

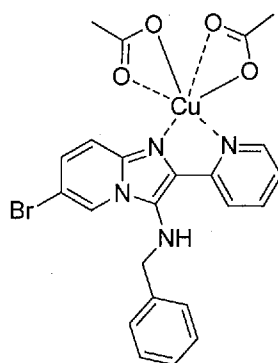
R₄ may be any one of the following group: benzyl and cyclohexyl.

Preferably, R₁, R₂ and R₃ may each be any one of the following group: hydrogen, bromine and chlorine.

It is to be understood that the position of R₃ may vary around an aromatic ring structure, and this is indicated by a line representing a bond projecting into said ring structure.

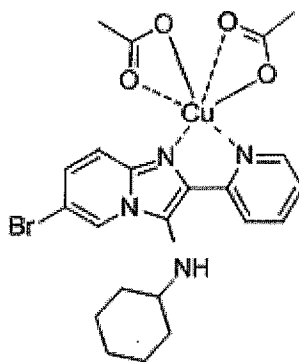
The copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue thereof may include at least one of the following group:

copper N-benzyl-6-bromo-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate



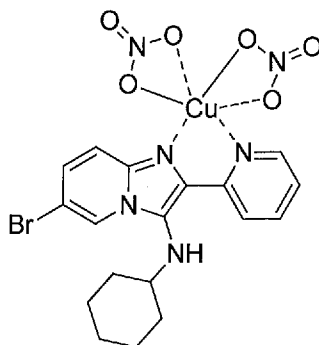
Formula (A1);

copper 6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate



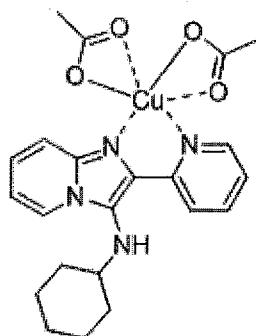
Formula (A2.1);

copper 6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine nitrate



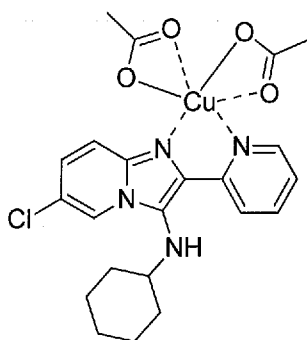
Formula (A2.2);

copper N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate



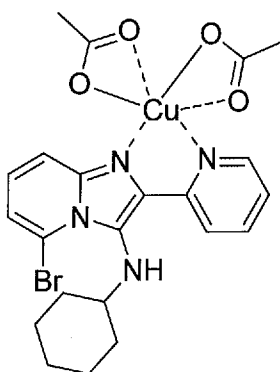
Formula (A3);

copper 6-chloro-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate



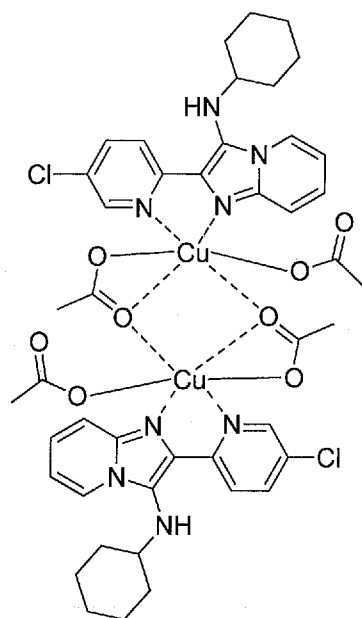
Formula (A4);

copper 5-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate



Formula (A5); and

copper 2-(5-chloropyridin-2-yl)-N-cyclohexylimidazo[1,2-a]pyridin-3-amine acetate



Formula (A6) (shown as a dimer).

In accordance with a **second aspect** of this invention there is provided a metal complex of an imidazo[1,2-a]pyridine ligand and/or a metal complex of an imidazo[1,2-a]pyridine ligand derivative and/or an analogue thereof, for use in treating cancer.

The cancer may include at least one of the following group: breast cancer, colorectal cancer, colon cancer, and leukemia.

The metal complex may further include at least one second ligand interacting with the metal. The interaction between the imidazo[1,2-a]pyridine ligand and the metal, and the interaction between the second ligand and the metal, may each be a molecular interaction, and may be at least one of the following group of molecular interactions: ionic, covalent, dative, coordinative, and van der Waals.

The at least one second ligand may be an oxygen containing ligand and/or a halogen interacting with the metal. Preferably, the at least one second ligand is an oxygen containing ligand.

The oxygen containing second ligand may include at least one of the following groups: nitrates, acetates, sulphates and phosphates. Typically, the oxygen containing ligand is nitrate and/or acetate.

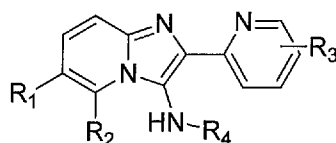
The metal may be a metal ion. The metal ion may be copper. The copper may be Cu¹⁺ and/or Cu²⁺. Preferably, the metal is Cu²⁺.

The metal complex may be a copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue. Said copper (II) complex may provide a distorted square planar geometry.

There is provided for the copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue to provide a distorted square planar geometry, wherein the oxygen containing second ligand provides at least one oxygen atom being in a position out of plane relative to the remainder of the complex.

The copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue may form dimers.

The imidazo[1,2-a]pyridine ligand derivative and/or analogue may be a compound of Formula A:



Formula (A)

wherein,

R₁, R₂ and R₃ may each be a hydrogen or a halogen; and

R₄ may be any one of the following group: benzyl and cyclohexyl.

Preferably, R₁, R₂ and R₃ may each be any one of the following group: hydrogen, bromine and chlorine.

It is to be understood that the position of R₃ may vary around an aromatic ring structure, and this is indicated by a line representing a bond projecting into said ring structure.

The copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue may include at least one of the following group:

Formula (A1) - copper N-benzyl-6-bromo-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

Formula (A2.1) - copper 6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

Formula (A2.2) - copper 6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine nitrate;

Formula (A3) - copper N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

Formula (A4) - copper 6-chloro-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

Formula (A5) - copper 5-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate; and

Formula (A6) - copper 2-(5-chloropyridin-2-yl)-N-cyclohexylimidazo[1,2-a]pyridin-3-amine acetate.

In accordance with a **third aspect** of this invention there is provided use of a metal complex of an imidazo[1,2-a]pyridine ligand and/or a metal complex of an imidazo[1,2-a]pyridine ligand derivative and/or an analogue thereof, in the manufacture of a medicament for treating cancer.

The use wherein the cancer may include at least one of the following group: breast cancer, colorectal cancer, colon cancer, and leukemia.

The use wherein the metal complex may further include at least one second ligand for interaction with the metal. The interaction between the imidazo[1,2-a]pyridine ligand and the metal, and the interaction between the second ligand and the metal, may each be a molecular interaction, and may be at least one of the following group of molecular interactions: ionic, covalent, dative, coordinative, and van der Waals.

The use wherein the at least one second ligand may be an oxygen containing ligand and/or a halogen interacting with the metal. Preferably, the at least one second ligand is an oxygen containing ligand.

The use wherein the oxygen containing second ligand may include at least one of the following groups: nitrates, acetates, sulphates and phosphates. Typically, the oxygen containing ligand is nitrate and/or acetate.

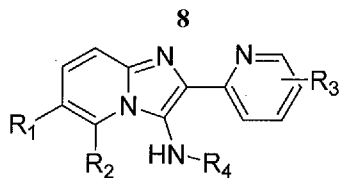
The use wherein the metal may be a metal ion. The metal ion may be copper. The copper may be Cu^{1+} and/or Cu^{2+} . Preferably, the metal is Cu^{2+} .

The use wherein the metal complex may be a copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue. Said copper (II) complex may provide a distorted square planar geometry.

The use wherein the copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue which provides a distorted square planar geometry, wherein the oxygen containing second ligand provides at least one oxygen atom being in a position out of plane relative to the remainder of the complex.

The use wherein the copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue may form dimers.

The use wherein the imidazo[1,2-a]pyridine ligand derivative and/or analogue may be a compound of Formula A:



Formula (A)

wherein,

R₁, R₂ and R₃ may each be a hydrogen or a halogen; and

R₄ may be any one of the following group: benzyl and cyclohexyl.

Preferably, R₁, R₂ and R₃ may each be any one of the following group: hydrogen, bromine and chlorine.

It is to be understood that the position of R₃ may vary around an aromatic ring structure, and this is indicated by a line representing a bond projecting into said ring structure.

The use wherein the copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue thereof may include at least one of the following group:

Formula (A1) - copper N-benzyl-6-bromo-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

Formula (A2.1) - copper 6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

Formula (A2.2) - copper 6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine nitrate;

Formula (A3) - copper N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

Formula (A4) - copper 6-chloro-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

Formula (A5) - copper 5-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate; and

Formula (A6) - copper 2-(5-chloropyridin-2-yl)-N-cyclohexylimidazo[1,2-a]pyridin-3-amine acetate.

In accordance with a **fourth aspect** of this invention there is provided a method of treating cancer comprising the step of administering a metal complex of an imidazo[1,2-a]pyridine ligand and/or a metal complex of an imidazo[1,2-a]pyridine ligand derivative and/or an analogue thereof to a human or animal in need thereof.

The method wherein the cancer may include at least one of the following group: breast cancer, colorectal cancer, colon cancer, and leukemia.

The method wherein the metal complex is any metal complex described in any one of the first, second and/or third aspects of the invention herein.

The method wherein the step of administering may be via parenteral or non-parenteral means.

There is further provided for a metal complex of an imidazo[1,2-a]pyridine ligand and/or a metal complex of an imidazo[1,2-a]pyridine ligand derivative and/or an analogue thereof according to a first aspect of this invention; a metal complex of an imidazo[1,2-a]pyridine ligand and/or a metal complex of an imidazo[1,2-a]pyridine ligand derivative and/or an analogue thereof for use in treating cancer according to a second aspect of this invention; use of a metal complex of an imidazo[1,2-a]pyridine ligand and/or a metal complex of an imidazo[1,2-a]pyridine ligand derivative and/or an analogue thereof in the manufacture of a medicament according to a third aspect of this invention; and a method of treating cancer according to a fourth aspect of this invention; substantially as herein described, illustrated and/or exemplified with reference to any one of the chemical representations, figures, and/or examples herein.

BRIEF DESCRIPTION OF THE FIGURES

Embodiments of the invention will be described below by way of example only and with reference to the accompanying figures in which:

- FIGURE 1** shows crystallographic data of a compound of Formula (A1);
- FIGURE 2** shows crystallographic data of a compound of Formula (A2.1);
- FIGURE 3** shows crystallographic data of a compound of Formula (A2.2);
- FIGURE 4** shows crystallographic data of a compound of Formula (A3);
- FIGURE 5** shows crystallographic data of a compound of Formula (A4);
- FIGURE 6** shows crystallographic data of a compound of Formula (A5);
- FIGURE 7** shows crystallographic data of a compound of Formula (A6);
- FIGURE 8** shows leucocyte cell viability when treated with 20 μ M of selected metal complexes of the Formula (A1) to (A6) for 24 hours;
- FIGURE 9** shows the effect of Formula (A2.1) on cell and nuclear morphology on MCF-7 and HL-60 cells after a 48 h exposure period indicates apoptotic and necrotic cell death. MCF-7 and HL-60 cells were treated with Formula (A2.1) at a concentration of 1.5 μ M and 2.0 μ M,

respectively. Images were captured at a 400 × magnification and scalebars represent 20 micron. Legend: N = normal nucleus; NN = necrotic nucleus; AN = apoptotic nucleus; LAN = late apoptotic nucleus;

FIGURE 10 The effect of Formulas (A2.2), (A3), and (A5) on the cell and nuclear morphology of MCF-7 cells shows the formation of cytoplasmic vacuoles, acidic granules, deformed- and apoptotic nuclei. Cells were treated with 10 μM of the test compounds for 24 h. Images were captured at a 400 × magnification and scalebars represent 20 micrometers. Legend: N = nucleus; CN = contracted nucleus; AN = apoptotic nucleus; DN = deformed nucleus; AG = acidic granules; F = filopodia; V = vacuoles;

FIGURE 11 shows the formation of cytoplasmic vacuoles do not co-localize with the acidic granules. MCF-7 cells were treated with 10 μM of Formula (A2.1) for 24 hours. Images were captured at a 400 x magnification and scalebars represent 20 micrometers. Legend: AG = acidic granules, V = Cytoplasmic vacuoles, N = nucleus; and

FIGURE 12 shows the effect of Formula (A5) on MCF-7 cells at a concentration of 5 μM for 24 hours indicates that the formation of apoptotic nuclei and acidic granules is a dose dependent effect. Images were captured at a 400 × magnification and scalebars represent 20 micrometers. Legend: AN = apoptotic nucleus; AG = acidic granules.

DETAILED DESCRIPTION

In general terms the content of the Summary, above, is repeated herein by way of reference only to avoid repetition. Any embodiments of the invention detailed hereunder are not intended to be limiting. The disclosure herein provides for metal complexes, preferably copper complexes, of imidazo[1,2-a]pyridine derivatives and/or analogues, and their use in the treatment of cancer, preferably breast cancer, colon cancer, colorectal cancer and/or leukemia.

Imidazo[1,2-a]pyridines are known to show bioactivity against a number of different and non-related medical conditions including insomnia, anxiety, cancer and HIV. Metal complexes of imidazo[1,2-a]pyridines have been investigated for use in fluorescent materials and LED applications.

The chemistry and biochemistry of copper in medicine, physiology and pathology teaches away from its use in developing anti-cancer pharmaceutical compositions. It has been shown that copper concentration in the human body is strictly regulated to avoid toxicity in cells. Typically, regulation at cellular, organ and systemic level is required to avoid unwanted radical-type reactions from occurring, which reactions

are aided by the redox activity of copper and its affinity for binding sites that should be occupied by other metals. An abnormal concentration of copper is toxic and harmful to cells, organs and the overall biological system.

Abnormal accumulation of copper is associated with several pathological states including neurodegenerative disorders (for example Alzheimer, Parkinson and Creutzfeld-Jakob), rheumatoid arthritis, gastrointestinal ulcers, epilepsy, diabetes, and cancer. It has been shown that cancer cells contain significantly greater amounts of copper than their healthy cell equivalents, suggesting that cancer cells do not regulate intracellular copper levels as strictly or effectively as healthy cells. These findings suggest that treating cancer cells (which contain abnormally greater amounts of copper) by providing more copper to the cancer cell, as per currently framed invention, will not be successful. This is in direct contrast to the invention described herein, and the Applicant was surprised at the anti-cancer activity of the copper complexes according to this invention.

The copper complexes of imidazo[1,2-a]pyridine derivatives and/or analogues according to this disclosure are surprisingly active against cancer cell lines, and/or induce apoptosis, paraptosis and/or autophagy of cancer cells. Specific effects on DNA morphology indicate that the nucleus may be a target organelle (see experimental detail below).

Cisplatin, is a known anti-cancer agent that has a square planar geometry. The square planar geometry of cisplatin is enabled by the electron configuration of platinum and its ions. Cisplatin and its derivatives are expensive to manufacture, have low solubility and are toxic to healthy cells with long term neurotoxic effects on surviving patients.

Copper (II) (Cu^{2+}) ions are an exception to the general rules governing electron configurations, and instead of providing the most stable structure with an electron configuration of $[\text{Ar}]4s^13d^8$, Cu^{2+} takes on the electron configuration of $[\text{Ar}]4s^03d^9$ and is stabilized through Jahn-Teller distortion. Since the nature of the distortion is not readily predictable, and will be dependent on several factors including for example the nature of the coordinating ligand, predicting stability and/or lability of copper (II) metal complexes is not readily possible. Even more unpredictable would be their biochemical properties.

The results described herein below indicate, without being limited to theory, that the complexes of imidazo[1,2-a]pyridine derivatives and/or analogues according to this invention may have distorted square planar geometries including out of plane ligands or portions of ligands being out of plane.

Leucocyte cell viability tests of the exemplified complexes (Formulas A1 to A6) indicate that said exemplified complexes may be non-lethal to non-cancerous cells. This is very advantageous, since not

only are these copper complexes cheaper to manufacture when compared to cisplatin, they may also be less toxic relative to cisplatin which has been shown to be extremely toxic to healthy non-cancerous cells.

The chemical structure of ligands is regarded as being of critical importance in regard to both metal complex formation and the physico-chemical properties of the formed metal complex. Herein below it is shown that the Cu²⁺ electron configuration and/or coordination environment also plays a critical role in providing anti-cancer activity for the copper (II) imidazo[1,2-a]pyridine complexes.

It is shown that the physico-chemical properties of the complex as a whole are not linked to the properties of its component reagents. For example, excess copper is often associated with cancer cells, however, providing a copper (II) imidazo[1,2-a]pyridine complex to cancer cells shows anti-cancer activity. Further, the anticancer activity of metal salts (Cu(Ac)₂, Cu(NO₃)₂ and CuCl₂) were also investigated and were found to be inactive against cancer cell lines used herein. Still further, Ligands A1, A2, A3, A4, A5 and A6 were all inactive against cancer. Consequently, the observed anti-cancer activity from copper complexes in accordance with this invention is wholly unexpected when considering the inactivity of the ligands and metal salt reagents separately.

In accordance with this invention the copper (II) complexes of imidazo[1,2-a]pyridine derivatives and/or analogues may be considered as an independent molecular species having distinct properties separate from its component reagents. The molecular interactions between the imidazo[1,2-a]pyridine derivative and/or analogue, the copper, and other organic ligands are such that the overall metal complex is a unique stable molecular species. The copper (II) electronic structure, the coordination environment around copper (II) and its molecular interactions and/or bond formation with bidentate imidazo[1,2-a]pyridine ligands and other oxygen containing ligands and/or halogen ligands provides for a unique anticancer complex indicating a nuclear mechanism of action. The unique molecular interactions and/or bond formation and/or coordination between ligands and metal provides for an independent molecular species with unique characteristics.

Predicting physico-chemical properties of chemical compounds is not readily possible, and the quest to be able to predict the nature of intermolecular forces in the design of property-specific supramolecular entities (such as the imidazo[1,2-a]pyridine copper complexes according to this disclosure) remains one of the greatest scientific challenges of our age (Ball, P., *Nature*, 1996, 381. pp.648-650 and Maddox, J., *Nature*, 1988. 335. pp. 201).

Imidazo[1,2-a]pyridines may have a plethora of different functional groups which may readily impact of their physico-chemical properties. Based on the sheer number of possibilities and lack of guiding selection principles it is difficult to predict which imidazo[1,2-a]pyridine derivative and/or analogue would, when

complexed with copper, provide for an anti-cancer agent, preferably an anti-breast cancer, anti-colon cancer and/or anti-leukemia agent. Similarly, the sheer number of known uses for imidazo[1,2-a]pyridines in biological applications suggests several independent and unpredictable mechanisms of action.

In accordance with a first aspect of this invention there is provided a metal complex of an imidazo[1,2-a]pyridine ligand and/or a metal complex of an imidazo[1,2-a]pyridine ligand derivative and/or an analogue thereof. The metal complex typically further includes at least one oxygen containing second ligand interacting with the metal. The interaction between the imidazo[1,2-a]pyridine ligand and the metal, and the interaction between the second ligand and the metal, may each be a molecular interaction, and may be at least one of the following group of molecular interactions: ionic, covalent, dative, coordinative, and van der Waals.

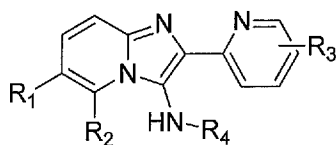
Certain non-limiting embodiments of the metal complexes are provided below.

The metal complex typically includes at least one oxygen containing second ligand interacting with the metal. The oxygen containing second ligand may include at least one of the following groups: nitrates, acetates, sulphates and phosphates. Typically, the oxygen containing second ligand is nitrate and/or acetate.

The metal is typically a metal ion. The metal ion is typically copper. The copper may be Cu^{1+} and/or Cu^{2+} . Preferably, the metal is Cu^{2+} . The metal complex may be a copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue. Said copper (II) complex may provide a distorted square planar geometry. The copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue which provides a distorted square planar geometry, wherein the oxygen containing ligand provides at least one oxygen atom being in a position out of plane relative to the square planar arrangement.

The copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue may form dimers. In this regard, the complex of Formula (A6) shows two distorted square planar copper (II) complexes forming a dimer. This is also seen in the accompanying crystallographic data herein below.

The imidazo[1,2-a]pyridine derivative ligand and/or analogue may be a compound of Formula A:



Formula (A)

wherein,

R₁, R₂ and R₃ may each be a hydrogen or a halogen; and

R₄ may be any one of the following group: benzyl and cyclohexyl.

Preferably, R₁, R₂ and R₃ may each be any one of the following group: hydrogen, bromine and chlorine.

It is to be understood that the position of R₃ may vary around an aromatic ring structure, and this is indicated by a line representing a bond projecting into said ring structure.

The copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue may include at least one of the following group:

Formula (A1) - copper N-benzyl-6-bromo-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

Formula (A2.1) - copper 6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

Formula (A2.2) - copper 6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine nitrate;

Formula (A3) - copper N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

Formula (A4) - copper 6-chloro-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

Formula (A5) - copper 5-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate; and

Formula (A6) - copper 2-(5-chloropyridin-2-yl)-N-cyclohexylimidazo[1,2-a]pyridin-3-amine acetate.

In accordance with a second aspect of this invention there is provided a metal complex of an imidazo[1,2-a]pyridine ligand and/or a metal complex of an imidazo[1,2-a]pyridine ligand derivative and/or an analogue thereof, for use in treating cancer, as described in the Summary above. The cancer may include at least one of the following group: breast cancer, colorectal cancer, colon cancer, and leukemia. The metal complexes according to this disclosure have been found to be active against several different cancer cell lines.

SYNTHESIS OF IMIDAZO[1,2-A]PYRIDINES:

Typically, imidazo[1,2-a]pyridines are synthesized according to the Groebke-Blackburn-Bienaymé multicomponent reaction, wherein functionalized imidazo[1,2-a]pyridines are synthesized in a single step from substituted 2-aminopyridines, isocyanides and aldehydes in the presence of an acid catalyst such as zinc(II)chloride or scandium triflate.

All the reagents and chemicals were purchased from Sigma-Aldrich, Merck or ACE Chemicals and used without further purification unless otherwise noted. ^1H NMR (nuclear magnetic resonance) and ^{13}C NMR were recorded using Bruker Advance-500 and Bruker Advance-300 spectrometers operating at 500 MHz and 300 MHz respectively. All spectra were recorded in CDCl_3 or MeOD with all chemical shift values reported in parts per million referenced against TMS (tetramethylsilane) as an internal standard. Coupling constants are given in Hertz (Hz). Infrared spectra were recorded on a Bruker Tensor 27 standard system spectrometer. The Groebke multicomponent coupling reactions were monitored by thin layer chromatography (TLC) which was performed on aluminium-backed MachereyNagel Alugram Sil G/UV254 plates pre-coated with 0.25 mm silica gel 60.

GENERAL PROCEDURE FOR THE GROEBKE MULTICOMPONENT REACTION:

The general procedure for the synthesis of imidazo[1,2-*a*]pyridines involved the combination of the appropriate 2-aminopyridine, isocyanide and pyridinecarbaldehyde in equal mole ratios in a round bottomed flask. To this Montmorillonite K-10 Clay was added (same mass as the 2-aminopyridine used) followed by ethanol as the solvent. The mixture was stirred under reflux conditions for 4-48 hours, in some cases with additional stirring at room temperature for a further 12 hours. The clay was filtered off through celite and washed with hot EtOAc (ethyl acetate). The filtrate was then concentrated *in vacuo* and the product purified by recrystallization or by column chromatography. ^{13}C and ^1H nuclear magnetic NMR studies were conducted.

SYNTHESIS OF METAL COMPLEXES OF IMIDAZO[1,2-A]PYRIDINES:

The relevant imidazo[1,2-*a*]pyridine was added to diethylether to form a solution. Equimolar amounts of an appropriate metal salt, for example copper(II)chloride (CuCl_2), copper(II)acetate ($\text{Cu}(\text{Ac})_2$) and copper(II)nitrate ($\text{Cu}(\text{NO}_3)_2$) were added to the solution and stirred for over 18 hours at room temperature. During this time a powder formed in the solution and this was collected by filtration and washed with diethylether to remove any unreacted imidazo[1,2-*a*]pyridine. The copper complexes were then dissolved in acetone, filtered and concentrated *in vacuo* to remove any unreacted copper salts. The powder was then crystallized slowly from suitable solvents to afford x-ray quality crystals. Infra-red (IR) studies were also conducted.

Formula (A1): Copper N-benzyl-6-bromo-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate:

N-benzyl-6-bromo-2-(pyridin-2-yl)imidazo[1,2-*a*]pyridin-3-amine (Ligand A1) was synthesized as follows. Combining 5-bromo-2-aminopyridine (2.304 g, 13.317 mmol), benzyl isocyanide (1.56 g, 13.317 mmol, 1 eq) and pyridinecarbaldehyde (1.27 ml, 13.317 mmol, 1 eq) with Montmorillonite K-10 Clay

(2.30 g) and following the general procedure with EtOH as the solvent resulted in 13 (2.037g, 40 %), which was collected as a yellow powder after recrystallization from ether. $R_f = 0.57$ (50% EtOAc/Hexane), M.p. = 121 °C, ^1H NMR (300 MHz, Chloroform- d) δ 8.53 – 8.49 (1H, m), 8.19 – 8.07 (2H, m), 7.74 (1H, td, $J = 7.8, 1.8$ Hz), 7.48 – 7.23 (6H, m), 7.19 – 7.10 (2H, m), 6.54 (1H, t, $J = 7.0$ Hz), 4.24 (2H, d, $J = 6.8$ Hz). ^{13}C NMR (75 MHz, CDCl_3) δ 154.5, 148.6, 139.2, 138.9, 136.6, 131.9, 131.3, 128.5, 128.1, 127.5, 126.9, 122.8, 121.5, 120.3, 118.5, 106.8, 51.4. Infra-red spectroscopy (IR) ($\nu_{\text{m}}, \text{cm}^{-1}$) = 3302, 3028, 2930, 2856, 1589, 1516, 1477, 1429, 1396, 1352, 1317, 1290, 1251, 1226, 1110, 1037, 995, 931, 790, 733, 798, 663, 628, 617. EIS-MS (m/z) calculated $M+H$ for $\text{C}_{17}\text{H}_{19}\text{N}_3\text{Br}$ 379.0560, found 379.0558. Anal. Calcd (%) for $\text{C}_{19}\text{H}_{15}\text{N}_4\text{Br}$: C 60.17, H 3.99, N 14.77. Found: C 60.442, H 5.337, N 14.575.

N-benzyl-6-bromo-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine (Ligand A1) (0.200 g, 0.527 mmol) and copper(II) acetate monohydrate (0.116 g, 0.570 mmol, 1.1 eq) were used as described above. After collection of the green powder, the product Formula (A1) (0.320 g, 99 %) was recrystallized by vapour diffusion from MeOD and ether. The structure of the complex was confirmed by single crystal X-ray crystallography. IR ($\nu_{\text{m}}, \text{cm}^{-1}$) = 3428, 3246, 3084, 3029, 2999, 2925, 1577, 1564, 1526, 1498, 1482, 1391, 1330, 1244, 1204, 1166, 1124, 1096, 1045, 1018, 975, 930, 842, 813, 785, 750, 704, 675, 647, 617. Anal. Calcd (%) for $\text{CuC}_{23}\text{H}_{21}\text{N}_4\text{O}_4\text{Br}$: C 49.25, H 3.77, N 9.99. Found: C 47.517, H 4.767, N 9.407.

It is interesting to note that Ligand A1 was not active against any cancer cell lines, however, copper (II) complex compound of Formula (A1) was active against all tested cancer cell lines (see Table 1). The Applicant did not expect that the complex of Formula (A1) would be active against all the tested cancer cell lines.

Formula (A2.1) Copper 6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate:

6-bromo-N-cyclohexyl-2-(pyridine-2-yl)imidazo[1,2-a]pyridine-3-amine (0.200 g, 0.539 mmol) (Ligand A2) was synthesized as follows: 2-Amino-5-bromopyridine (2.00 g, 11.56 mmol), 2-pyridinecarboxaldehyde (1.24 g, 11.56 mmol, 1 eq), cyclohexyl isocyanide (1.26 g, 11.56 mmol, 1 eq) and Montmorillonite K-10 Clay (2.00 g) were combined and the general procedure followed. The resulting mixture was purified by recrystallization from an ether/methanol mixture to afford **Ligand A2** as yellow needles (1.4 g, 33 %). $R_f = 0.50$ (50% EtOAc/Hexane), M.p. = 135 - 136 °C, ^1H NMR (500 MHz, CDCl_3): $\delta_{\text{H}} = 8.55$ (1H, m), 8.15 (1H, dt, $J = 8.1$ and 1.1 Hz), 8.09 (1H, dd, $J = 1.9$ and 0.9 Hz), 7.75 (1H, td, $J = 7.8$ and 1.8 Hz), 7.41 (1H, dd, $J = 9.5$ and 0.9 Hz), 7.17 – 7.09 (2H, m), 6.21 (1H, d, $J = 9.9$ Hz),

3.07 (1H, m), 1.93 – 1.84 (2H, m), 1.76 – 1.66 (2H, m), 1.57 (1H, dd, $J = 9.7$ and 4.4 Hz), 1.37 – 1.17 (5H, m). ^{13}C NMR (126 MHz, CDCl_3): $\delta_{\text{C}} = 154.8, 148.5, 139.2, 136.7, 131.5, 131.4, 126.7, 123.1, 121.5, 120.5, 118.6, 106.7, 55.6, 34.2, 25.9, 25.0$. IR ($\nu_{\text{m}}, \text{cm}^{-1}$) = 3338, 2925, 2918, 2858, 2856, 1612, 1554, 1506, 1456, 1390, 1303, 1298, 1247, 1205, 1159, 1126, 1085, 1024, 912, 887, 844, 779, 736, 702, 667, 634. EIS-MS (m/z) calculated for $\text{C}_{18}\text{H}_{20}\text{BrN}_4$, (M+H), 371.0871, found 371.0871. Anal. Calcd (%) for $\text{C}_{18}\text{H}_{19}\text{N}_4\text{Cl}$: C 58.23, H 5.16, N 15.09. Found: C 58.540, H 5.098, N 14.857.

Ligand A2 and copper(II) acetate monohydrate (0.106 g, 0.539 mmol, 1.00 eq) were combined following the general procedure described above. The product of Formula (A2.1) purified by recrystallization from CH_2Cl_2 to afford the complex of Formula (A2.1). The structure of the complex was confirmed by single crystal X-ray crystallography (0.708 g, 99%). IR ($\nu_{\text{m}}, \text{cm}^{-1}$) = 3444, 3252, 3076, 2925, 2856, 1571, 1500, 1389, 1329, 1245, 1098, 1047, 1020, 784, 755, 674, 619. Anal. Calcd (%) for $\text{CuC}_{22}\text{H}_{25}\text{N}_4\text{O}_4\text{Br}$: C 47.79, H 4.56, N 10.13. Found: C 47.83, H 4.37, N 10.18.

It is interesting to note that Ligand A2 was not active against any cancer cell lines, however, copper (II) complex compound of Formula (A2.1) was active against all tested cancer cell lines (see Table 1). The Applicant did not expect that the complex of Formula (A2.1) would be active against all the tested cancer cell lines.

Formula (A2.2): Copper 6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine nitrate:

6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine (Ligand A2) was synthesized as described previously.

The combination of an ethereal solution of Ligand A2 (0.300 g, 0.808 mmol) with $\text{Cu}(\text{NO}_3)_2$ (0.188g, 0.808 mmol, 1 eq) according to the general procedure resulted in the formation of a lime green powder, Formula (A2.2) (0.439 g, 97 %) that was collected by filtration. A slow evaporation recrystallization from a MeOH/DCM solution provided suitable crystals for SCXRD (single crystal x-ray diffraction) which confirmed the structure. IR ($\nu_{\text{m}}, \text{cm}^{-1}$) = 3327, 3094, 2924, 2854, 1610, 1568, 1479, 1452, 1410, 1302, 1275, 1246, 1161, 1113, 1097, 1069, 1009, 970, 893, 872, 808, 783, 750, 708, 675, 652. Anal. Calcd (%) for $\text{CuC}_{18}\text{H}_{19}\text{N}_6\text{O}_6\text{Br}$: C 38.69, H 3.43, N 15.04. Found: C 39.269, H 3.637, N 14.483.

It is interesting to note that Ligand A2 was not active against any cancer cell lines, however, copper (II) complex compound of Formula (A2.2) was active against all tested cancer cell lines (see Table 1). The

Applicant did not expect that the complex of Formula (A2.2) would be active against all the tested cell lines.

Formula (A3): Copper N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate:

N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine (Ligand A3) was synthesized as follows: 2-Aminopyridine (1.00 g, 10.626 mmol), 2-pyridinecarboxaldehyde (1.14 g, 10.626 mmol, 1 eq), cyclohexyl isocyanide (1.16 g, 10.626 mmol, 1 eq) and Montmorillonite K-10 Clay (1.2 g) were combined and the general procedure followed. The resulting mixture was purified by recrystallization from an ether/DCM mixture to afford Ligand A3 as yellow crystals (2.31 g, 74 %). $R_f = 0.53$ (50% EtOAc/Hexane), $M.p. = 123-124\text{ }^{\circ}\text{C}$, $^1\text{H NMR}$ (300 MHz, CDCl_3): $\delta_H = 8.54$ (1H, m), 8.18 (1H, dt, $J = 8.1$ Hz and $J = 1.1$ Hz), 8.01 – 7.94 (1H, m), 7.73 (1H, td, $J = 7.7$ and $J = 1.7$ Hz), 7.52 (1H, d, $J = 9.1$ Hz), 7.18 – 6.98 (2H, m), 6.73 (1H, td, $J = 6.7$ Hz and $J = 1.1$ Hz), 6.25 (1H, s), 3.10 (1H, d, $J = 9.9$ Hz), 1.89 (2H, dd, $J = 10.2$ Hz and $J = 4.5$ Hz), 1.77 – 1.65 (2H, m), 1.55 (1H, d, $J = 3.9$ Hz), 1.39 – 1.14 (5H, m). $^{13}\text{C NMR}$ (75 MHz, CDCl_3): $\delta_C = 155.1, 148.3, 140.7, 136.4, 131.2, 130.2, 123.2, 123.0, 121.0, 120.2, 117.8, 111.6, 55.5, 34.0, 25.8, 25.0$. IR (ν_{max} , cm^{-1}) = 3262, 2925, 2848, 1590, 1555, 1477, 1403, 1346, 1229, 1095, 1073, 892, 792, 760, 734, 694, 639. EIS-MS (m/z) calculated for $\text{C}_{18}\text{H}_{21}\text{N}_4$, (M+H), 293.1766, found 293.1766.

Combining Ligand A3 (0.300 g, 1.074 mmol) and copper(II) acetate monohydrate (0.214 g, 1.074 mmol, 1.00 eq) according to the general procedure resulted in the formation of a green powder (0.455 g, 89%), the Formula (A3). After vapour diffusion recrystallization from MeOD and diethyl ether and characterization by SXCRD the green powder was found to be the Formula (A3). IR (nm cm^{-1}) $\frac{1}{4}$ 3302, 3265, 3107, 3082, 3049, 2930, 2857, 1582, 1506, 1479, 1381, 1327, 1292, 1247, 1151, 1091, 1047, 1010, 941, 926, 850, 775, 758, 673, 646, 621. Anal. Calcd (%) for $\text{CuC}_{22}\text{H}_{26}\text{N}_4\text{O}_4$: C 55.74, H 5.53, N 11.82. Found: C 55.82, H 5.34, N 11.49.

It is interesting to note that Ligand A3 was not active against any cancer cell lines, however, copper (II) complex compound of Formula (A3) was active against all tested cancer cell lines (see Table 1). The Applicant did not expect that the complex of Formula (A3) would be active against all the tested cancer cell lines.

Formula (A4): Copper 6-chloro-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate:

6-chloro-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine (Ligand A4) was synthesized as follows. 2-amino-5-chloropyridine (1.00 g, 7.78 mmol), 2-pyridinecarboxaldehyde (0.833 g, 7.78 mmol, 1 eq), cyclohexyl isocyanide (0.857 g, 7.78 mmol, 1 eq) and Montmorillonite K-10 Clay (1.0 g) were combined in ethanol. Column chromatography was used to purify the compound with 20% EtOAc/Hexane as an eluent to afford a yellow powder, 9 (2.27 g, 89 %). $R_f = 0.57$ (50% EtOAc/Hexane), M.p. = 118-119 °C, ^1H NMR (300 MHz, CDCl_3): $\delta_{\text{H}} = 8.60 - 8.52$ (1H, m), 8.15 (1H, dt, $J = 8.0$ Hz and $J = 1.1$ Hz), 7.98 (1H, dd, $J = 2.0$ Hz and $J = 0.9$ Hz), 7.74 (1H, dd, $J = 8.7$ Hz and $J = 6.8$ Hz), 7.46 (1H, d, $J = 9.5$ Hz), 7.13 (1H, m), 7.08 – 6.91 (1H, m), 6.21 (1H, s), 3.07 (1H, s), 1.88 (2H, d, $J = 11.1$ Hz), 1.78 – 1.62 (2H, m), 1.56 (1H, d, $J = 6.0$ Hz), 1.45 – 1.13 (5H, m). ^{13}C NMR (75 MHz, CDCl_3): $\delta_{\text{C}} = 154.7, 148.3, 139.0, 136.5, 131.48, 131.46, 124.5, 121.3, 120.7, 120.3, 120.0, 118.2, 55.4, 34.0, 25.8, 24.9$. IR ($\nu_{\text{m}}\text{.cm}^{-1}$) = 3305, 2930, 2853, 1590, 1476, 1400, 1324, 1287, 1232, 1201, 1184, 1115, 1085, 1046, 943, 891, 807, 786, 740, 702, 626. EIS-MS (m/z) calculated for $\text{C}_{18}\text{H}_{20}\text{ClN}_4$, (M+H), 327.1376, found 327.1376. Anal. Calcd (%) for $\text{C}_{18}\text{H}_{19}\text{N}_4\text{Cl}$: C 66.15, H 8.56, N 17.14. Found: C 66.819, H 4.239, N 16.949.

6-chloro-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine (Ligand A4) (0.500 g, 1.530 mmol) and $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ (0.305 g, 1.530 mmol, 1 eq) were combined following the general procedure. A dark green powder precipitated from the reaction and this was collected by gravity filtration and washed with ether to afford Formula (A4). The structure of the complex of Formula (A4) was confirmed by single crystal X-ray crystallography (0.789 g, 99 %). IR ($\nu_{\text{m}}\text{.cm}^{-1}$) = 3379, 3282, 3282, 3067, 2930, 2858, 1610, 1568, 1528, 1502, 1477, 1400, 1331, 1292, 1242, 1151, 1128, 1097, 1078, 1047, 1022, 877, 822, 787, 756, 725, 679, 621. Anal. Calcd (%) for $\text{CuC}_{22}\text{H}_{25}\text{N}_4\text{O}_4\text{Cl}$: C 51.97, H 4.96, N 11.02. Found: C 50.463, H 5.673, N 10.573.

It is interesting to note that Ligand A4 was not active against any cancer cell lines, however, copper (II) complex compound of Formula A4 was active against all tested cancer cell lines (see Table 1). This was unexpected.

Formula (A5): Copper 5-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate:

5-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine (Ligand A5) was synthesized as follows. 2-Amino-6-bromopyridine (2.00 g, 11.56 mmol), 2-pyridinecarboxaldehyde (1.238 g, 11.56 mmol, 1 eq), cyclohexyl isocyanide (1.262 g, 11.65 mmol, 1 eq) and Montmorillonite K-10 Clay (1.0 g)

were combined and the general procedure followed. The resulting mixture was purified by column chromatography with 20% EtOAc/Hexane as an eluent to afford a viscous golden oil, 10 (2.976 g, 69 %). $R_f = 0.68$ (50% EtOAc/Hexane), $^1\text{H NMR}$ (300 MHz, CDCl_3): 8.57 (1H, m), 8.18 (1H, dt, $J = 8.1, 1.1$ Hz), 7.82 – 7.70 (1H, m), 7.49 (1H, dt, $J = 8.6, 1.0$ Hz), 7.21 – 7.12 (1H, m), 6.98 – 6.84 (2H, m), 6.28 (1H, s), 3.08 (1H, s), 1.91 (2H, s), 1.69 (2H, d, $J = 23.3$ Hz), 1.56 (1H, s), 1.19 – 1.08 (5H, m). $^{13}\text{C NMR}$ (75 MHz, CDCl_3): 154.6, 148.2, 142.9, 136.5, 133.2, 123.6, 121.5, 121.0, 118.8, 117.3, 112.3, 60.3, 32.8, 26.0, 25.6. IR ($\text{v}_{\text{m}}\cdot\text{cm}^{-1}$) = 3268, 2928, 2852, 1589, 1552, 1470, 1428, 1400, 1298, 1225, 1144, 1074, 976, 891, 768, 721. EIS-MS (m/z) calculated for $\text{C}_{18}\text{H}_{20}\text{BrN}_4$, ($\text{M}+\text{H}$), 371.0871, found 371.0871.

The general procedure was followed using 5-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine (Ligand A5) (1.776 g, 4.784 mmol) and $\text{Cu}(\text{OAc})_2\cdot\text{H}_2\text{O}$ (0.955 g, 4.784 mmol, 1 eq). A green powder, Formula (A5) resulted. The structure of the complex was confirmed by single crystal X-ray crystallography (2.238 g, 85 %) following suitable crystal formation by slow evaporation from MeOD. IR ($\text{v}_{\text{m}}\cdot\text{cm}^{-1}$) = 3414, 3287, 3080, 2924, 2855, 1576, 1485, 1391, 1334, 1306, 1256, 1159, 1099, 1047, 1022, 964, 930, 785, 758, 679, 648, 621. Anal. Calcd (%) for $\text{CuC}_{22}\text{H}_{25}\text{N}_4\text{O}_4\text{Br}$: C 47.79, H 4.56, N 10.13. Found: C 44.640, H 4.239, N 9.169.

It is interesting to note that Ligand A5 was not active against any cancer cell lines, however, copper (II) complex compound of Formula (A5) was active against all tested cancer cell lines (see Table 1). The Applicant did not expect that the complex of Formula (A5) would be active against all the tested cancer cell lines.

Formula (A6): Copper 2-(5-chloropyridin-2-yl)-N-cyclohexylimidazo[1,2-a]pyridin-3-amine acetate:

2-(5-chloropyridin-2-yl)-N-cyclohexylimidazo[1,2-a]pyridin-3-amine (Ligand A6) was synthesized as follows: The combination of 2-aminopyridine (0.332 g, 3.532 mmol), 5-chloro-2-pyridinecarboxaldehyde (0.500 g, 3.532 mmol, 1 eq), cyclohexyl isocyanide (0.386 g, 3.532 mmol, 1 eq) and Montmorillonite K-10 Clay (0.5 g) according to the general procedure resulted in the formation of Ligand A6 (0.997 g, 86 %) as a yellow powder following purification by column chromatography with a 20% EtOAc/Hexane eluent. $R_f = 0.54$ (50% EtOAc/Hexane), $\text{M.p.} = 139\text{--}140^\circ\text{C}$, $^1\text{H NMR}$ (300 MHz, CDCl_3): $\delta_{\text{H}} = 8.50$ (1H, dt, $J = 2.5$ and 0.8 Hz), 8.13 (1H, dt, $J = 8.6$ and 0.8 Hz), 7.96 (1H, m), 7.71 (1H, m), 7.50 (1H, m), 7.09 (1H, m), 6.75 (1H, m), 5.93 (1H, d, $J = 9.8$ Hz), 3.06 (1H, m), 1.94 – 1.83 (2H, m), 1.73 (2H, d, $J = 7.8$ Hz), 1.57 (1H, s), 1.40 – 1.15 (5H, m). $^{13}\text{C NMR}$ (75 MHz, CDCl_3): $\delta_{\text{C}} = 153.5, 147.3, 141.0, 136.4, 131.4, 129.7, 129.0, 123.7, 123.2, 121.3, 118.0, 111.9, 55.8, 34.3, 25.9, 25.1$. IR ($\text{v}_{\text{m}}\cdot\text{cm}^{-1}$) = 3303, 2925, 2850, 1579, 1541, 1471, 1446, 1363, 1346, 1288, 1230, 1199, 1139, 1107, 1089, 1008, 923, 891, 842, 798, 752, 738,

704, 638, 621. **EIS-MS (m/z)** calculated for $C_{18}H_{20}ClN_4$, (M+H), 327.1376, found 327.1376. **Anal. Calcd (%)** for $C_{18}H_{19}N_4Cl$: C 66.15, H 8.56, N 17.14. Found: C 66.859, H 6.073, N 16.892.

The general procedure was followed using Ligand A6 (0.300 g, 0.918 mmol) and $Cu(OAc)_2 \cdot H_2O$ (0.183 g, 0.918 mmol, 1 eq). The resulting green powder (0.408 g, 50 %) was found to be Formula A6 following crystallization by slow evaporation from MeOD. **IR (ν_{max} , cm^{-1})** = 3510, 3221, 3111, 3053, 2930, 2855, 1595, 1504, 1483, 1423, 1375, 1325, 1246, 1146, 1121, 1097, 1069, 1032, 1013, 928, 854, 826, 756, 681, 667, 617. **Anal. Calcd (%)** for $CuC_{22}H_{25}N_4O_4Cl$: C 51.97, H 4.96, N 11.02. Found: C 50.421, H 4.857, N 10.444.

It is interesting to note that Ligand A6 was not active against any cancer cell lines, however, copper (II) complex compound of Formula (A6) was active against all tested cancer cell lines (see Table 1). The Applicant did not expect that the complex of Formula (A6) would be active against all the tested cancer cell lines.

CRYSTAL STRUCTURE DETERMINATION:

Suitable ligand crystals for single crystal X-ray diffraction were grown from deuterated methanol unless otherwise stated. However, complex of Formula (A2.2) was crystallized from deuterated chloroform. Following mounting of the crystals on a glass fibre, the intensity data for the crystals were collected on a Bruker APEX II CCD area detector diffractometer with graphite monochromated Mo- K_α radiation (50 kV, 30 mA) at 173K. The collection method involved ω -scans having a width of 0.5° . Data reduction was carried out using SAINT+ version 6.02.6 software and SADABS was used to make empirical absorption corrections. The crystal structures were solved through direct methods using *SHELXS-97*. Non-hydrogen atoms were first refined isotropically followed by anisotropic refinement by full matrix least-squares calculations based on F^2 using *SHELXL-97*. C-bound H atoms were first located in the difference map, then positioned geometrically and allowed to ride on their respective parent atoms, with thermal displacement parameters 1.2 times of the parent C atom. Where possible, the coordinates and isotropic displacement parameters of the N-bound and O-bound H atoms involved in hydrogen bonding interactions were allowed to refine freely. Diagrams and publication material were generated using *WinGX* and *ORTEP-3*. Crystallographic data of each crystal of Formulas (A1) to (A6) appears as Figures 1 to 7.

EVALUATION OF ANTI-CANCER ACTIVITY:

Maintenance of cancer cell lines

The four cancer cell lines used in this study originates from breast cancer (MCF-7), colorectal cancer (HT-29) and leukaemia (K562 and HL-60). All cell lines were obtained from the National Cancer Institute (NCI) and were authenticated by short tandem analysis performed by Inqaba Biotec. In addition, screening for mycoplasma infections are routinely performed in our laboratory and cells are observed daily by phase contrast microscopy to ensure the absence of microbial infection. The breast and colorectal cancer cell lines were maintained as a monolayer in culture flasks whereas the leukemic cell lines are maintained in suspension culture. MCF-7, HT-29 cells were maintained in Dulbecco's Modified Eagles's Medium (DMEM) supplemented with 10% (v/v) of foetal bovine serum (FBS). The K562 and HL-60 cells were maintained in RPMI 1640 media supplemented with 10 % (v/v) FBS. Cells were maintained under asptic conditions at 37 °C in a humidified incubator under a normal atmosphere supplemented with 5% CO₂. All procedures were carried out under aseptic conditions.

Cell viability assay

Cell viability was determined by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay. Briefly, cells were seeded at predetermined cell numbers (dependent on cell type) in a 96 well plate and allowed to adhere overnight, for the adherent cell lines, or exposed immediately to the test compounds in the case of the leukaemic cell lines. All cells were exposed to the test compounds for a total of 48 hours in a humidified incubator at 37 °C and 5% CO₂. At 46 hours, 40 µl of MTT (5mg/mL in PBS) was added to each well and allowed to incubate for two hours at 37°C and a 5% CO₂. This was followed by centrifugation at 400 x g for 5 minutes and aspiration of the media and MTT. The insoluble formazan crystals were solubilized by the addition of 200 µl of dimethylsulfoxide per well. The plates were shaken at 1000 rpm in a plate shaker for five minutes to ensure complete solubilisation of the crystals. The absorbance of each well was measured in a Labsystems iEMS micro-plate reader at 540 nm and a reference wavelength of 690 nm. All data points were collected in quadruplicate and each experiment was repeated at least four times and the percentage cell viability was calculated by expressing the absorbance of treated cells as a percentage of untreated control cells.

The isolation of leukocytes from human blood

Whole blood was collected from human volunteers in heparin containing blood collection tubes. White blood cells (leucocytes) were isolated under sterile conditions from whole blood by differential

centrifugation by using the Histopaque -1077 solution (Sigma-Aldrich). Briefly, a volume of blood was carefully layered (without mixing the two phases) over an equal volume of Histopaque -1077 solution in a 50 mL centrifuge tube and centrifuged for 30 mins at $250 \times g$ at room temperature. The leucocytes collect at the plasma/ Histopaque-1077 interphase and erythrocytes and granulocytes sediment to the bottom of the tube. Following centrifugation the upper phase containing plasma and platelets were aspirated and discarded. The leukocyte containing interphase was collected and washed three times with 10 mL of phosphate buffered saline and centrifugation at $250 \times g$ for 10 minutes at room temperature. The leukocytes were then re-suspended in 10 mL RPMI 1640 media containing 20% v/v FBS. This was followed by counting the cells on a haemocytometer after the addition of trypan blue to determine the number and viability of the cells. The leukocytes were seeded at a density of 40 000 cells/well and exposed to test compounds for 24 hours before the determination of their viability as described in the previous section.

Cell morphology

The adherent cell lines (MCF-7, HT-29) were grown on sterile glass coverslips which were placed in 6 well culture plates. Cells were seeded at a density of 50 000 cells/ coverslip and allowed to grow overnight before the start of treatment. The following day the media was replaced with fresh media containing the appropriate compound for testing. Cells were typically incubated for 24 hours at 37 °C in a humidified incubator with 5% CO₂.

The cells were viewed under phase contrast microscopy (Olympus inverted microscope) to determine if cells have detached from the glass surface and had to be harvested from the culture media by centrifugation at $250 \times g$ for separate staining. These cells were washed twice with phosphate buffered saline (PBS) before the addition of the staining solution. The coverslips were gently irrigated twice with 3 mL of PBS after the culture media was removed. The coverslips were then flooded with 150 µl of a mixture containing acridine orange (10 µg/ml), ethidium bromide (10 µg/ml) and Hoechst 33342 (5 µg/ml). This was followed by incubation at room temperature in the dark for 30 minutes. The slides were then washed with PBS and placed cell side downwards on a microscope slide. The cells were viewed with an Olympus BX41 epifluorescent microscope under 100 and 400 times magnification using the appropriate filters for acridine orange and Hoechst 33342. Ethidium bromide fluoresces red under the acridine orange filter. Images were captured with a DP72 digital camera at an ISO of 200 and a fixed exposure time for each fluorochrome. Following capturing, the images were optimized for contrast and brightness using the Olympus Cell Sens software package. Phase contrast and Hoechst stained images were combined to obtain merged images using the Olympus Cell Sense software package.

EVALUATION OF ANTI-CANCER ACTIVITY:

All anti-cancer experiments were conducted using the powder samples and not the crystalline forms.

The imidazo[1,2-*a*]pyridines and their corresponding metal complexes were tested to investigate their cytotoxicity against human cancer cell lines originating from colorectal and breast carcinomas as well as leukaemia. The HT-29 cell lines represent a colorectal cancer cell line. In addition, the compounds were tested against one breast cancer cell line (MCF-7) and two leukaemic cell lines (K562 and HL-60).

Initial testing involved exposing the relevant cancer cells to the imidazo[1,2-*a*]pyridine ligands, copper salts, zinc complexes thereof, as well as the copper complexes thereof, at a final concentration of 100 μM at 37 °C for 48 hours. Cell viability was measured with tetrazolium based MTT assay. Compounds were considered to be active when the viability of the cells had decreased by 80% when compared to the negative control. These compounds were further tested to determine the 50% inhibitory concentration IC_{50} values (see Table 1 below) which is an indication of the relative potency of a compound. IC_{50} values below about 20 μM were considered active against cancer cells. IC_{50} values greater than about 20 μM were considered inactive against cancer cells. IC_{50} values below about 10 μM were considered very active against cancer cells. The inhibitory effect of these compounds was compared to that of camptothecin, an inhibitor of topoisomerase 1 and a known inducer of apoptosis. Camptothecin is also the parent compound of irinotecan and topotecan, anticancer agents used in the management of colorectal-, ovarian and small cell lung cancers.

The copper complexes for Formulas A1 to A6 showed significant anti-cancer activity (with all IC_{50} values well below 20 μM and frequently below 15 μM and below 10 μM) across a range of cancer cell lines.

The anticancer activity of metal salt precursors (for example, $\text{Cu}(\text{OAc})_2$, $\text{Cu}(\text{NO}_3)_2$ and CuCl_2) were also investigated and were found to be inactive against the cancer cell lines used in this study.

All the Ligands A1 to A6 were tested for anti-cancer activity. All ligands were inactive against cancer cells, as seen in Table 1. The Applicant was therefore very surprised that the copper complexes according to the invention showed anti-cancer activity and/or improved anti-cancer activity when compared to the ligand(s) alone. In light of the absence of anti-cancer activity from the ligand(s), the Applicant did not expect the copper complexes according to this invention to show any anti-cancer activity.

Furthermore, when Ligands A1 to A6 were complexed with zinc, the resulting zinc metal complexes showed no anti-cancer activity. The Applicant was therefore surprised that the copper complexes according to the invention showed anti-cancer activity.

For example, a zinc complex including Ligand A2, namely zinc 6-bromo-N-cyclohexyl-2-(pyridine-2-yl)imidazo[1,2-a]pyridine-3-amine dichloride, showed no anti-cancer activity whatsoever at a concentration of 100 μ M against the tested cancer cell lines. However, a copper complex including Ligand A2, namely Formula A2.1, showed the most potent anti-cancer activity of all the tested compounds. This is particularly surprising in light of the fact that it has been shown that cancer cells contain significantly greater amounts of copper relative to their healthy cell equivalents, and the Applicant did not reasonably expect that providing the cancer cell with more copper would have an anti-cancer effect.

TABLE 1 – IC₅₀ values for compounds and complexes (μ M)

Compound	HT-29	MCF-7	K562	HL-60
Camptothecin	8.0 $\pm$ 0.8	0.32 $\pm$ 0.01	2.6 $\pm$ 0.24	0.005 $\pm$ 0.001
Ligand A1	NA	NA	NA	NA
Ligand A2	NA	35.4 $\pm$ 0.4	36.20 $\pm$ 4.1	NA
Ligand A3	NA	NA	NA	NA
Ligand (A4)	NA	23 $\pm$ 3	NA	29.2 $\pm$ 0.7
Ligand (A5)	NA	NA	NA	NA
Ligand (A6)	NA	NA	NA	NA
Formula A1	6.2 $\pm$ 0.1	3.7 $\pm$ 0.5	7.7 $\pm$ 0.5	6.5 $\pm$ 1.0
Formula A2.1	0.79 $\pm$ 0.02	0.62 $\pm$ 0.03	1.9 $\pm$ 0.9	2.2 $\pm$ 0.5
Formula A2.2	6.8 $\pm$ 0.08	1.1 $\pm$ 0.3	9 $\pm$ 1	12 $\pm$ 4

Formula A3	0.8±0.2	1.1±0.6	3±1	3±1
Formula A4	8.1±0.2	3.8±0.9	3.0±0.4	5±2
Formula A5	1.6±0.3	1.20±0.08	6.0±0.4	2.1±0.3
Formula A6	7.4±0.4	9.2±0.9	10.6±0.7	±2

NA - IC₅₀ values were not determined as compounds were not found to be significantly active at 100 µM

Leucocytes prepared from human blood were treated with a selection of imidazo[1,2-*a*]pyridines and copper complexes of Formulas (A1) to (A6) at 20 µM for 24 hours as shown in Figure 8. Compounds causing leukocytes to have cell viability lower than 50% after 24 hours were considered to be potentially toxic. Copper complexes of Formulas (A1), (A2.2), (A3), (A4), (A5) and (A6) showed leucocyte viability above 50 % and so are considered nontoxic. It must be noted that many cancer chemotherapeutic agents, for example cisplatin, are used successfully in the treatment of cancer despite their toxicity towards non-cancerous cells and that a decrease in the white blood cell count is a common feature of successful cancer chemotherapeutic regimens.

Effects on cancer cell morphology

The morphology of the cells was evaluated using the vital stains acridine orange (AO), Hoechst 33342 (HO) and ethidium bromide (EB). Changes to the cell morphology making use of epifluorescent microscopy provided valuable clues regarding the mechanism of cell death. Acridine orange stains healthy nuclei and cell cytoplasm an emerald green colour and is also be used to visualise the distribution of lysosomes within a cell which are stained red. More recently it has been used as a preliminary indicator of autophagy. Ethidium bromide enters cells only when membranes are damaged and stains nuclei a bright orange red indicating necrotic cell death or late apoptosis depending on the staining pattern of the nuclei. Hoechst 33342 is a specific stain for nuclei and can distinguish between apoptotic and non-apoptotic nuclei, showing an increase in fluorescent intensity and specific changes to the structure of the nucleus which include chromatin condensation and fragmentation.

Figure 9 shows the changes induced by the copper-imidazo[1,2-*a*]pyridine complex of Formula (A2.1) when MCF-7 and HL-60 cells were exposed to a concentration of the test compound equivalent to an IC₇₅

value for the respective cell lines. There was evidence of both apoptotic and necrotic changes in both cell lines with late apoptotic/necrotic changes being more prominent in the HL-60 cells. A purely necrotic nucleus stains a uniform red/orange colour (see Figure 9 panel d, labelled NN) whereas a late apoptotic nucleus will show evidence of fragmentation as seen in Figure 9 panel j, (labelled LAN). Many MCF-7 cells partially detached from the growth surface and assumed a rounded shape while remaining anchored to the growth surface by filopodia, indicated with an F, (Figure 9, panels b and f). Some of these detached, rounded cells showed a fragmented nucleus which is consistent with apoptosis (AN) while others showed necrotic changes (NN) as shown in Figure 9, panel d. The HL-60 cells (Figure 9, panels k-l), showed that some cells were in a late apoptotic stage as evidenced by the globular nature of the Hoechst and/or acridine orange stained nuclei.

The effects of the copper imidazo[1,2-a]pyridines complexes of Formulas (A2.2), (A3), and (A5) on the morphology of MCF-7 cells are shown in Figure 10. MCF-7 cells were exposed to a concentration of 10 μ M of each compound for 24 h. The cells were stained with a combination of Hoechst 33342, acridine orange and ethidium bromide as described above. None of the cells showed the characteristic red/orange staining of the nuclei viewed using the UV filter cube that is observed with ethidium bromide entering the cells, indicating that the cell membranes were intact and that there was no necrosis present. The untreated MCF-7 cells (Figure 10, panels a,b,c,d) stained in a typical manner, showing the somewhat rounded morphology of these cells and a tendency of the cells to grow on top of each other, accounting for the cells on the image that are not in sharp focus. A prominent feature of the three test compounds of Formulas (A2.2), (A3), and (A5) was the formation of cytoplasmic vacuoles (V) in the perinuclear space (Figure 10, panels e,f,i,j). Moreover, cells treated with both Formula (A2.2) and Formula (A3) caused the appearance of acidic granules (AG) which did not co-localize with the cytoplasmic granules (Figure 10, panels k and g) but appears to co-exist in the perinuclear space as shown in Figure 11 where MCF-7 cells were treated with 10 μ M of Formula (A2.1) for 24 h. The extensive formation of cytoplasmic vacuoles has been associated with endoplasmic reticulum stress and paraptosis. Paraptosis is a type of programmed cell death that is associated with extensive cytoplasmic vacuolisation, expression of phosphatidylserine on the cell membrane (annexin V positive) but no activation of caspases, or the formation of apoptotic bodies. In addition, the formation of the acidic granules detected with the acridine orange indicate the potential of the compounds to induce autophagy which may lead to autophagic cell death. Cells treated with Formula (A5) (Figure 12, panels m and n) showed rounding of the cells and detachment from the growth surface. Some cells remain attached to the growth surface by filopodia (F). Although not clearly visible due to the rounded shape of the cells, the complex of Formula (A5) also induced cytoplasmic vacuole formation, and at a lower dose (5 μ M), shown in Figure 12, there is a clear indication of the formation of acidic granules.

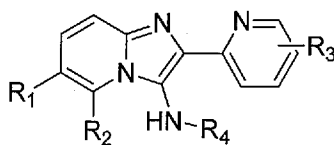
Hoechst 33342 stain revealed profound changes in the typical nuclear morphology of the treated cells. The copper imidazo[1,2-a]pyridine complexes of Formula (A3) and Formula (A5) caused a high percentage of nuclei to become deformed indicating the likelihood that the nucleus is the target organelle of these compounds with some early indicators for apoptosis. MCF-7 cells treated with a lower concentration of Formula (A5) (5 μ M) appears to favour fragmentation off the nucleus indicating that 29 at a lower concentrations cause apoptosis (Figure 12). The lower concentration also caused the formation of acidic granules in a high percentage of cells which were not evident when the cells were treated with 10 μ M (Figures 10 and 12). Cells treated with Formula (A3) also showed the presence of apoptotic nuclei (Figure 10, panel h and p). The complex of Formula (A2.2) showed the presence of contracted nuclei (CN) and a loss of the normal round or oval shaped nuclei (block arrow) in Figure 10, panel l, which is indicative of pre-apoptotic changes. Morphological analysis indicated changes in cell morphology that included apoptosis, paraptosis and autophagy. Specific effects on DNA morphology indicated that the nucleus may be a target organelle. The effect on normal leucocytes is encouraging indicating a potential sparing effect on normal cells.

The Applicant has surprisingly shown that the metal complexes according to the first aspect of this invention described above are active against cancer. The Applicant has found that by providing cancer cells (known to harbor excessive amounts of copper) with more copper complexes is lethal to cancer cells. Further, the ligands and metal salts used to manufacture the copper complexes of the invention individually showed no anti-cancer activity. The results are surprising and unexpected.

While the invention has been described in detail with respect to specific embodiments and/or examples thereof, it will be appreciated that those skilled in the art, upon attaining an understanding of the foregoing may readily conceive of alterations to, variations of and equivalents to these embodiments. Accordingly, the scope of the present invention should be assessed as that of the claims and any equivalents thereto, which claims are appended hereto.

CLAIMS:

1. A copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or an analogue thereof, for use in treating cancer.
2. The copper (II) complex for use in treating cancer according to Claim 1, wherein the metal complex further comprises at least one second ligand selected from the following groups: oxygen containing ligands and halogens.
3. The copper (II) complex for use in treating cancer according to Claim 2, wherein the at least one second ligand is an oxygen containing second ligand and is at least one selected from the following group: nitrates, acetates, sulphates and phosphates.
4. The copper (II) complex for use in treating cancer according to Claim 2 or 3, wherein said copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue provides a distorted square planar geometry.
5. The copper (II) complex for use in treating cancer according to Claim 4, wherein said copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue provides a distorted square planar geometry, and wherein the oxygen containing second ligand provides at least one oxygen atom being in a position out of plane.
6. The copper (II) complex for use in treating cancer according to any one of Claims 1 to 5, wherein the imidazo[1,2-a]pyridine ligand derivative and/or analogue is a compound of Formula A:



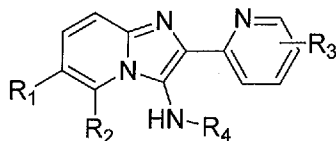
Formula (A)

wherein,

R₁, R₂ and R₃ is each a hydrogen or a halogen; and

R₄ is selected from any one of the following group: benzyl and cyclohexyl.

7. The copper (II) complex for use in treating cancer according to Claim 6, wherein R_1 , R_2 and R_3 is any one of the following group: hydrogen, bromine and chlorine.
8. The copper (II) complex for use in treating cancer according to any one of Claims 1 to 7, wherein said copper (II) complex is any one of the following group:
- Formula (A1) - copper N-benzyl-6-bromo-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate ;
- Formula (A2.1) - copper 6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;
- Formula (A2.2) - copper 6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine nitrate;
- Formula (A3) - copper N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;
- Formula (A4) - copper 6-chloro-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;
- Formula (A5) - copper 5-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate; and
- Formula (A6) - copper 2-(5-chloropyridin-2-yl)-N-cyclohexylimidazo[1,2-a]pyridin-3-amine acetate.
9. The copper (II) complex for use in treating cancer according to any one of Claims 1 to 8, wherein the cancer is at least one of the following group: breast cancer, colorectal cancer, colon cancer, and leukemia.
10. A copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue wherein the imidazo[1,2-a]pyridine ligand derivative and/or analogue is a compound of Formula A:



Formula (A)

wherein,

R₁, R₂ and R₃ is each a hydrogen or a halogen; and

R₄ is selected from any one of the following group: benzyl and cyclohexyl.

11. The copper (II) complex according to Claim 10, wherein R₁, R₂ and R₃ is any one of the following group: hydrogen, bromine and chlorine.
12. The copper (II) complex of an imidazo[1,2-a]pyridine ligand derivative and/or analogue according to Claim 11, wherein said copper (II) complex is any one of the following group:

Formula (A1) - copper N-benzyl-6-bromo-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

Formula (A2.1) - copper 6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

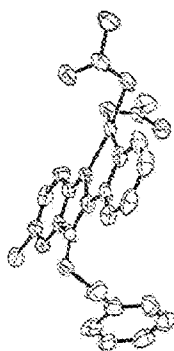
Formula (A2.2) - copper 6-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine nitrate;

Formula (A3) - copper N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

Formula (A4) - copper 6-chloro-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate;

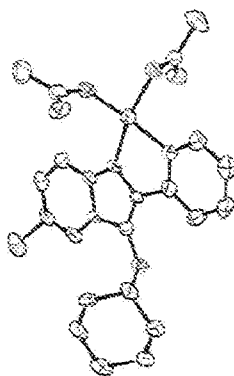
Formula (A5) - copper 5-bromo-N-cyclohexyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-3-amine acetate; and

Formula (A6) - copper 2-(5-chloropyridin-2-yl)-N-cyclohexylimidazo[1,2-a]pyridin-3-amine acetate.



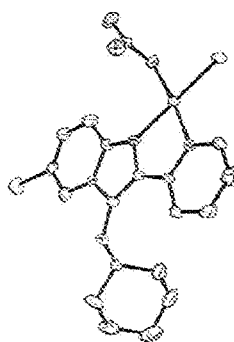
CCDC Number	1500263
Identification Code	14j_cdkjd10_253_0a
Empirical formula	C ₂₃ H ₂₁ Br Cu N ₄ O ₄
Formula weight	560.89
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	P 2/c
Unit cell dimensions	a = 10.3293(5) Å
	b = 9.6968(5) Å
	c = 25.3960(12) Å
	a = 90°
	b = 91.8710(10)°
	g = 90°
Volume	2542.3(2) Å ³
Z	4
Density (calculated)	1.465 Mg/m ³
Absorption coefficient	2.465 mm ⁻¹
F(000)	1132
Crystal size	0.31 x 0.193 x 0.156 mm ³
Theta range for data collection	1.60 to 26.45°
Index ranges	-12 ≤ h ≤ 12, -12 ≤ k ≤ 12, -31 ≤ l ≤ 31
Reflections collected	27558
Independent reflections	5226 [R(int) = 0.0543]
Completeness to theta = 28.00°	99.80%
Absorption correction	Integration
Max. and min. transmission	0.7637 and 0.6402
Refinement method	Full-matrix least-squares on F ²
Data / restraints parameters	522 / 0 / 300
Goodness-of-fit on F ²	1.023
Final R indices [I > 2σ(I)]	R ₁ = 0.0442, W R ₂ = 0.1106
R indices (all data)	R ₁ = 0.0614, W R ₂ = 0.1148
Largest diff. peak and hole	1.123 and -0.422 e.Å ⁻³

FIGURE 1



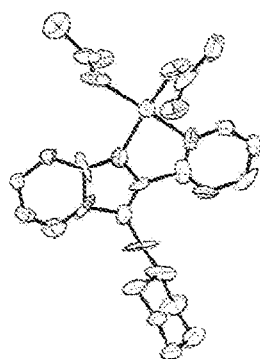
CCDC Number	1500256
Identification Code	12j_cdkjd8_bacuac_0a
Empirical formula	C ₂₄ H ₂₉ Br Cl ₄ Cu N ₄ O ₄
Formula weight	722.76
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P -1
Unit cell dimensions	a = 10.0655(7) Å
	b = 12.1779(8) Å
	c = 12.8618(8) Å
	α = 94.595(4)°
	β = 104.690(4)°
	γ = 101.168(4)°
Volume	1482.21(17) Å ³
Z	2
Density (calculated)	1.620 Mg/m ³
Absorption coefficient	2.482 mm ⁻¹
F(000)	730
Crystal size	0.719 x 0.272 x 0.212 mm ³
Theta range for data collection	1.65 to 24.91°
Index ranges	-11 ≤ h ≤ 11, -14 ≤ k ≤ 14, -15 ≤ l ≤ 15
Reflections collected	14786
Independent reflections	5004 [R(int) = 0.0892]
Completeness to theta = 28.00°	96.70%
Absorption correction	Integration
Max. and min. transmission	0.73827 and 0.3243
Refinement method	Full-matrix least-squares on F ²
Data / restraints parameters	5004 / 1 / 348
Goodness-of-fit on F ²	0.967
Final R indices [I > 2σ(I)]	R ₁ = 0.0447, wR ₂ = 0.1025
R indices (all data)	R ₁ = 0.0935, wR ₂ = 0.1207
Largest diff. peak and hole	1.025 and -0.6232 e.Å ⁻³

FIGURE 2



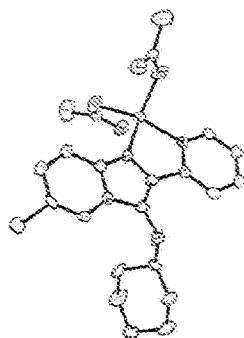
CCDC Number	1500260
Identification Code	15j_cdkjd9_35r_0a
Empirical formula	C ₂₀ H ₂₇ Br Cl Cu N ₅ O ₅
Formula weight	596.37
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P -1
Unit cell dimensions	a = 9.3726(2) Å
	b = 9.6188(2) Å
	c = 13.6046(2) Å
	α = 96.4040(10)°
	β = 96.7890(10)°
	γ = 92.1850(10)°
Volume	1208.66(4) Å ³
Z	2
Density (calculated)	1.639 Mg/m ³
Absorption coefficient	2.708 mm ⁻¹
F(000)	606
Crystal size	0.40 x 0.32 x 0.18 mm ³
Theta range for data collection	1.52 to 28.00°
Index ranges	-12 ≤ h ≤ 12, -12 ≤ k ≤ 12, -17 ≤ l ≤ 17
Reflections collected	44667
Independent reflections	5845 [R(int) = 0.0537]
Completeness to theta = 28.00°	100.00%
Absorption correction	Integration
Max. and min. transmission	0.6414 and 0.4105
Refinement method	Full-matrix least-squares on F ²
Data / restraints parameters	58454 / 0 / 309
Goodness-of-fit on F ²	1.092
Final R indices [I > 2σ(I)]	R ₁ = 0.0236, W R ₂ = 0.0626
R indices (all data)	R ₁ = 0.0302, W R ₂ = 0.0640
Largest diff. peak and hole	0.332 and -0.529 e.Å ⁻³

FIGURE 3



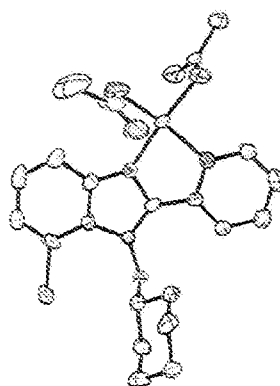
CCDC Number	1500264
Identification Code	15j_cdkjd3_46r_0a
Empirical formula	C ₂₃ H ₃₀ Cu N ₄ O ₅
Formula weight	506.05
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P 2 ₁ /n
Unit cell dimensions	a = 12.8791(4) Å
	b = 13.5066(4) Å
	c = 14.0648(5) Å
	α = 90°
	β = 99.563(2)°
	γ = 90°
Volume	2412.61(14) Å ³
Z	4
Density (calculated)	1.393 Mg/m ³
Absorption coefficient	0.946 mm ⁻¹
F(000)	1060
Crystal size	0.303 x 0.141 x 0.140 mm ³
Theta range for data collection	1.986 to 26.436°
Index ranges	-16 ≤ h ≤ 15, -16 ≤ k ≤ 16, -17 ≤ l ≤ 17
Reflections collected	22139
Independent reflections	49765 [R(int) = 0.0780]
Completeness to theta = 28.00°	100.00%
Absorption correction	Integration
Max. and min. transmission	0.9141 and 0.7836
Refinement method	Full-matrix least-squares on F ²
Data / restraints parameters	4976 / 360 / 584
Goodness-of-fit on F ²	1.123
Final R indices [I > 2σ(I)]	R ₁ = 0.0756, W R ₂ = 0.1761
R indices (all data)	R ₁ = 0.1065, W R ₂ = 0.1860
Largest diff. peak and hole	0.603 and -0.412 e.Å ⁻³

FIGURE 4



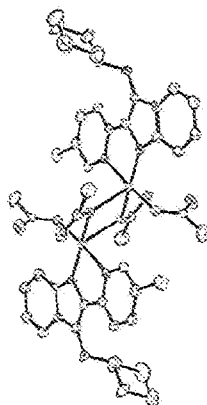
CCDC Number	1500262
Identification Code	15j_cdkjd2_47rb_0a
Empirical formula	C ₂₂ H ₂₅ Cl Cu N ₄ O ₄
Formula weight	508.46
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P 2(1)/n
Unit cell dimensions	a = 12.0120(4) Å
	b = 14.6964(5) Å
	c = 13.2423(4) Å
	$\alpha = 90^\circ$
	$\beta = 100.852(2)^\circ$
	$\gamma = 90^\circ$
Volume	2295.90(13) Å ³
Z	4
Density (calculated)	1.471 Mg/m ³
Absorption coefficient	1.104 mm ⁻¹
F(000)	1052
Crystal size	0.25 x 0.18 x 0.12 mm ³
Theta range for data collection	2.09 to 28.00°
Index ranges	-15 ≤ h ≤ 15, -19 ≤ k ≤ 19, -17 ≤ l ≤ 14
Reflections collected	24889
Independent reflections	5535 [R(int) = 0.0545]
Completeness to theta = 28.00°	99.90%
Absorption correction	Integration
Max. and min. transmission	0.8909 and 0.7907
Refinement method	Full-matrix least-squares on F ²
Data / restraints parameters	5535 / 0 / 295
Goodness-of-fit on F ²	1.048
Final R indices [I > 2σ(I)]	R ₁ = 0.0459, WR ₂ = 0.1212
R indices (all data)	R ₁ = 0.0687, WR ₂ = 0.1312
Largest diff. peak and hole	1.995 and -0.559 e.Å ⁻³

FIGURE 5



CCDC Number	1500258
Identification Code	15j_cdkjd5_47r_0a
Empirical formula	C ₂₃ H ₂₆ Br Cl ₃ Cu N ₄ O ₄
Formula weight	672.28
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P -1
Unit cell dimensions	a = 9.6013(4) Å
	b = 10.9802(4) Å
	c = 13.4165(6) Å
	α = 77.920(2)°
	β = 86.350(2)°
	γ = 85.579 (2)°
Volume	1377.35(10) Å ³
Z	2
Density (calculated)	1.621 Mg/m ³
Absorption coefficient	2.571 mm ⁻¹
F(000)	678
Crystal size	0.18 x 0.17 x 0.14 mm ³
Theta range for data collection	1.55 to 26.02°
Index ranges	-9 ≤ h ≤ 11, -13 ≤ k ≤ 13, -14 ≤ l ≤ 16
Reflections collected	11817
Independent reflections	5102 [R(int) = 0.1000]
Completeness to theta = 28.00°	93.80%
Absorption correction	Integration
Max. and min. transmission	0.5712 and 0.4978
Refinement method	Full-matrix least-squares on F ²
Data / restraints parameters	5102 / 0 / 331
Goodness-of-fit on F ²	0.999
Final R indices [I > 2σ(I)]	R ₁ = 0.0546, WR ₂ = 0.1432
R indices (all data)	R ₁ = 0.0847, WR ₂ = 0.1558
Largest diff. peak and hole	1.796 and -0.517 e.Å ⁻³

FIGURE 6



CCDC Number	1500261
Identification Code	15j_cdkjd4_36r_0a
Empirical formula	C ₄₆ H ₅₈ Cl ₂ Cu ₂ N ₈ O ₁₀
Formula weight	1081
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P -1
Unit cell dimensions	a = 9.6782(2) Å
	b = 11.6063(3) Å
	c = 13.0369(3) Å
	α = 68.3700(10)°
	β = 82.4450(10)°
	γ = 67.5930 (10)°
Volume	1258.44(5) Å ³
Z	1
Density (calculated)	1.426 Mg/m ³
Absorption coefficient	1.014 mm ⁻¹
F(000)	562
Crystal size	0.48 x 0.23 x 0.19 mm ³
Theta range for data collection	1.68 to 28.00°
Index ranges	-12 ≤ h ≤ 12, -15 ≤ k ≤ 15, -17 ≤ l ≤ 17
Reflections collected	33862
Independent reflections	6075 [R(int) = 0.0621]
Completeness to theta = 28.00°	100.00%
Absorption correction	Integration
Max. and min. transmission	0.4544 and 0.2007
Refinement method	Full-matrix least-squares on F ²
Data / restraints parameters	6075 / 0 / 315
Goodness-of-fit on F ²	1.068
Final R indices [I > 2σ(I)]	R ₁ = 0.0323, W R ₂ = 0.0865
R indices (all data)	R ₁ = 0.0402, W R ₂ = 0.0894
Largest diff. peak and hole	0.373 and -0.465 e.Å ⁻³

FIGURE 7

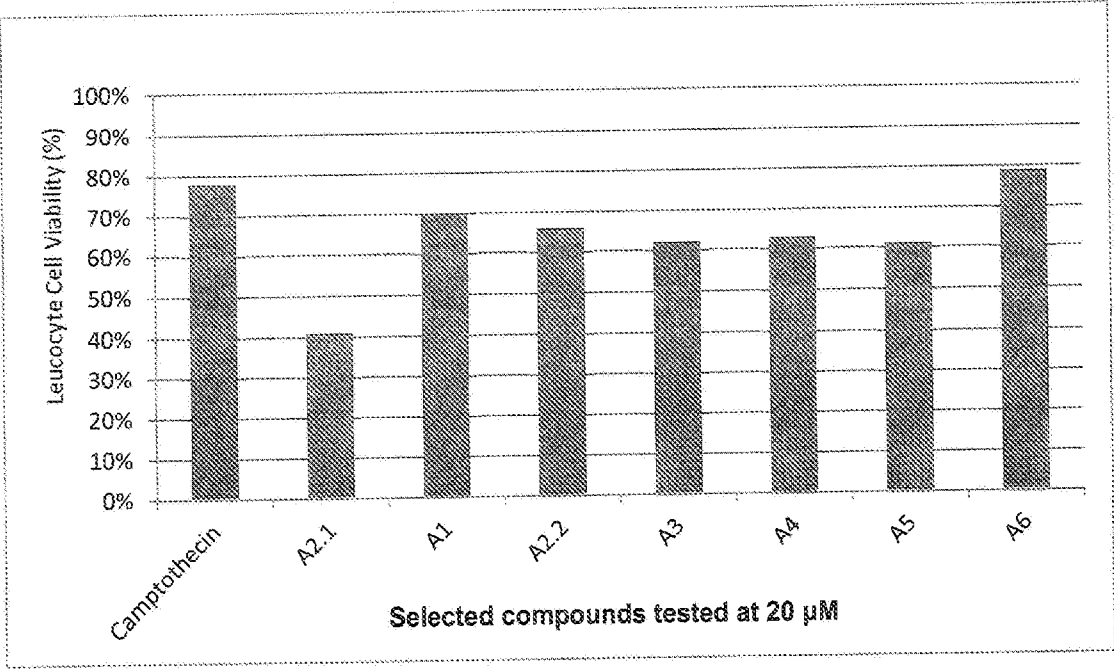


FIGURE 8

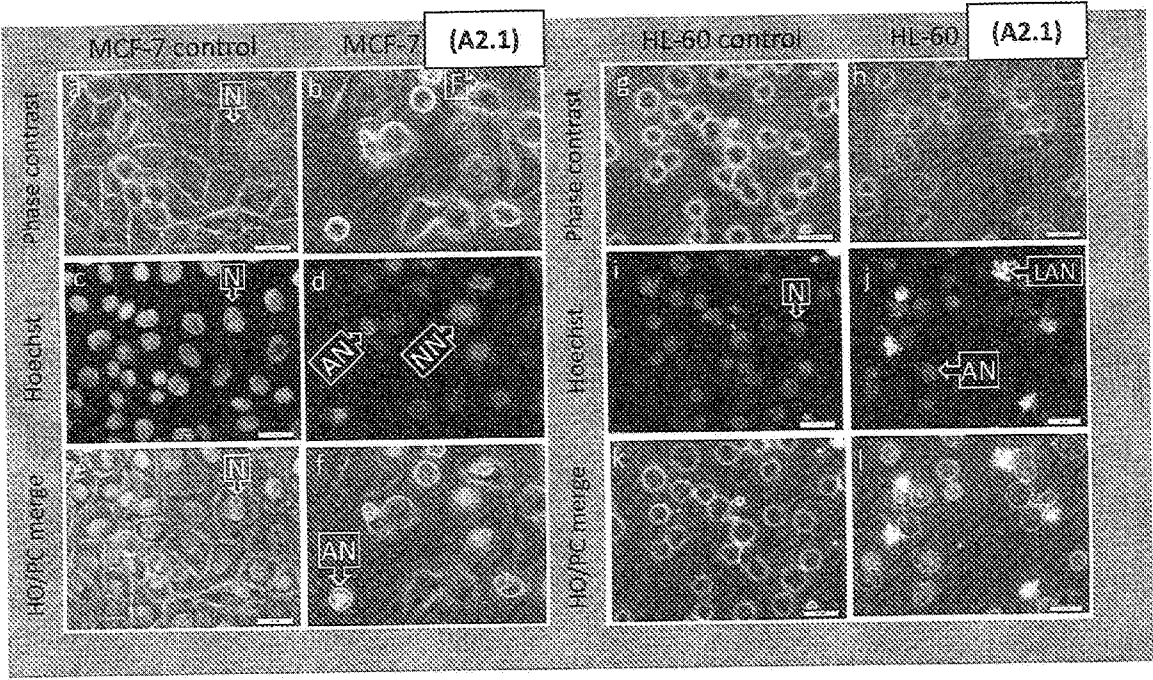


FIGURE 9

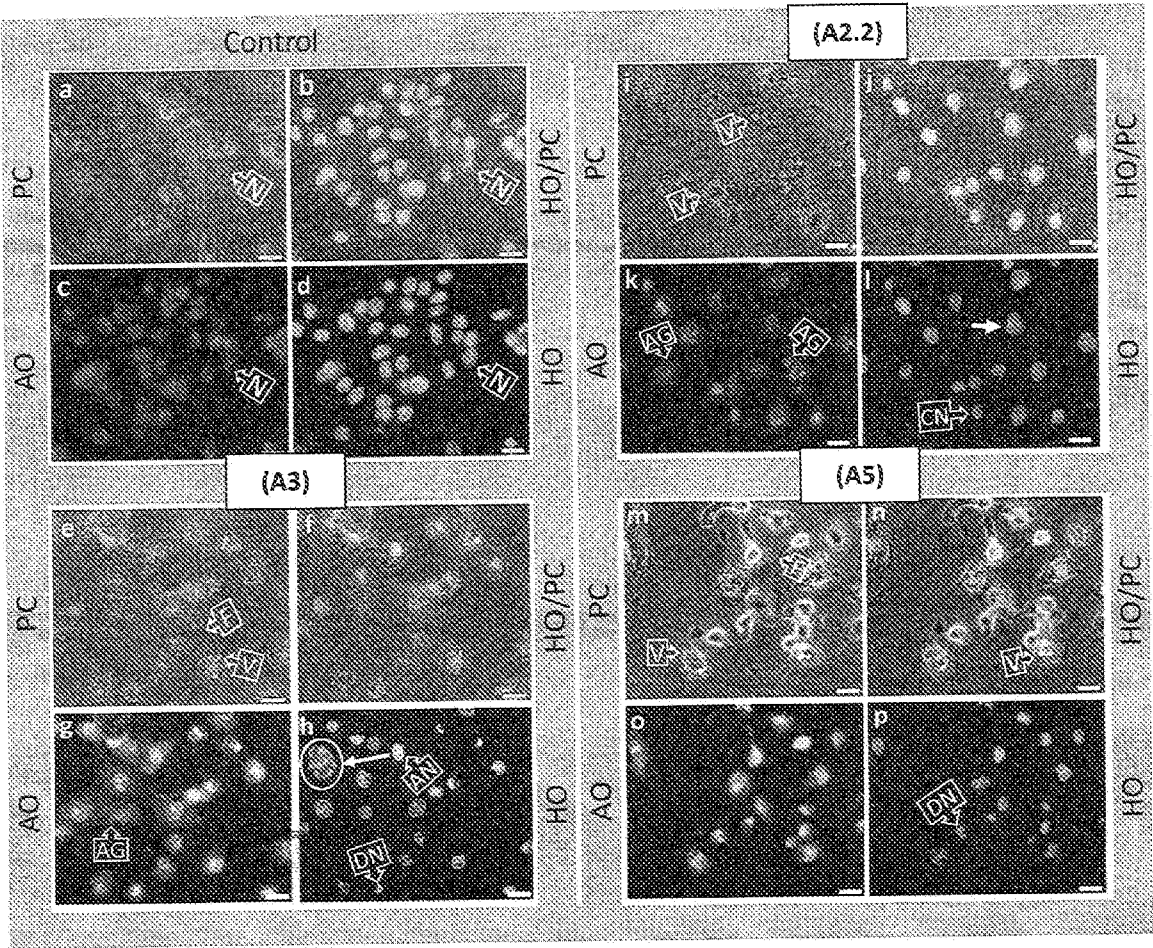


FIGURE 10

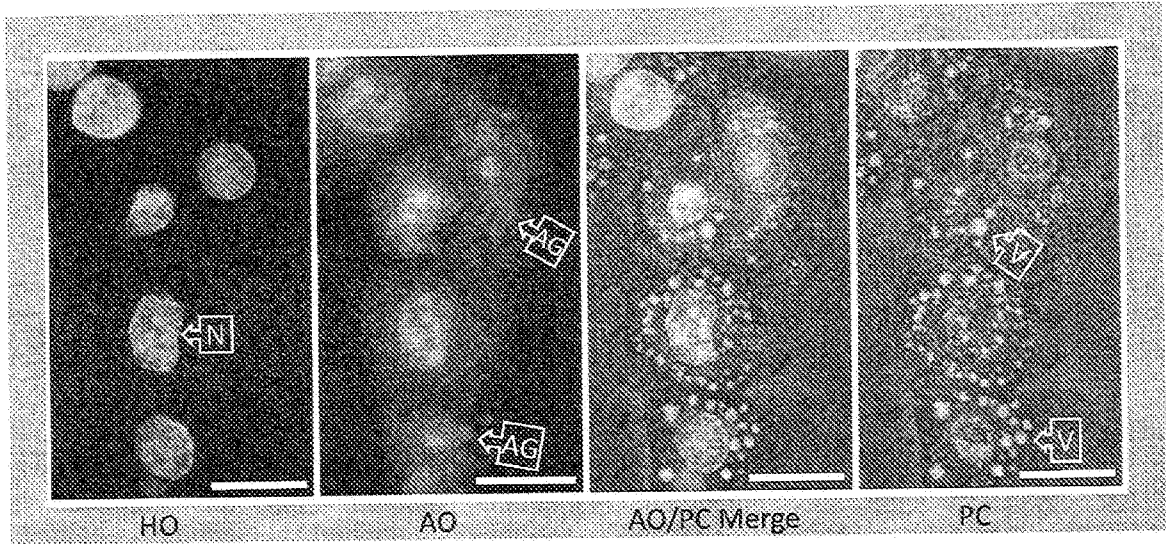


FIGURE 11

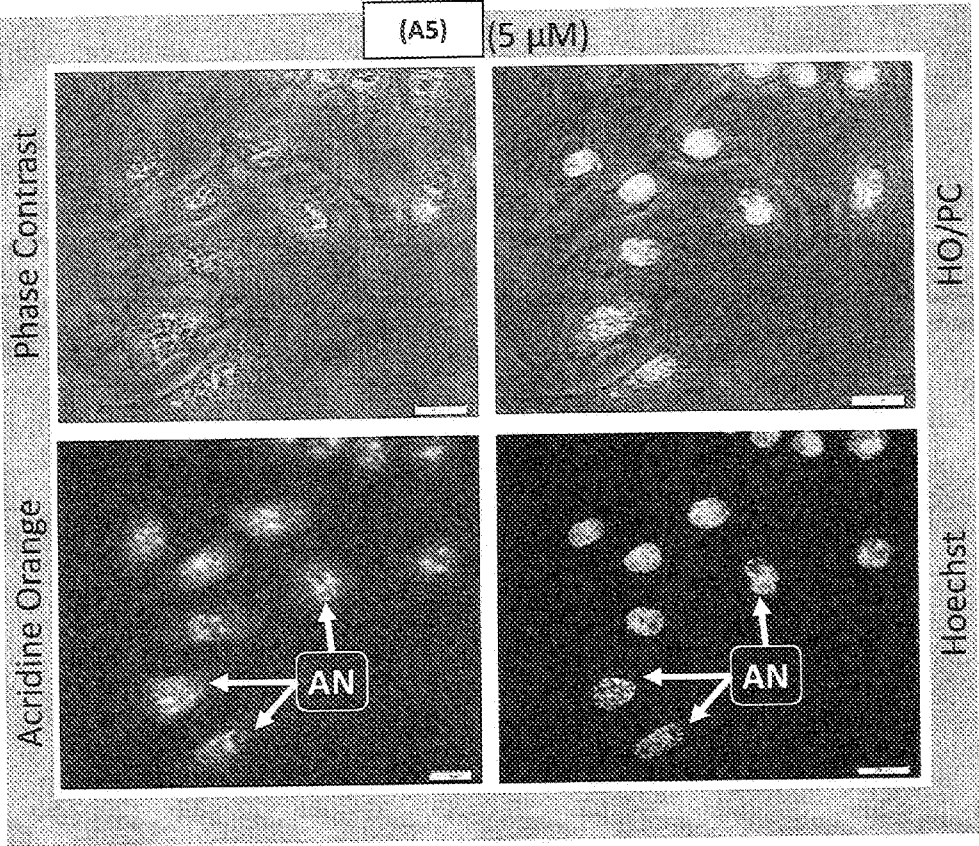


FIGURE 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2017/052122

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P35/00 C07F1/08 A61K31/437
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07F A61P A61K

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Taurai Flloyd Kurebwa: "The Effect of imidazo[1,2-a]pyridine amines on MCF-7 and MDA-MB-231 Breast Cancer Cells", A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Masters of Science in Medicine (Pharmacology), 1 January 2015 (2015-01-01), pages 1-142, XP055381320, Retrieved from the Internet: URL: http://mobile.wiredspace.wits.ac.za/bitstream/handle/10539/21597/kurebwa_Flloyd_Taurai_2015.pdf?sequence=1&isAllowed=y [retrieved on 2017-06-14] * Chapter 3.6 * figures 3.19, 3.20 tables 3.1, 3.9-3.11, 4.1, 4.2 -/--	1-12



Further documents are listed in the continuation of Box C.



See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search

14 June 2017

Date of mailing of the international search report

28/06/2017

Name and mailing address of the ISA/

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Authorized officer

Eberhard, Michael

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2017/052122

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	----- NICOLA MARGIOTTA ET AL: ") with a ligand targeting the translocator protein (TSP0)", DALTON TRANSACTIONS: THE INTERNATIONAL JOURNAL FOR INORGANIC, ORGANOMETALLIC AND BIOINORGANIC CHEMISTRY, vol. 43, no. 43, 1 January 2014 (2014-01-01), pages 16252-16264, XP055380771, GB ISSN: 1477-9226, DOI: 10.1039/C4DT01540A abstract * Charts 1, 2 *	1-12
A	----- NICOLA MARGIOTTA ET AL: "Platinum(II) Complexes with Bioactive Carrier Ligands Having High Affinity for the Translocator Protein", JOURNAL OF MEDICINAL CHEMISTRY, vol. 53, no. 14, 22 July 2010 (2010-07-22), pages 5144-5154, XP055380773, ISSN: 0022-2623, DOI: 10.1021/jm100429r figure 1	1-12
A	----- JOLANTA SAWICKA ET AL: "Synthesis and coordination ability of substituted imidazo-pyridines, structural analogues of oxine. Influence of copper(II) and nickel(II) ions on toxicity of the organic ligand", JOURNAL OF INORGANIC BIOCHEMISTRY, vol. 44, no. 2, 1 November 1991 (1991-11-01), pages 117-125, XP055380776, US ISSN: 0162-0134, DOI: 10.1016/0162-0134(91)84024-4 abstract table 1 ----- -/--	1-12

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2017/052122

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	DAM JEAN ET AL: "Synthesis of copper and zinc 2-(pyridin-2-yl)imidazo[1,2-a]pyridine complexes and their potential anticancer activity", EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, EDITIONS SCIENTIFIQUE ELSEVIER, PARIS, FR, vol. 126, 11 November 2016 (2016-11-11), pages 353-368, XP029885675, ISSN: 0223-5234, DOI: 10.1016/J.EJMECH.2016.10.041 the whole document	1-12
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2017/052122

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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