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(54) **Title**: APTAMER CONJUGATES WITH N-HETEROCYCLIC CARBENE METAL COMPLEXES FOR TARGETED DRUG DELIVERY

(57) **Abstract**: An aptamer-N-heterocyclic-carbene metal complex conjugate (aptamer-NHCM conjugate) or an aptamer-bis-N-heterocyclic-carbene metal complex conjugate (aptamer-bis-NHCM conjugate) includes an aptamer coupled through a hydrolytically stable bond to an N-heterocyclic-carbene metal complex (NHCM) or a bis-N-heterocyclic-carbene metal complex (bis-NHCM). The aptamer-NHCM conjugate is prepared where the chosen aptamer displays selective binding to a cell specific receptor, such that the cytotoxic NHCM can be directed specifically to cells responsible for a target disease (e.g., a specific cancer type). A method of preparing the aptamer-N-heterocyclic-carbene metal complex conjugate involves installing a coupling group to an N-heterocyclic-carbene metal complex that can specifically bond with a functional group on an aptamer; the bond, covalent or non-covalent, is stable hydrolytically in the absence of an environment that promotes intentional cleavage of the bond.

DESCRIPTION

APTAMER CONJUGATES WITH N-HETEROCYCLIC CARBENE METAL COMPLEXES FOR TARGETED DRUG DELIVERY

CROSS-REFERENCE TO A RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application Serial No. 62/019,960, filed July 2, 2014, the disclosure of which is hereby incorporated by reference in its entirety, including all figures, tables and drawings.

The Sequence Listing for this application is labeled "Seq-List.txt" which was created on July 2, 2015 and is 1 KB. The entire contents of the sequence listing is incorporated herein by reference in its entirety.

BACKGROUND OF INVENTION

Malignant neoplasms accounted for approximately 7.6 million deaths worldwide in 2008, and as the population ages, that number projects to 13.2 million by 2030. Patient responses to specific drugs vary widely and it is unreasonable to expect current drug therapies to combat the expected increase in cancer deaths. Personalized medicine through the use of individualized biomarkers provides a potential means of combating this problem. The biomarkers, proteins expressed by the specific cancer cell, even to the patient's particular cancer, are exploited to detect and deliver chemotherapeutics. Polyclonal and monoclonal antibodies perform this task rather well, but drawbacks exist due to the complexity and expense of antibody production in addition to the difficulty of conjugating the antibodies to active cytotoxic agents. Even if individual biomarkers can be exploited to deliver chemotherapeutic agents to the cancer cell, multiple drugs must be engineered to kill the many different varieties and patient-specific cancers.

A potential approach is to exploit cancer-specific biomarkers to deliver a drug that is highly cytotoxic to all cells in all patients. Thus, only one highly cytotoxic drug must be synthesized, as long as it is specifically delivered to the cancer cell and then excreted. Cell-specific aptamers with a high affinity for leukemia, liver, lymphoma, colon, and most recently breast cancer, are known. Aptamers are very attractive drug delivery agents because they have low molecular weights, are formed by a relatively easy and reproducible DNA

synthesis, display high binding affinities and molecular specificities, are easy modified, have fast tissue penetration, have low toxicity, are tunable in binding affinity, and are easy stored. Presently a number of diagnostic kits and imaging reagents using aptamers are in production.

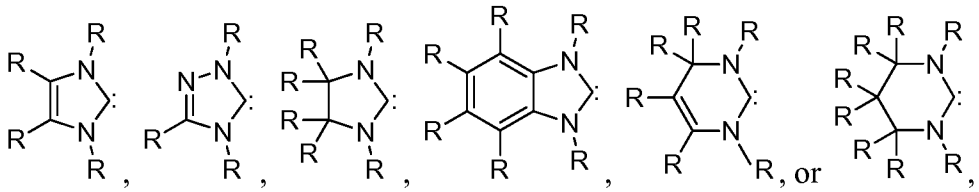
No single cytotoxic drug has been identified, although many metal ions are highly cytotoxic to cancer. Hence, a metal ion is an obvious choice for an all-inclusive drug candidate. Moreover, since 1935 gold complexes have served as effective aurotherapeutic agents. Auranofin, used to treat rheumatoid arthritis, is also highly active against cervical carcinoma (HeLa) cells in vitro and effective against *cis*-platin resistant cancer cells. Numerous derivatives of gold complexes demonstrate high activity. However, as with most heavy metals including *cis*-platin, accumulation in the body causes significant negative side effects. One mode of accumulation is metal ion complex degradation, whereby a metal-aqua complex is formed and is difficult to excrete. Auranofin contains an Au-$P(CH_2CH_3)_3$ gold bonded ligand which has low stability. As a result, Auranofin accumulates in the body, which is detrimental. In contrast, *N*-heterocyclic carbene gold (NHCAu) complexes have the remarkable stability engendered by the Au-carbon bond. NHCAu complexes are stable towards water, acidic solutions, and heat--important qualities for a good drug candidate.

NHCAu complexes have a significant advantage because aquation and deposition of Au ions in the body can be minimized. Because NHC ligands are highly modular, the rapid and easy modification of steric and electronic properties of the metal complexes can fine-tune to the complex's cytotoxicity. Examples of NHCAu complexes that show activity are those in FIG. 1, which exhibit IC50 values as low as 0.21 μM for breast carcinoma (MDA MB231). For example, the chiral di-gold complex **5** of FIG. 1 exhibits activity towards cervical carcinoma (HeLa) with an IC50 = 8.7 μM. Unfortunately, the NHCAu complexes are also toxic to normal healthy cells, as in the case of **5** where an IC50 = 4.6 μM for healthy embryonic kidney cells (HEK) is observed.

Currently, cancer-specific aptamer-NHCAu conjugates or other aptamer-N-heterocyclic carbene metal (NHCM) conjugates are unknown but they have the potential to avoid toxicity to normal cells, while still being highly effective for the destruction of cancer cells. Thus, to prevent heavy metal accumulation in the patient and the undesired side effects, aptamer-NHCM conjugates where the gold or alternative metal ion remains attached to the aptamer are desirable.

BRIEF SUMMARY

Embodiments of the invention are aptamer-N-heterocyclic-carbene metal complex conjugates (aptamer-NHCM conjugates) and aptamer-*bis*-N-heterocyclic-carbene metal complex conjugates (aptamer-*bis*-NHCM conjugates). This design couples an aptamer to one or more N-heterocyclic-carbene metal complexes (NHCMs) and/or one or more *bis*-N-heterocyclic-carbene metal complexes (*bis*-NHCMs) through hydrolytically stable bonds between the NHCM and the aptamer. The NHCMs or *bis*-NHCMs can be Au, Ag, Pt, Pd, Ru, Ni, or Cu complexes that include at least one N-heterocyclic-carbene ligand of the structure:



wherein R-groups are independently H, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_2-C_{18} alkynyl, C_6-C_{14} aryl, C_7-C_{18} arylalkyl, C_8-C_{18} arylalkenyl, C_8-C_{18} arylalkynyl, C_1-C_{18} alkoxy, C_6-C_{14} aryloxy, C_7-C_{18} arylalkyloxy, C_2-C_{18} alkenyloxy, C_2-C_{18} alkynyloxy, C_8-C_{18} arylalkenyloxy, C_8-C_{30} arylalkynyloxy, C_2-C_{30} alkylester, C_7-C_{15} arylester, C_8-C_{30} alkylarylester, C_3-C_{18} alkenylester, C_3-C_{18} alkynylester, C_3-C_{18} di- or poly-ether, C_3-C_{18} di- or poly-etherester, C_3-C_{18} di- or poly-ester, C_3-C_{18} di- or poly-amine, C_4-C_{18} di- or poly-ene and optionally substituted or multiply substituted with any of Cl, Br, I, F, OH, R'$_2$N, R'SO_2, R'SO, R'S, R'$_3$Si, R'O, NH_2, C(O)OH, N_3, C≡CH, vicinal disubstituted with C(O)OC(O), a cyclic conjugated diene, any salts derived therefrom or any condensation or addition derivative substituent therefrom, wherein R' can be C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_2-C_{18} alkynyl, C_6-C_{14} aryl, C_7-C_{18} arylalkyl, C_8-C_{18} arylalkenyl, C_8-C_{18} arylalkynyl, C_1-C_{18} alkoxy, C_6-C_{14} aryloxy, C_7-C_{18} arylalkyloxy, C_2-C_{18} alkenyloxy, C_2-C_{18} alkynyloxy, C_8-C_{18} arylalkenyloxy, C_8-C_{30} arylalkynyloxy, C_2-C_{30} alkylester, C_7-C_{15} arylester, C_8-C_{30} alkylarylester, C_3-C_{18} alkenylester, C_3-C_{18} alkynylester, C_3-C_{18} di- or poly-ether, C_3-C_{18} di- or poly-etherester, C_3-C_{18} di- or poly-ester, C_3-C_{18} di- or poly-amine, C_4-C_{18} di- or poly-ene, wherein at least one of the R groups of the N-heterocyclic-carbene comprises the condensation or addition derivative substituent comprising the coupling unit that forms the stable bond between the N-heterocyclic-carbene metal complex and the aptamer, wherein the condensation or addition derivative substituent is derived from condensation or addition of a -NH_2 and -C(O)OH, -N_3, and -C≡CH; -NH_2 and

vicinal disubstituted -C(O)OC(O)-, or homocyclic or heterocyclic conjugated diene and -C≡CH or –HC=CH_2. The aptamer is chosen to bind to a cancer cell specific receptor, such as a G-protein coupled receptor, epidermal growth factor receptor, tyrosine kinase receptor mutation variant III, or protein tyrosine kinase receptor 7. The coupling unit can include an amide -NHC(O)-, a 1,4-substituted triazole -N_3C_2H-; an imide [-$C(O)]_2N$-, a bicycle[2.2.1]heptane -C_7H_8-, a substituted bicycle[2.2.1]heptane, a 7-oxabicyclo[2.2.1]heptane -C_6H_6O-, a substituted 7-oxabicyclo[2.2.1]heptane, a 7-azabicyclo[2.2.1]heptane -C_6H_7N-, a substituted 7-azabicyclo[2.2.1]heptane, or a succinimide thioether.

Additionally or alternatively, a *bis*-N-heterocyclic-carbene metal complex can be included such that a plurality of N-heterocyclic-carbene ligands complex one or more metals and are coupled to an aptamer. N-heterocyclic-carbene ligands can be coupled through a framework that limits the degree of conformational freedom and disposes the ligands in a favorable orientation to complex a single metal ion. The framework can be, but is not necessarily, a portion of the coupling unit for attachment of the aptamer. In an embodiment of the invention, the framework and N-heterocyclic-carbenes can be a *trans*-9,10-dihydro-9,10-ethanoanthracene-11,12-di-*N*-heterocyclic-carbene.

An embodiment of the invention is the treatment of a disease by administering the aptamer-NHCM conjugate in a pharmaceutically effective amount to a patient with a cancer or a microbial infection. The aptamer-NHCM conjugate binds to the disease causing cells via the specific cell surface receptor present on the disease causing cells.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 shows exemplary NHCAu complexes that exhibit cytotoxicity at the micromolar level.

FIG. 2 shows reaction schemes to produce a carboxylic acid containing NHC-gold complex capable of conjugating to an aptamer, according to an embodiment of the invention.

FIG. 3 shows a reaction scheme to generate a silver salt from a carboxylate alkyl substituted imidazole zwitterion and converting the NHCAg complex into the NHCAu complex prior to conjugation with an aptamer, according to an embodiment of the invention.

FIG. 4 shows a reaction scheme to couple the carboxylic acid containing NHC-gold complex of FIG 2 with the coupling reagents 1-ethyl-3-[3-

dimethylaminopropyl]carbodiimide hydrochloride (EDC) and the conjugation with an amine functionalized aptamer, according to an embodiment of the invention.

FIG. 5 shows a reaction scheme where a 1-dimethylaminonaphthalene-5-sulfonamidopolymixin (dansp) flourophore functionalized NHC-gold complex is conjugated to an amine functionalized aptamer, according to an embodiment of the invention.

FIG. 6 shows a reaction scheme for the preparation of the imidazolium chloride salt **10** via alkylation with benzyl chloroacetate.

FIG. 7 shows a reaction scheme for generation of a benzyl protected silver(I) complex **11** followed by transmetalation with $(CH_3)_2SAuCl$ to yield the benzyl ester protected monocarbene gold(I)-NHC complex **12**, according to an embodiment of the invention.

FIG. 8 shows a reaction scheme to form alkyne functionalized heterocycles **13-15**.

FIG. 9 shows a reaction scheme for the deprotonation and metalation of ligand **13** with silver(I)oxide to form silver complex **16**.

FIG. 10 shows composite ^{1}H-NMR spectra of the reaction of FIG. 9 over time.

FIG. 11 shows a reaction scheme for the transmetalation of complex **16** to gold(I) complex **17.**

FIG. 12 shows a reaction scheme for the generation of a ligand conjugate **28** and an NHC-gold conjugate **21**, according to an embodiment of the invention.

FIG. 13A is a plot of cell viability in the presence of **21** aptamer-NHC-gold conjugate and FIG. 13B in the presence of **28**.

FIG. 14 is a reaction scheme for the preparation of **27** , a fluorescent drug bound NHC-gold complex for conjugation with an amine functionalized aptamer, according to an embodiment of the invention

FIG. 15 is an emission spectrum of compound **27** under excitation at 389 nm.

FIG. 16 is an emission spectra of FITC in water under excitation at 389 nm.

FIG 17 is a reaction scheme for conjugation of a fluorescent dye bound NHC-gold complex with an amine functionalized sgc8-aptamer, according to an embodiment of the invention.

FIG. 18 is an emission spectra of the conjugate between fluorescent dye bound NHC-gold complex with an amine functionalized sgc8-aptamer, according to an embodiment of the invention as isolated by HPLC.

BRIEF DESCRIPTION OF THE SEQUENCES

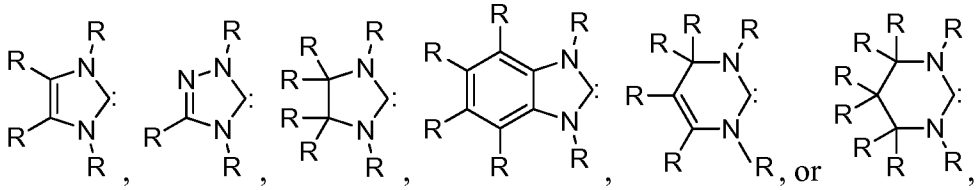
SEQ ID NO: 1: Sequence of sgc8c, an example of an aptamer.

DETAILED DISCLOSURE

Embodiments of the invention are aptamer-N-heterocyclic-carbene metal complex conjugates (aptamer-NHCM conjugates) or aptamer- *bis*-N-heterocyclic-carbene metal complex conjugates (aptamer-*bis*-NHCM conjugates). These include aptamer-N-heterocyclic-carbene gold complex conjugates (aptamer-NHCAu conjugates) or other aptamer-NHCM metal complex conjugates, for targeting cancer cells. The aptamer-NHCM conjugates are constructed to ensure that the gold or alternative metal ion complex remains intact and bound to the aptamer. In this manner, the decomposition of the metal complex is avoided along with heavy metal accumulation side effects. The conjugation of the gold, or other metal ion complex, to the aptamer is through an inert and highly stable bond (e.g., an amide bond). An embodiment of the invention involves a method to couple an aptamer containing or adapted to contain a functionality that can bond with a NHCM complex containing the complementary functionality. In one embodiment of the invention, the aptamer functionality is an amine and the complementary functionality of the NHCM complex is a carboxylic acid. In another embodiment of the invention, the NHCM complex has amine functionality and an aptamer has a carboxylic acid coupling functionality. In other embodiments of the invention, the aptamer is modified to attach a complementary functionality onto the aptamer and the NHCM complex is equipped with a complementary functionality for the formation of at least one bond by a "click" reaction or other high conversion addition or condensation reactions. For example, the functionalities can be $-N_3$ and -C≡CH for a Huisgen cycloaddition, an –ene or an –yne, a conjugated diene, a cyclic diene or a heterocyclic diene for a Diels-Alder cycloaddition, a primary amine and a dicarboxylic acid anhydride for an imidization, a maleimide and a thiol for a succinimide thioether, or any other complementary reactive functionalities for a high yield, stoichiometric reaction to couple the units upon combining the aptamer and NHCM or *bis*-NHCM.

FIG. 2 shows two alternative reactions sequences to synthesize an exemplary carboxylic acid containing NHC-gold complex where an alkyl imidazole reacts with a chloro substituted ester to yield an imidazolium salt in high yield. Subsequently, metalation followed or preceded by ester hydrolysis forms the NHCAu complex with a carboxylic acid

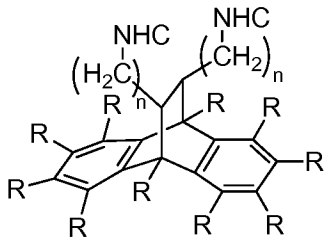
group. Other *N*-heterocyclic carbenes can be substituted for the exemplary imidazole containing carbene Au complex, shown for an exemplary NHCAu complex in FIG. 2, or used to prepare any alternative NHC-metal (NHCM) complex and ultimately the aptamer-NHCM conjugates. The carbene can be of the structure:



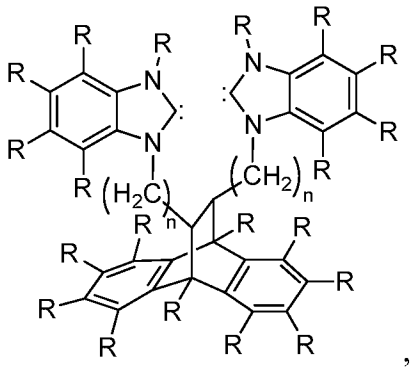
where R-groups are independently H, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_2-C_{18} alkynyl, C_6-C_{14} aryl, C_7-C_{18} arylalkyl, C_8-C_{18} arylalkenyl, C_8-C_{18} arylalkynyl, C_1-C_{18} alkoxy, C_6-C_{14} aryloxy, C_7-C_{18} arylalkyloxy, C_2-C_{18} alkenyloxy, C_2-C_{18} alkynyloxy, C_8-C_{18} arylalkenyloxy, C_8-C_{30} arylalkynyloxy, C_2-C_{30} alkylester, C_7-C_{15} arylester, C_8-C_{30} alkylarylester, C_3-C_{18} alkenylester, C_3-C_{18} alkynylester, C_3-C_{18} di- or poly-ether, C_3-C_{18} di- or poly-etherester, C_3-C_{18} di- or poly-ester, C_3-C_{18} di- or poly-amine, C_4-C_{18} di- or poly-ene and optionally substituted or multiply substituted with any of Cl, Br, I, F, OH, R'_2N, $R'SO_2$, R'SO, R'S, R'_3Si, R'O, NH_2, C(O)OH, N_3, C≡CH, vicinal disubstituted with C(O)OC(O), a cyclic conjugated diene, or any salts derived therefrom, wherein R' can be C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_2-C_{18} alkynyl, C_6-C_{14} aryl, C_7-C_{18} arylalkyl, C_8-C_{18} arylalkenyl, C_8-C_{18} arylalkynyl, C_1-C_{18} alkoxy, C_6-C_{14} aryloxy, C_7-C_{18} arylalkyloxy, C_2-C_{18} alkenyloxy, C_2-C_{18} alkynyloxy, C_8-C_{18} arylalkenyloxy, C_8-C_{30} arylalkynyloxy, C_2-C_{30} alkylester, C_7-C_{15} arylester, C_8-C_{30} alkylarylester, C_3-C_{18} alkenylester, C_3-C_{18} alkynylester, C_3-C_{18} di- or poly-ether, C_3-C_{18} di- or poly-etherester, C_3-C_{18} di- or poly-ester, C_3-C_{18} di- or poly-amine, C_4-C_{18} di- or poly-ene, where, optionally, one of the R groups of one of the N-heterocyclic-carbenes contains the requisite coupling functional group to a complementary functionality necessary for covalent attachment to the aptamer, where the coupling functional group is -NH_2, -C(O)OH, -N_3, -C≡CH; vicinal disubstituted -C(O)OC(O)-, or homocyclic or heterocyclic conjugated diene, where R and R' groups are linear, branched, cyclic, polycyclic or any combination thereof, and where the N-heterocyclic-carbene is achiral, racemic mixture, partially resolved enantiomer, resolved enantiomer, or a mixture of diastereomers.

In an embodiment of the invention, the N-heterocyclic-carbene metal complex is a *bis*-N-heterocyclic-carbene metal complex, where a plurality of N-heterocyclic-carbene ligands and one or more metals are coupled to an aptamer. In an embodiment of the

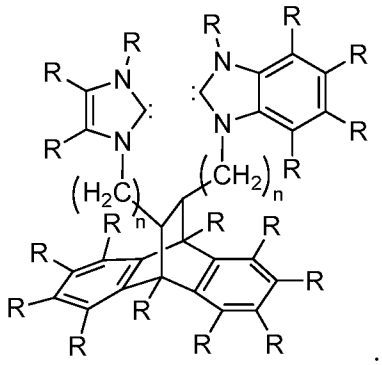
invention, N-heterocyclic-carbene ligands can be positionally fixed through a framework that limits the degree of conformational freedom to dispose the carbene ligands in a favorable orientation and proximity relative to each other to ligate a single metal ion. The framework can be, but is not necessarily, a portion of the coupling unit. In an embodiment of the invention, the framework and N-heterocyclic-carbenes can be a *trans*-9,10-dihydro-9,10-ethanoanthracene-11,12-di-oligomethylene-*N*-heterocyclic-carbene:



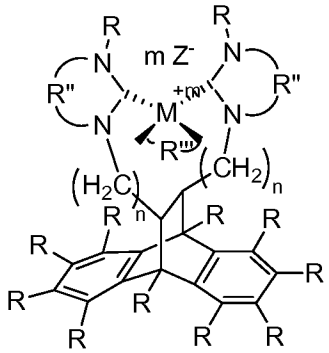
where NHC indicates any of the NHC carbenes above, independently chosen, where one of the nitrogens of each of the two NHCs is bound to the terminal methylene of the oligomethylene links of the bicyclo framework, and where n is 1 to 4. For purposes of the invention, the oligomethylene can be of a single methylene unit and does not require a plurality of methylene units to fulfill the definition of an oligomethylene. The R groups of the framework are independently defined as the R groups of the NHC carbenes, above. The *trans*-9,10-dihydro-9,10-ethanoanthracene-11,12-di-oligomethylene-*N*-heterocyclic-carbene can be a symmetric *bis*-NHC, such as:



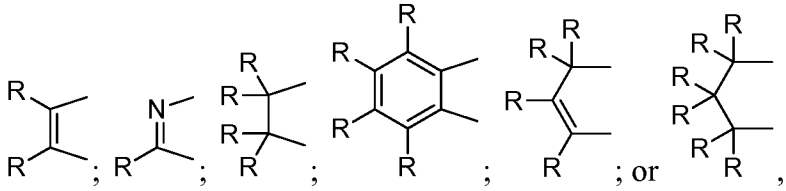
, or an asymmetric *bis*-NHC, such as:



The *bis*-NHC metal complex, can have the structure:



wherein n and R are defined as above, R" is independently:



wherein anion Z is Cl^-, Br^-, I^-, BF_4^-, PF_6^-, SbF_6^-, $^-OSO_2CF_3$, or $^-OSO_2C_6H_5$, wherein M is Au, Ag, Rh, Ir, Pd, Pt, Ru, Ni, Cu, or other transition metal, m is 0 to 3, and wherein R"' is an optional ancillary bidentate diene ligand selected from the group consisting of norbornene, substituted norbornene, 1,5-cyclooctadiene, or substituted 1,5-cyclooctadiene.

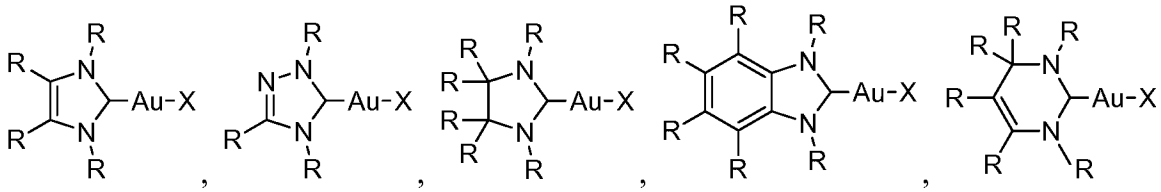
Although FIG. 2 shows an ethyl ester of the carboxylic acid, in other embodiments of the invention, alternative esters can be used, particularly those whose conversion to the carboxylic acid is carried out under mild conditions. These include, but are not limited to, benzyl and silyl esters such as trimethylsilyl, triethylsilyl, or *t*-butyldimethylsilyl esters, which are liberated by hydrolysis or fluoride substitution, esters of fluorinated phenols, for example pentafluorophenoxy esters, as well as alkyl esters prone to elimination by ester pyrolysis. Other embodiments of the invention can employ a precursor other than an ester that yield a carboxylic acid, for example, but not limited to, a mixed carboxylic acid anhydride, a carboxylic acid halide, or other functionality that can be hydrolyzed to form a

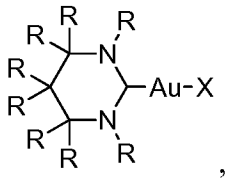
carboxylic acid. Additional embodiments of the invention include other functionalities than those of a carboxylic acid derivative for formation of the carboxylic acid. These include, but not limited to, a nitrile, which can be hydrolyzed, or an aldehyde or alcohol which can be oxidized to form the carboxylic acid. In another embodiment of the invention, the carboxylic acid, or its carboxylate salt, can be used directly in the synthesis of the NHCAu complex without an intermediate ester or other carboxylic acid precursor functionality.

In addition to the route towards the NHCAu complex shown in FIG. 2, several alternative approaches to the synthesis of a carboxylic acid appended NHCAu complex can be carried out, including: cleavage of a C=C bond in electron rich alkenes as disclosed in Ozdemir *et al.*, *Applied Organometallic Chemistry* **2004,** *18* (7), 318-322; generation of a free carbene by deprotonation of the imidazolium precursor with a strong base as disclosed in Bohler *et al.*, *New Journal of Chemistry* **2002,** *26* (10), 1291-1295; transmetalation of a deprotonated azole followed by protonation or alkylation of the gold azolyl compound as disclosed in Raubenheimer *et al.*, *Journal of Organometallic Chemistry* **2001,** *617* (1), 170-181; in situ deprotonation of an imidazolium salt with a weak base as disclosed in Poyatos *et al.*, *Inorganic Chemistry* **2003,** *42* (8), 2572-2576; and transmetalation from a silver-NHC complex prepared by a reaction of an imidazolium precursor with Ag_2O as disclosed in Wang *et al.*, *Organometallics* **1998,** *17* (5), 972-975 and Ozdemir *et al.*, *Molecules* **2010,** *15* (4), 2203-2210, all of these references are incorporated herein by reference.

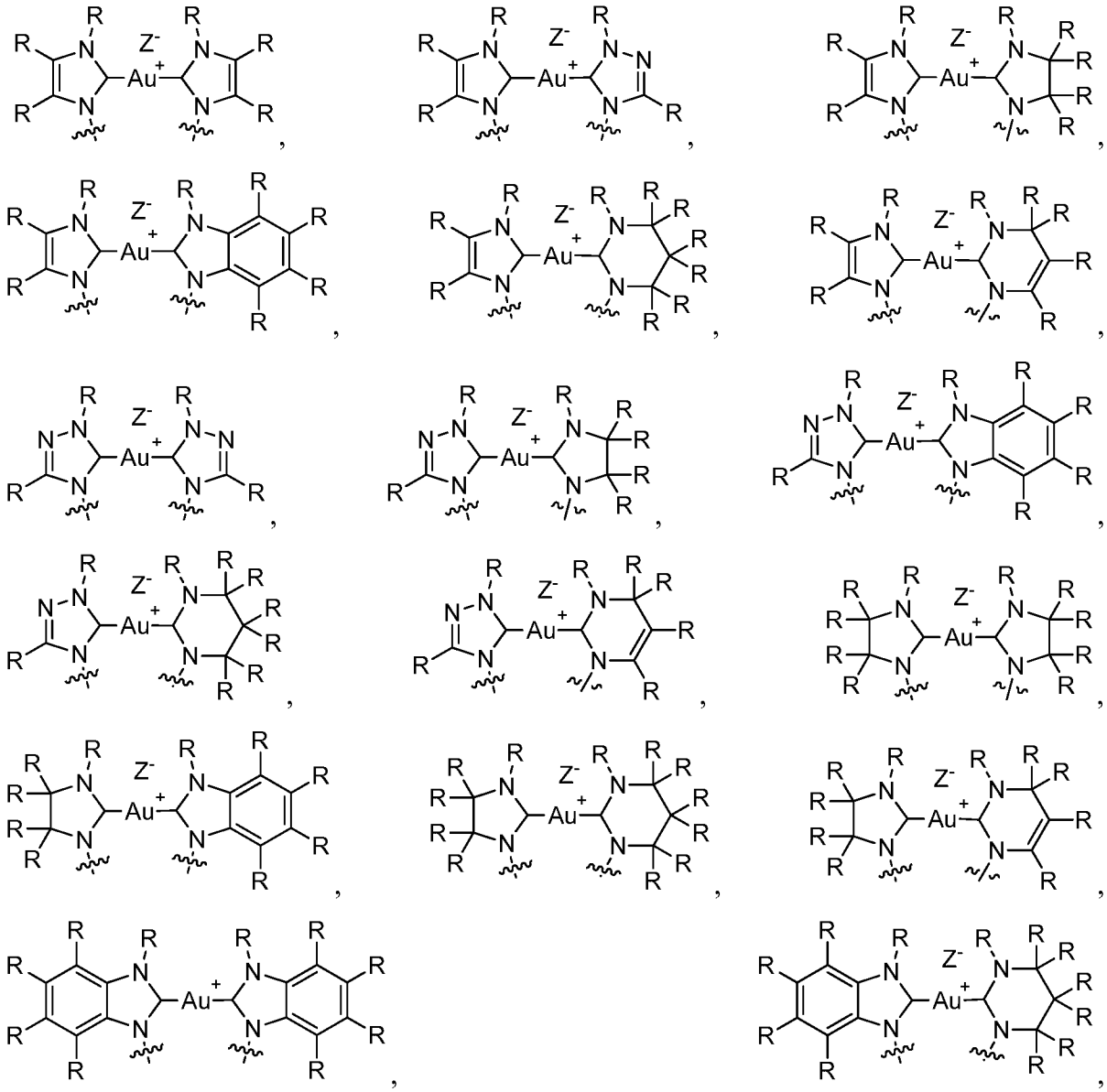
Of these routes, a gold(I)-NHC comlex is readily synthesized via transmetalation from a corresponding silver(I)-NHC complex or bis-NHC-silver(I) halide salt by treatment with an appropriate gold(I) source. Carboxylate functionalized silver(I)-NHC complexes can be readily prepared by the method disclosed in Moore *et al.*, *Organometallics* **2006,** *25* (21), 5151-5158, which is incorporated herein. FIG. 3 shows a reaction scheme to generate a silver salt from a carboxylic acid alkyl substituted imidazole zwitterion and subsequently convert the NHCAg complex to the NHCAu complex.

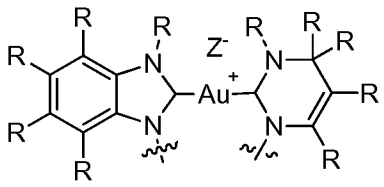
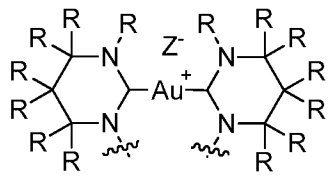
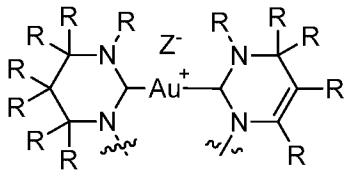
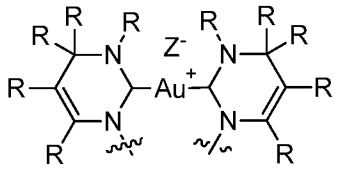
An NHCAu complex can comprise any of the structures:


, or


,

and a *bis*-NHCAu complex can comprise any of the structures:




,

,

, or


wherein X is Cl, Br, I or OH, Z is Cl^-, Br^-, I^-, BF_4^-, PF_6^-, SbF_6^-, $^-OSO_2CF_3$, or $^-OSO_2C_6H_5$, and, independently, R-groups are independently H, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_2-C_{18} alkynyl, C_6-C_{14} aryl, C_7-C_{18} arylalkyl, C_8-C_{18} arylalkenyl, C_8-C_{18} arylalkynyl, C_1-C_{18} alkoxy, C_6-C_{14} aryloxy, C_7-C_{18} arylalkyloxy, C_2-C_{18} alkenyloxy, C_2-C_{18} alkynyloxy, C_8-C_{18} arylalkenyloxy, C_8-C_{30} arylalkynyloxy, C_2-C_{30} alkylester, C_7-C_{15} arylester, C_8-C_{30} alkylarylester, C_3-C_{18} alkenylester, C_3-C_{18} alkynylester, C_3-C_{18} di- or poly-ether, C_3-C_{18} di- or poly-etherester, C_3-C_{18} di- or poly-ester, C_3-C_{18} di- or poly-amine, C_4-C_{18} di- or poly-ene and optionally substituted or multiply substituted with any of Cl, Br, I, F, OH, R'_2N, $R'SO_2$, R'SO, R'S, R'_3Si, R'O, NH_2, C(O)OH, N_3, C≡CH, vicinal disubstituted with C(O)OC(O), a cyclic conjugated diene, any salts derived therefrom or any condensation or addition derivative substituent therefrom, wherein R' can be C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_2-C_{18} alkynyl, C_6-C_{14} aryl, C_7-C_{18} arylalkyl, C_8-C_{18} arylalkenyl, C_8-C_{18} arylalkynyl, C_1-C_{18} alkoxy, C_6-C_{14} aryloxy, C_7-C_{18} arylalkyloxy, C_2-C_{18} alkenyloxy, C_2-C_{18} alkynyloxy, C_8-C_{18} arylalkenyloxy, C_8-C_{30} arylalkynyloxy, C_2-C_{30} alkylester, C_7-C_{15} arylester, C_8-C_{30} alkylarylester, C_3-C_{18} alkenylester, C_3-C_{18} alkynylester, C_3-C_{18} di- or poly-ether, C_3-C_{18} di- or poly-etherester, C_3-C_{18} di- or poly-ester, C_3-C_{18} di- or poly-amine, C_4-C_{18} di- or poly-ene, wherein at least one of the R groups of the N-heterocyclic-carbene comprises the condensation or addition derivative substituent that comprises the coupling unit with the stable bond between the N-heterocyclic-carbene metal complex and the aptamer, where the condensation or addition derivative substituent is derived from condensation or addition of a $-NH_2$ and -C(O)OH, $-N_3$ and -C≡CH; $-NH_2$ and vicinal disubstituted -C(O)OC(O)-, homocyclic or heterocyclic conjugated diene and -C≡CH or $–HC=CH_2$, or –SH and a maleimide, wherein R and R' groups are linear, branched, cyclic, polycyclic or any combination thereof, and wherein the N-heterocyclic-carbene is achiral, a racemic mixture, partially resolved enantiomer, resolved enantiomer, resolved diastereomers, or a mixture of diastereomers. The undefined bonding to a nitrogen of the N-heterocyclic

carbene can be to an R group or can be attached to a framework, such as to the terminal position of the oligomethylene *trans*-9,10-dihydro-9,10-ethanoanthracene-11,12-di-oligomethylene-*N*-heterocyclic-carbene.

In other embodiments of the invention, the Au metal can be substituted by Ag, Pt, Pd, Ru, Ni, or Cu, where the additional X, Z^-, and/or NHC ligands lacking a site for coupling with an aptamer can be included in the complex as required by the oxidation state of the metal ion in the complex, as the metal ion can be in any oxidation state.

Aptamers are polynucleotide or polypeptide molecules that bind to a specific target molecule. Non-limiting examples of aptamers include: DNA aptamers; RNA aptamers; XNA (nucleic acid analogs or artificial nucleic acids) aptamers; and polypeptide aptamers. Examples of XNA include, but are not limited to, polypeptide nucleic acid (PNA), morpholino and locked nucleic acid (LNA), glycol nucleic acid (GNA), and threose nucleic acid (TNA).

NHCM can be conjugated to aptamers in a covalent or a non-covalent manner. NHCM can be covalently conjugated to aptamers directly or via molecular linkers. Various molecular linkers are known to a person of ordinary skill in the art, and certain non-limiting examples are described in "Easy molecular bonding crosslinking technology" published by Thermo Scientific (2012), the contents of which are herein incorporated by reference in its entirety.

In an embodiment of the invention, the covalent bond between the aptamer and NHCM is acid labile so that the covalent bond is broken between the NHCM and the conjugated aptamer as the NHCM and aptamer are internalized by the target cells and subjected to the acidic environment of the endosomes, thereby releasing the NHCMs into the target cells. An example of acid labile cross-linking involves the use of N-(Epsilon-Maleimidocaproic Acid) hydrazide to conjugate NHCM with aptamers. Additional examples of acid labile covalent binding are known to a person of ordinary skill in the art and such embodiments are within the purview of the current invention.

In another embodiment of the invention, the NHCM is conjugated to the aptamer in a non-covalent manner. Non-limiting examples of non-covalent binding between NHCM and aptamers include electrostatic binding, π-binding, van der Waals interactions, and hydrophobic binding between the NHCM and the aptamers.

Aptamer conjugated NHCM complexes can be used to specifically deliver the NHCM complexes to target cells, e.g., cancer cells. The aptamers can selectively recognize target cells through specific cell surface proteins, and direct the tethered NHCM complexes to the target cells. NHCM complexes specifically targeted and delivered to target cells can kill the target cells (e.g., cancer cells), without affecting the non-targeted cells (e.g., normal cells). As such, the current invention provides compositions and methods for treating a disease, e.g., cancer, in a subject. The aptamer is capable of binding to a cell surface receptor present on a target cell. The cell surface receptor can be specific to the target cells, *i.e.*, the cell surface receptor is present only on the surfaces of target cells and is absent from the surfaces of non-target cells, or the surface receptor is present on the surfaces of target cells at a high level and is present on the surfaces of non-target cells at a significantly lower level than that of the target cells. For example, the aptamer can bind to a cell surface receptor specific to cancer cells, *i.e.*, a cell surface receptor which is present only on the surfaces of cancer cells and is absent from the surfaces of normal cells or is present on the surfaces of cancer cells at high level and is present on the surfaces of normal cells at a significantly lower level. The aptamer can bind to a cell surface receptor specific to an infectious agent, *i.e.*, a cell surface receptor which is present only on the surfaces of infectious agents and is absent from the surfaces of normal cells or is present on the surfaces of infectious agents at high level and is present on the surfaces of normal cells at a significantly lower level.

Accordingly, the diseases that can be treated, according to compositions and methods of the invention, include cancer, microbial infections and other diseases where the disease causing cells exhibit presence of a specific cell surface receptor that can be exploited to target the diseased cells. Various cancers that can be treated, according to an embodiment of the invention, are well known to a person of ordinary skill in the art and such cancers are within the purview of the current invention. The microbial infections can be viral, fungal, bacterial, protozoan, or prion mediated.

To treat a disease, according to an embodiment of the invention, an aptamer can be selected for its capability to bind to all or most of the target cells in a subject without binding to all or most of the non-target cells. An aptamer can bind to a target cell through a target cell specific receptor. For example, to treat cancer according to an embodiment of the invention, the aptamer can be selected for its ability to bind to most or all of the cancer cells without binding to all or most of the normal cells through a receptor specific for cancer cells.

Non-limiting examples of cancer cell specific receptors include, G-protein coupled receptors, epidermal growth factor receptor, tyrosine kinase receptor mutation variant III, and protein tyrosine kinase receptor 7. Additional examples of receptors specific to cancer cells and corresponding aptamers are well known to a person of ordinary skill in the art and such embodiments are within the purview of this invention. For example, Meyer *et al.* (2011), Cell-specific aptamers as emerging therapeutics, Journal of Nucleic Acids, Volume 2011, Article ID 904750, teaches cancer cell receptor specific aptamers, the contents of which are herein incorporated by reference in its entirety, particularly, page 5, section under "Cell specific aptamers for therapy" continuing on to pages 6 to 13.

Examples of aptamers and corresponding diseases that can be treated using the aptamers are given in Marolt *et al.*, Generating aptamers for cancer diagnosis and therapy, *Clin. Exp. Pharmacol.*, (2012) 2:111, the contents of which are herein incorporated by reference in its entirety, particularly, page 3, table 1; page 3, section under "Aptamers in cancer cell diagnostics and Treatment" continuing on to page 4; page 4, section under "Aptamers used in alimentary neoplasm, continuing on to pages 5 and 6; and Figures 1 and 2. Other examples of aptamers and corresponding diseases that can be treated using the aptamers are given in Meyer *et al.* (discussed above). A skilled artisan can identify additional diseases and corresponding aptamers that can be treated according to the compositions and methods of the current invention and such diseases are within the purview of the current invention.

The subjects that can be treated according to the methods of the current invention can be a mammal, for example, a human, porcine, canine, rodent, feline, or bovine.

The pharmaceutically effective amount of the aptamer-polynucleotide stem complex-drug conjugate depends on the type of disease to be treated and the type of drug conjugated to the complex as well as the tolerance of the subject for the treatment.

The disease treatment according to the current invention can also be administered in combination with one or more other treatments. For example, cancer in a subject can be treated by administering the NHCM-aptamer conjugate of the current invention in combination with chemotherapy and/or radiotherapy.

In an exemplary embodiment of the invention, the leukemia specific aptamer **Sgc8c** with a primary amine modification is coupled with a carboxylic acid functional NHCAu complex. Conjugation of a NHCM complex to the aptamer can be carried out with an

aptamer, for example, but not limited to, the leukemia-specific aptamer **Sgc8c** (5'-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA-3' SEQ ID NO: 1). For example, the coupling reagents 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), as shown in FIG. 4, and *N*-hydroxysulfosuccinimide (Sulfo-NHS) can be used to couple the carboxylic acid functionalized NHCAu complex to the primary amine modified aptamer.

Standard phosphoramidite solid phase DNA synthesis is completely automated and highly reproducible, and DNA oligonucleotides are easily purified. Aptamer synthesis by solid phase phosphoramidite chemistry is amenable to synthetic modifications. By simply inserting any commercially available phosphoramidite modified with the requisite functional group onto either the 3' or 5' end of the oligonucleotide sequence, an aptamer can be generated that is complementary for coupling to a NHCM complex.

In an embodiment of the invention, an NHCM complex can have an R group that is a fluorescent dye or other functionality that yields an aptamer-NHCM conjugate that can act as a delivery-therapeutic-detection agent, where the dye is a fluorophore, to facilitate *in vivo* detection. For example, as shown in FIG. 5, a 1-dimethylaminonaphthalene-5-sulfonamidopolymixin (dansp) flourophore functionalized NHC-gold complex can be formed and conjugated in the manner of FIG. 4 to form the aptamer-NHCAu conjugate delivery-therapeutic-detection agent. The dansp moiety fluoresces at 532 nm upon excitation at 365 nm. Other fluorescent dyes and molecules can be adapted for use as the R group, including, but not limited to, pyrenylmethyl, anthracenylmethyl, or any other fluorescent moiety. Alternatively or additionally, the NHCM complex can have an R group that comprises a polyether group or other group that can aid in crossing a cancer cell membrane and/or increasing the aptamer-NHCM complexes solubility in aqueous media.

METHODS AND MATERIALS

Compounds **1** and **7** were prepared according to reported procedures. Unless stated otherwise all syntheses and manipulations were performed under aerobic conditions. All starting reagents were obtained from either Sigma Aldrich or STREM chemicals and used as received without further purification. $(CH_3)_2SAuCl$, Ag_2O, glycine were obtained from Sigma Aldrich and used without further purification. Sgc8c-Aptamer was synthesized with a DNA synthesizer according to literature procedures.

All NMR spectra were collected on either a Varian Mercury Broad Band 300 MHz or Varian Inova 500 MHz spectrometer. Chemical shifts are reported in δ (ppm) with the solvent peak referenced as an internal reference. The DOSY experiments used bipolar pulse pair stimulated echo with convection compensation. The gradient length was 1 ms and the diffusion time 100 ms; the gradient strength was arrayed in 15 steps squarely spaced from 2 to 53 Gauss/cm. Spectra were collected in 16 scans with an acquisition time of 3.6 s and a relaxation delay of 3 s.

Electrospray Ionization Mass Spectrometry (ESI-MS) data for both positive and negative modes were obtained according to the following procedure. Samples were dissolved in methylene chloride or in water and directly injected into an auto-sampler, where they were subjected to ESI with methanol as the mobile phase. Ions were detected with an Agilent 6210 TOF-MS instrument and the data was processed using MassHunterTMsoftware.

Imidazolium chloride salt **10** was prepared via alkylation with benzyl chloroacetate, as shown in FIG. 6. Generating the benzyl protected silver(I) complex **11** in situ followed by transmetalation, as shown in FIG. 7, with $(CH_3)_2SAuCl$ provides complex **12**, which is confirmed by ^{1}H-NMR spectroscopy. The ions detected in positive mode ESI-MS analysis support the assignment of **12** as the benzyl ester protected monocarbene gold(I)-NHC complex **12**.

Alkyne functionalized heterocycles **13-15**, were prepared under the conditions shown in FIG. 8, where relatively harsh conditions are required to drive the reaction. Using a relatively high temperature (120 °C) and long reaction time (3 days), the method permitted the assembly of ligands **13-15** in yields of 63.4, 80.2, and <25%, respectively. ^{1}H-NMR spectra of **13-15** reveal diagnostic downfield chemical shifts of 10.0-10.5 ppm, corresponding to the deshielded C_2 proton flanked by two nitrogen atoms of the imidazol-2-ylidene ring. ^{13}C-NMR spectra of these ligands exhibit resonances attributable to the C_2 carbons between 135.7 ppm and 137.9 ppm. The terminal alkynyl protons of ligands **14** and **15** appear as triplets (J = 2.2-2.7 Hz) due to long range coupling with the methylene protons distal to the heterocyclic ring and adjacent to the triple bond. This long range coupling is still present for compound **13** but is not viewable by ^{1}H-NMR spectroscopy due to signal overlap with a large singlet at 2.05 ppm from the mesityl methyl groups.

Deprotonation and metalation of ligand **13**, as shown in FIG. 9, with 0.70 equivalents of silver(I)oxide required long reaction times. Aliquots of the reaction mixture taken

periodically throughout the duration of the reaction and subjected to ^{1}H-NMR spectroscopic analysis, as shown in FIG. 10, provided a method of monitoring the reaction progress. The ligand's C_2 imidazolium proton, resonating at 10.15 ppm, decreases over time concomitant with the growth of a set of resonances in the aromatic and aliphatic regions corresponding to complex **16**. Total conversion of **13** to the silver(I) species **16** requires stirring at room temperature for three full days in the absence of light. Precipitation of complex **16** as a fine tan powder resulted from passing the reaction mixture through celite, reducing the filtrate by vacuum, and adding an excess of ether. The ^{1}H-NMR spectrum of **16** provides evidence that the C_2 proton of **13** was removed. Though the lack of resonances downfield of 10 ppm in the ^{1}H-NMR spectrum of **16** indicates a complex form, the weak and seemingly absent, chemical shift in the range of 170 ppm and 190 ppm (commonly reported for NHC silver(I)-carbene complexes) in the ^{13}C-NMR spectrum of complex **16**, prompted further characterization.

Data suggest that the more labile (*exchanging*) a silver-carbene bond is on the NMR time scale, the broader the ^{13}C NMR signal of the carbene carbon. In the case of complex **16**, the weak, almost indistinguishable signal, is the result of broadening and baseline saturation caused by a relatively labile silver-carbene bond. To establish the structure of **16** in solution, gradient heteronuclear multiple bond coherence (gHMBC) experiments were performed to elucidate C-H connectivities and confirm peak assignments. .Long range coupling between the carbene carbon (182.9 ppm) of the imidazol-2-ylidene ring and the proximal methylene protons of the of the alkyne (4.38 ppm) *N*-substituent as well as the C4 and C5 protons of the imidazolium ring (6.92 and 7.36 ppm). The lack of a >200 ppm ^{13}C-NMR chemical shift in conjunction with a detected resonance at 182.9 ppm, precludes the possibility of a free carbene and supports the assignment of a silver(I)-NHC complex.

Transmetalation of complex **16** to gold(I) proceeds rapidly upon treatment of the silver(I) species with 1.2 eq of chlorodimethylsulfide gold(I), as shown in FIG. 11. Solid AgCl immediately precipitates as the gold(I) ion coordinates and displaces silver(I) from the carbene carbon of the NHC ligand. Complete conversion of **16** to **17** requires only 15 minutes, as monitored by ^{1}H and ^{13}C-NMR spectroscopy. A diagnostic gold(I)-carbene ^{13}C-NMR resonance of 172.0 ppm appears, along with new chemical shifts across the remainder of the spectrum.

Transmetalation proceeds rapidly and reaction times exceeding 1 h lead to the appearance of colloidal gold accompanied by a purple color change. Although this color

change is indicative of complex decomposition or possible product conversion, no observable demetalation products (*i.e.*, free ligand resonances) are observed spectroscopically; in fact, both the ^{1}H and ^{13}C-NMR spectra still indicate the presence of only one clean product in solution. An explanation for this phenomenon is that the decomposition products are comprised exclusively of NMR silent and/or chloroform insoluble species. To probe whether the appearance of colloidal gold forms as a consequence of a monocarbene to biscarbene conversion, or an oxidation state change from gold(I) to gold(III), a succinct NMR study was performed. The reaction of FIG. 11 was repeated on an NMR scale with 30 mg of **16**. ^{1}H and ^{13}C NMR spectra obtained at variable time intervals indicate the initial transmetalation from silver(I) to gold(I) occurs with a clear ^{13}C-NMR signal shift from ~184 to 172 ppm. The data support immediate formation of a single species that does not undergo subsequent mono- to bis-NHC conversion or metal ion oxidation processes.

Synthesis of compound 10

1-Mesityl-3-(2-benzyleacetyl) imidazolium chloride **10** was prepared by adding compound **1'** (0.640 g, 3.44 mmol), a magnetic stir bar, and 4 mL of dry toluene to a 250 mL round bottom flask. The mixture was allowed to stir for 2 min, whereupon benzyl chloroacetate **2'b** (0.53 mL, 3.44 mmol) was added dropwise. The system was placed under Ar and heated to 110 °C for 6 h while stirring. The mixture was then allowed to cool and left to stir for an additional 12 h at room temperature. The solvent was removed in vacuo and the resulting colorless solid was triturated with ether (1.02 g, 79.9%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ = 10.41 (s, 1H), 7.94 (m, 1H), 7.34-7.32 (5H, m), 7.08 (m, 1H), 6.95 (s, 2H), 5.98 (s, 2H), 5.18 (s, 2H), 2.32 (s, 3H), 2.01, (s, 6H). ^{13}C-NMR ($CDCl_3$, 125 MHz). δ = 166.4 (C=O), 141.3, 139.7 (NCN), 139.7, 134.30, 134.3, 130.6, 129.7, 128.7, 128.6, 124.4, 122.3, 68.38, 50.57, 21.04, 17.39.

Synthesis of compound 12

1-Mesityl-3-(2-benzyleacetyl)imidazolium gold(I) chloride **12** was prepared via an *in situ* transmetalation reaction. 0.201g of **10** and 0.150 g of Ag_2O were suspended in 5 mL of dichloromethane and allowed to stir for 24 h in the dark. The resulting suspension was passed through a celite pad and fine frit filter directly into a stirring suspension of 0.075g of $(CH_3)_2SAu(I)Cl$ in 5 mL of dicholoromethane. The reaction mixture was allowed to stir at room temperature for 3.5 h in the dark. The mixture was then passed through a celite pad and medium frit to yield an amber colored filtrate. The solvent was concentrated and then treated

with an excess of pentanes to precipitate a fine off-white powder. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ = 7.38 (m, 5H), 7.23 (d, *J* = 1.32 Hz, 1H), 6.95 (s, 2H), 6.92 (d, *J* = 1.32 Hz, 1H), 5.24 (s, 2H), 5.14 (s, 2H), 2.32 (s, 3H), 1.99, (s, 6H). ESI-MS (positive ion, calculated for M = $C_{21}H_{22}N_2O_2AuCl$). 1155.1945 m/z $[2M+Na]^+$, 1097.2374 m/z $[2M-Cl]^+$, 589.0926 m/z $[M+Na]^+$, 531.1339 m/z $[M-Cl]^+$.

Hydrogenolysis Reaction

Compound **12** (30 mg) was dissolved in a 1:1 mixture of methanol: ethyl acetate (10 mL). This mixture was added to a solution of 1:1 methanol: ethyl acetate (5 mL) containing 5 mg of Pd/C. The reaction mixture was left to stir at room temperature under a balloon of H_2 for 24 h. After the allotted time, the solution was passed through a celite pad and fine frit. The solvent was removed in vacuo and a ^{1}H-NMR of the residue was obtained ($CDCl_3$, 300 MHz) and indicated the presence of only starting material **12**.

Synthesis of compound 13

1-Mesityl-3-(1-butyne)imidazolium bromide **13** was prepared by dissolving 1.11 g (5.95 mmol) of 1-mesitylimidazole in 10 mL of toluene in a 100 mL round bottom flask. To this stirring solution, 0.84 mL (8.92 mmol) of cold 4-bromo-1-butyne was added dropwise. The reaction mixture was then placed under an ice water cooled condenser, heated to 120 °C, and allowed to stir at this temperature for 3 d. After the reaction was complete an off-white residue formed a film on the inside of the reaction vessel. 20 mL of diethyl ether was added directly to the reaction mixture and the contents were stirred vigorously for 3 h at room temperature. The suspension was filtered and the solid was collected on a Buchner funnel and washed with 3 x 10 mL of diethyl ether. The resulting off-white solid was then dried under vacuum for 24 h (0.70 g, 63.4%). Heteronuclear multiple bond coherence (gHMBC) was applied to determine C-H connectivities and confirm peak assignments. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ = 10.15 (s, 1H), 8.23 (s, 1H), 7.17 (s, 1H), 6.97 (s, 2H), 4.89 (t, *J* = 5.85 Hz, 2H), 2.99 (dt, *J* = 5.85 Hz, *J* = 1.95 Hz, 2H), 2.31 (s, 3H), 2.05 (s, 6H), 2.05 (t, *J* = 1.95 Hz, 1H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ = 141.3, 137.9 (NCN), 134.2, 130.6, 129.8, 123.9, 122.7, 79.06, 72.54, 48.56, 21.07, 21.04, 17.56. ESI-MS (positive ion, calculated for M = $C_{16}H_{19}BrN_2$). 355.0402 m/z $[M+Cl]^+$, 319.0802 m/z $[M+H]^+$, 239.1554 m/z $[M-Br]^+$.

Synthesis of compound 14

1-Benzyl-3-(1-butyne)imidazolium bromide **14** was prepared by a similar synthetic procedure as **13** using 1.00 g (6.32 mmol) of 1-benzyl-imidazole, 0.71 mL (7.59 mmol) of 4-

bromo-1-butyne, and 15 mL of toluene. The resulting off-white powder was collected on a Buchner funnel and dried under vacuum for 24 h (1.48 g, 80.2%). Heteronuclear multiple bond coherence (gHMBC) was applied to determine C-H connectivities and confirm peak assignments. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ = 10.45 (s, 1H), 7.75 (s, 1H), 7.45 (d, J = 1.6 Hz, 2H), 7.42 (s, 1H), 7.34 (d, J = 1.6 Hz, 2H), 7.33 (d, J = 1.6 Hz, 1H), 5.57 (s, 2H), 4.51 (t, J = 6.3 Hz, 2H), 2.84 (dt, J = 6.3 Hz, J = 2.6 Hz, 2H), 2.08 (t, J = 2.6 Hz, 1H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ = 137.1 (NCN), 132.8, 129.5, 129.4, 128.9, 122.8, 121.6, 78.69, 72.90, 53.35, 48.42, 20.79. ESI-MS (positive ion, calculated for M = $C_{14}H_{15}BrN_2$). 327.0070 m/z $[M+Cl]^+$, 291.0484 m/z $[M+H]^+$, 211.1238 m/z $[M+Br]^+$.

Synthesis of compound 15

1-*tert*-Butyl-3-(1-butyne)imidazolium bromide **15** was prepared by a similar synthetic procedure as **13** but with a slightly modified work-up. The synthesis was performed using 1.11 mL (8.05 mmol) of 1-(tert-butyl)-imidazole, 1.13 mL (12.1 mmol) of 4-bromo-1-butyne, and 10 mL of toluene. After the reaction was complete, the mixture was allowed to cool to room temperature and 20 mL of diethyl ether was added directly to the reaction vessel. The mixture was stirred for 10 min and the supernatant was decanted. The off-white residue was resuspended in 10 mL of fresh diethyl ether, stirred for 10 min and the supernatant was decanted (this was repeated 2 more times). After the final decanting, the solid was suspended in 2 mL of diethyl ether, transferred directly to a vial. The remaining solvent was removed in vacuo and the resulting off-white, very hygroscopic solid was dried under vacuum for 24 h before transferring to an Ar filled glovebox for storage (0.453 g, 21.9%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ = 10.47 (s, 1H), 7.75 (s, 1H), 7.55 (s, 1H), 4.62 (t, J = 6.3 Hz, 2H), 2.88 (dt, J = 6.3 Hz, J = 2.2 Hz, 2H), 2.07 (t, J = 2.2 Hz, 1H), 1.68 (s, 9H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ = 135.7 (NCN), 123.0, 119.2, 79.07, 72.38, 60.38, 48.04, 29.99, 20.77.

Synthesis of compound 16

1-Mesityl-3-(1-butyne)imidazol-2-ylidene silver(I) bromide **16** was prepared by deprotonation and direct metalation with silver(I) oxide. Inside an Ar filled glovebox, 303.0 mg (0.949 mmol) of compound **13** was added to a vial and dissolved in 4 mL of chloroform. 151.1 mg (0.652 mmol) of silver(I) oxide was added directly as a solid to this stirring solution. The reaction vessel was wrapped in aluminum foil and allowed to stir for 3 d at room temperature. The reaction mixture was passed through a pad of celite atop a filter paper fitted glass pipette to remove any insoluble gray particulates. The amber colored filtrate was

reduced in vacuo to 1 mL whereupon an excess of diethyl ether (~5 mL) was added to precipitate an off-white solid. The supernatant was decanted, the solid was resuspended in 2 mL of diethyl ether, and the supernatant was decanted again (this was repeated 2 more times with 2 x 2mL of diethyl ether). The solid was dried completely under vacuum to give an off-white solid (264.5 mg, 65.4%). Heteronuclear multiple bond coherence (gHMBC) was applied to determine C-H connectivities and confirm peak assignments. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ = 7.36 s, 1H), 6.94 (s, 2H), 6.92 (s, 1H), 4.38 (t, *J* = 6.3 Hz, 2H), 2.76 (dt, *J* = 6.3 Hz, *J* = 1.7 Hz, 2H), 2.33 (s, 3H), 2.04 (t, *J* = 1.7 Hz, 1H), 1.94 (s, 6H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ = 182.9 ($C_{carbene}$), 139.5, 135.4, 134.7, 129.4, 122.5, 121.4, 79.72, 72.17, 50.16, 21.87, 21.06, 17.63.

Synthesis of compound 17

1-Mesityl-3-(1-butyne)imidazol-2-ylidene gold(I) halide **17** was prepared by a transmetalation reaction from compound **16**. The following steps were performed inside an Ar filled glovebox and taking care to minimize light exposure. 152.3 mg (0.357 mmol) of compound **16** was dissolved in 4 mL of chloroform. To this stirring mixture, 126.3 mg (0.429 mmol) of chlorodimethylsulfide gold(I) was added directly as a solid over the course of 2 min whereupon a white solid immediately precipitated. The reaction vessel was capped and left to stir in the dark at room temperature for 1 h. The cloudy grayish purple reaction mixture was passed through a celite pad atop a filter paper fitted glass pipette in an attempt to remove the colloidal gold. The filtrate was reduced to 1 mL under vacuum and an excess of diethyl ether was added to precipitate a bright white solid. The supernatant was decanted and the solid was resuspended in diethyl ether (3 mL). This was repeated two more times (2 x 3mL diethyl ether). An accurate yield could not be obtained due to intractable colloidal gold. Heteronuclear multiple bond coherence (gHMBC) was applied to determine C-H connectivities and confirm peak assignments. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ = 7.31 (d, *J* = 1.39 Hz, 1H), 6.94 (s, 2H), 6.86 (d, *J* = 1.39 Hz, 1H), 4.41 (t, *J* = 6.35 Hz, 2H), 2.84 (dt, *J* = 6.35 Hz, *J* = 2.58 Hz, 2H), 2.31 (s, 3H), 2.07 (t, *J* = 2.58 Hz, 1H), 2.00 (s, 6H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ = 172.0 ($C_{carbene}$), 139.7, 134.7, 134.7, 129.4, 121.8, 121.3, 79.52, 72.02, 49.68, 21.40, 21.08, 17.73.

Synthesis of 19

Inside an Ar filled glovebox, solid ligand **18** (260 mg, 0.6 mmol, 1 equiv.) and Ag_2O (97.32 mg, 0.4 mmol, 0.7 equiv.) were dissolved in dichloromethane (10 mL) and stirred for

2 days to provide the NHC–Ag complex **19** in situ. To the reaction mixture was added $(CH_3)_2SAuCl$ (176.73 mg, 0.6 mmol, 1 equiv.) and stirred for 1 h. Then the reaction mixture was filtered through Celite®. The filtrate was collected and reduced under vacuum to 1 ml. Diethyl ether was added to precipitate a pale yellow powder. The solid was collected by filtration and dried under vacuum for 2 h to provide the NHC–Au complex **19** (112.5 mg, Yield =45%). ^{1}H NMR (300 MHz, CDCl3): δ =8.18 (s, 2 H, *H*Ar), 7.29-7.53 (m, 6 H, *H*Ar), 5.84 (s, 2 H, N-C*H*$_2$), 4.13 (s, 3 H, N-C*H*$_3$) ppm. ^{13}C NMR ($CDCl_3$, 500 MHz): δ = 179.6 (N*C*N), 161.9 (*C*OO), 141.5 (*C*-Ar), 136.1 (*C*-F), 134.4 (*C*-F), 134.1 (*C*-Ar), 132.7 (*C*-F), 132.7 (*C*-Ar), 131.5(C-Ar), 127.2 (C-Ar), 125.1 (*C*-Ar), 125.0 (*C*-Ar), 119.9 (*C*-Ar), 116.6 (*C*-Ar), 111.7 (*C*-Ar), 52.3 (N-*C*-Ar), 35.4 (N-*C*H$_3$) ppm. ^{19}F NMR ($CDCl_3$, 235 MHz): δ = –152.77 (t, $^3J_{F,F}$ = 19.1 Hz, 2 F, Ar*F*), –158.05 (t, $^3J_{F,F}$ = 23.2 Hz, 1 F, Ar*F*),–162.55 (dd, $^3J_{F,F}$ = 19.1, 23.2 Hz, 2 F, Ar*F*) ppm. Anal. Calcd for $C_{22}H_{13}AuClF_5N_2O_2$ (664.03 g mol^{-1}): C: 39.75%; H: 1.97%; N: 4.21%, Found: C: 39.89%; H: 2.10% N: 4.42%.

Synthesis of aptamer-drug conjugate 1-(methyl)-3-(4-*N*-sgc8c-aptamer-carbamoylbenzyl)benzo[*d*]imidazolium gold(I) chloride 21

In a 2 ml synthesis tube, complex **19** (6mg, 9μmol) was dissolved in 1 ml THF, and triethylamine (1mg, 9μmol) was added drop wise. After 10 min of shaking, 1ml aqueous solution of aptamer **20** (0.1μmol) was added to the tube, and the mixture was shaken for 24 h. Then the reaction mixture was separated and purified by HPLC and gel electrophoresis.

Synthesis of 1-(anthracen-9-ylmethyl)-1*H*-benzo[*d*]imidazole (24)

The mixture of benzimidazole **22** (354 mg, 3 mmol), tetrabutylammonium bromide (TBAB) (96mg, 0.3 mmol), THF (10 mL) and 50% aqueous K2CO3 (10 mL) was stirred. 9-(chloromethyl)anthracene **23** (678mg, 3 mmol) was added in portions. Then, the mixture was heated to 70 °C for 40 h. After heating the mixture was cooled to room temperature and extracted by dichloromethane (DCM). The organic layer was dried with $NaSO_4$. The solvent was removed in vacuo to give compound **24** as a yellow solid. (700mg, Yield= 76%). ^{1}H NMR (300 MHz, CDCl3): δ =8.63 (s, 1 H, N-C*H*-N), 8.10 (s, 4 H, *H*Ar), 7.82-7.37 (m, 9 H, *H*Ar), 6.21 (s, 2 H, N-C*H*$_2$). ^{13}C NMR ($CDCl_3$, 500 MHz): δ = 143.93 (N*C*N), 142.14 (*C*-Ar), 134.18 (*C*-Ar), 131.40 (*C*-Ar), 129.69 (*C*-Ar), 129.48 (*C*-Ar), 127.52 (*C*-Ar), 125.38 (*C*-Ar), 122.92(C-Ar), 122.41 (C-Ar), 120.50 (*C*-Ar), 109.49(*C*-Ar), 41.34 (N-*C*H$_2$) ppm.

Synthesis of 26

To a solution of **24** (500mg, 1.6 mmol) in dry THF (10 mL) was added dropwise a solution of **25** (610mg, 1.6 mmol) in THF (10 mL). After complete addition, the mixture was refluxed for 48 h during which time a yellow precipitate formed. The solvent was decanted from the precipitate and the solid was washed with THF (3x10 mL) and then dried in vacuo to give a yellow solid **26** (504 mg, 51% yield). ^{1}H NMR (300 MHz, CDCl3): δ =12.03 (s, 1 H, N-C*H*-N), 8.57-8.64 (m, 3 H, *H*Ar), 8.15 (m, 4 H, *H*Ar), 7.67-7.41 (m, 9 H, *H*Ar), 7.13 (m, 1 H, *H*Ar), 6.89 (s, 2H, CH_2- anthracene), 6.07 (s, 2H, CH_2-Ar) ppm. ^{19}F NMR ($CDCl_3$, 300 MHz): δ = –152.38 (t, $^{3}J_{F,F}$ = 19.1 Hz, 2 F, Ar*F*), –157.53 (t, $^{3}J_{F,F}$ = 23.2 Hz, 1 F, Ar*F*),–162.09 (dd, $^{3}J_{F,F}$ = 19.1, 23.2 Hz, 2 F, Ar*F*) ppm.

DART-MS (positive ion, calculated for M = $C_{36}H_{22}BrF_5N_2O_2$). 609.1612 m/z [M + H]+.

Synthesis of 1-(anthracen-9-ylmethyl)-3-(4-N-sgc8c-aptamer-carbamoylbenzyl) benzo[d]imidazolium gold(I) chloride 27

Inside an Ar filled glovebox, solid ligand **26** (100 mg, 0.16 mmol, 1 equiv.) and Ag_2O (26 mg, 0.11 mmol, 0.7 equiv.) were dissolved in DCM (5 mL) and stirred for 2 days to provide the NHC–Ag complex in situ. To the reaction mixture was added $(CH_3)_2SAuCl$ (50 mg, 0.16 mmol, 1 equiv.) and stirred for 1 h. Then, the reaction mixture was filtered through Celite®. The filtrate was collected and reduced under vacuum to 0.5 ml. Diethyl ether was added to precipitate a pale yellow powder. The solid was collected by filtration and dried under vacuum for 2 h to provide the NHC–Au complex **27** (98 mg, Yield =71%). ^{1}H NMR (300 MHz, CDCl3): δ = 8.63-8.53 (m, 3 H, *H*Ar), 8.13 (m, 4 H, *H*Ar), 7.63-7.49 (m, 6 H, *H*Ar), 7.15 (m, 2 H, *H*Ar), 6.84 (s, 2H, N-CH_2- anthracene), 5.92 (s, 2H, N-CH_2-Ar) ppm. ^{13}C NMR ($CDCl_3$, 500 MHz): δ = 180.55 (N*C*N), 161.9 (*C*OO), 141.5 (*C*-Ar), 136.1 (*C*-F), 134.4 (*C*-F), 134.1 (*C*-Ar), 132.7 (*C*-F), 132.7 (*C*-Au), 161.87(*C*OO), 141.54 (C-Ar), 133.44 (*C*-Ar), 131.42 (*C*-Ar), 129.87 (*C*-Ar), 127.64 (*C*-Ar), 127.37 (*C*-Ar), 125.35 (*C*-Ar), 124.64 (*C*-Ar), 123.20 (*C*-Ar), 122.03 (*C*-Ar), 112.98 (*C*-Ar), 111.46 (*C*-Ar), 52.36 (N-*C*-Antracene), 47.90 (N-*C*H_2-Ar) ppm. ^{19}F NMR ($CDCl_3$, 235 MHz): δ = –152.32 (t, $^{3}J_{F,F}$ = 19.1 Hz, 2 F, Ar*F*), –157.66 (t, $^{3}J_{F,F}$ = 23.2 Hz, 1 F, Ar*F*),–162.15 (dd, $^{3}J_{F,F}$ = 19.1, 23.2 Hz, 2 F, Ar*F*) ppm. DART-MS (positive ion, calculated for M = $C_{36}H_{22}AuClF_5N_2O_2$). 858.1236 m/z [M + NH_4]+.

Synthesis and biology assay of Aptamer-drug Conjugates

A NH_2-modified sgc8c aptamer was synthesized and purified based on that reported in Shangguan *et* al. *Clin. Chem.* **2007**, *53*(6), 1153–5. As shown in Fig. 12, aptamer **20** was treated with 100 equivalent of the NHC-Au complex **19** and 100 equivalent trimethylamine in THF/Water (1/1) at room temperature. After two days, the reaction mixture was purified by gel electrophoresis and HPLC. The conjugation reaction between the ligand **18** and sgc8c aptamer **20** was conducted under the same conditions to give conjugate **28**.

Synthesis of an anthracene dye-tagged NHC-Au complexes

As a tool for monitoring the uptake of the metal-aptamer conjugate and for confirming successful conjugation to the aptamer, a dye was tagged to the Au complex, as indicated in Fig. 14

As shown in Fig. 14, 9-(chloromethyl)anthracene **23** and benzimidazole **22** were stirred in THF and aqueous K_2CO_3 solution (1:1) for 2 days, forming the yellow-brown solid **24**. Then compound **25** was added to produce ligand precursor **26** as a yellow solid. The metallation reaction gave compound **27** as a yellow powder. Compound **27** has a blue fluorescence under 365 nm UV light. The luminescent spectrum of the NHC-Gold compound **27** was measured by dissolving 1 mg of **27** in 1 ml acetonitrile, as shown in Fig. 15.

Synthesis of a fluorescein dye-tagged aptamer

The coupling of fluorescein isothiocyanate (FITC) to NH_2-modified sgc8c aptamer was carried out. The FITC-sgc8c aptamer exhibits yellow-green fluorescence under 365 nm UV light, as does FITC shown in Fig. 16.

Synthesis of aptamer-dye-drug NHC-Au conjugate

FITC-sgc8c aptamer on the solid support bead were treated with 100 equivalent of triethylamine and 100 equivalent of compound **27** in THF as indicated in Fig. 17. The reaction was carried out for 18 h at room temperature, after which excess **27** and triethylamine were washed from the solid support beads. The beads, which have the aptamer and reaction products attached thereto, were incubated with 2 ml ammonium hydroxide and methylamine solution (1:1) at 65 °C for 20 min to release the conjugate. The crude product was purified by high performance liquid chromatography.

Pure FITC-sgc8c aptamer, has a retention time by HPLC of 6 min. After reaction, a concentrated component peak for the aptamer-dye-drug NHC-Au conjugate has a retention time of 24 min. This component was collected and dried. In the spectrum, shown in Fig. 18, emission peaks for FITC and the emission peak of **27** are observed.

Cell Viability Studies

Cell culture: Cell lines CEM (T cell leukiemia) and Ramos (Burkitt's Lymphoma) were cultured according to ATCC specifications in RPMI-1640 medium. The medium was supplemented with 10% fetal bovine serum (Invitrogen) and the cells were incubated at 37 °C in 5% CO_2.

Cytotoxicity studies using MTS assay: CEM and Ramos cells were treated at the following concentrations of compound **9** that were prepared by dilution of an aqueous solution of 20 mM **9**: 0.5 μM, 1.0 μ, 3.5 μ, 5.0 μ, 7.5 μ, 10 μ, 15 μ, and 25 μM, respectively, for 48 h at 37 °C. In addition to treatments, two negative controls, cells in media and water (0.01%) treated cells, were carried out. Approximately 30 μL of 10,000 freshly collected CEM cells were added to each well of a 96-well plate. Similarly, Ramos cells were added to a separate 96-well plate. Both plates were incubated at 37 °C in 5% CO_2 for 24 h before beginning experiments. After 24 h incubation, 30 μL of each concentration of **9** was added to eight wells of each of the 96-well plates and to a third plate with wells containing no cells (30 μL of media for background measurements). Each of the three plates was subjected to treatment and incubated for 48 h at 37 °C in 5% CO_2. After 48 h, 30 μL of MTS dye, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), were added to each well. The assay was allotted 4 h at 37 °C for development. After incubation with the MTS dye, the 490 nm absorbance was read on a Tecan plate 110 reader for each well. Each cell measurement had the treatment background subtracted before analysis. Quantitative and statistical analyses were done using the Origin 8.5 software. The same procedures were used to study compound **7** to assess whether the proligand displayed any cytotoxicity.

A cell viability assay to measure the cytotoxicity of the aptamer-drug conjugate **21** and aptamer-ligand conjugate **28** were performed, as indicated in Fig. 13. The aptamer-drug conjugate **21** efficiently killed 50% CCRF-CEM cells in 24 h at a concentration of 600 nM, which is a much higher efficiency than complex **19**. The IC_{50} for the aptamer-ligand conjugate **28** is only 1200 nM.

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

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

CLAIMS

We claim:

1. An aptamer-N-heterocyclic-carbene metal complex conjugate (aptamer-NHCM conjugate), comprising:

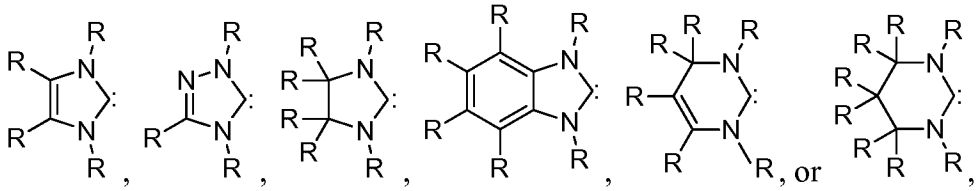
an aptamer;

at least one N-heterocyclic-carbene metal complex (NHCM) or *bis*-N-heterocyclic-carbene metal complex (*bis*-NHCM): and

a coupling unit, wherein the coupling unit comprises a hydrolytically stable.

2. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 1, wherein the N-heterocyclic-carbene metal complex or *bis*-N-heterocyclic-carbene metal complex comprises a metal selected from Au, Ag, Pt, Pd, Ru, Ni, or Cu.

3. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 1, wherein the N-heterocyclic-carbene metal complex or the *bis*-N-heterocyclic-carbene metal complex comprises at least one N-heterocyclic-carbene of the structure:

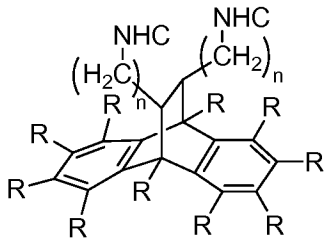


wherein R-groups are independently H, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_2-C_{18} alkynyl, C_6-C_{14} aryl, C_7-C_{18} arylalkyl, C_8-C_{18} arylalkenyl, C_8-C_{18} arylalkynyl, C_1-C_{18} alkoxy, C_6-C_{14} aryloxy, C_7-C_{18} arylalkyloxy, C_2-C_{18} alkenyloxy, C_2-C_{18} alkynyloxy, C_8-C_{18} arylalkenyloxy, C_8-C_{30} arylalkynyloxy, C_2-C_{30} alkylester, C_7-C_{15} arylester, C_8-C_{30} alkylarylester, C_3-C_{18} alkenylester, C_3-C_{18} alkynylester, C_3-C_{18} di- or poly-ether, C_3-C_{18} di- or poly-etherester, C_3-C_{18} di- or poly-ester, C_3-C_{18} di- or poly-amine, C_4-C_{18} di- or poly-ene and optionally substituted or multiply substituted with any of Cl, Br, I, F, OH, R'_2N, $R'SO_2$, R'SO, R'S, R'_3Si, R'O, NH_2, C(O)OH, N_3, C≡CH, vicinal disubstituted with C(O)OC(O), a cyclic conjugated diene, any salts derived therefrom or any condensation or addition derivative substituent therefrom, wherein R' can be C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_2-C_{18} alkynyl, C_6-C_{14} aryl, C_7-C_{18} arylalkyl, C_8-C_{18}

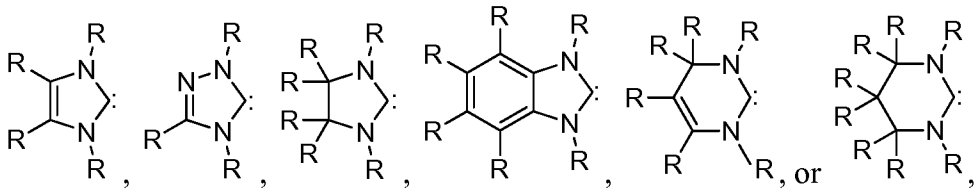
arylalkenyl, C_8-C_{18} arylalkynyl, C_1-C_{18} alkoxy, C_6-C_{14} aryloxy, C_7-C_{18} arylalkyloxy, C_2-C_{18} alkenyloxy, C_2-C_{18} alkynyloxy, C_8-C_{18} arylalkenyloxy, C_8-C_{30} arylalkynyloxy, C_2-C_{30} alkylester, C_7-C_{15} arylester, C_8-C_{30} alkylarylester, C_3-C_{18} alkenylester, C_3-C_{18} alkynylester, C_3-C_{18} di- or poly-ether, C_3-C_{18} di- or poly-etherester, C_3-C_{18} di- or poly-ester, C_3-C_{18} di- or poly-amine, C_4-C_{18} di- or poly-ene, wherein at least one of the R groups comprises the condensation or addition derivative substituent comprising the coupling unit forming the stable bond between the N-heterocyclic-carbene metal complex and the aptamer, wherein the condensation or addition derivative substituent is derived from condensation or addition of a -NH_2 and -C(O)OH, -N_3, and -C≡CH; -NH_2 and vicinal disubstituted -C(O)OC(O)-, or homocyclic or heterocyclic conjugated diene and -C≡CH or –HC=CH_2, wherein R and R' groups are linear, branched, cyclic, polycyclic or any combination thereof, and wherein the N-heterocyclic-carbene is achiral, a racemic mixture, partially resolved enantiomer, resolved enantiomer, resolved diastereomers, or a mixture of diastereomers.

4. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 1, wherein the *bis*-N-heterocyclic-carbene metal complex further comprises a framework that positionally fixes the relative proximity and orientation of the N-heterocyclic carbenes of the *bis*-N-heterocyclic-carbene metal complex.

5. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 4, wherein the framework and N-heterocyclic carbenes comprise a *trans*-9,10-dihydro-9,10-ethanoanthracene-11,12-di-oligomethylene-*N*-heterocyclic-carbene:



where NHC is an N-heterocyclic carbene, wherein the N-heterocyclic carbenes are independently selected from:



wherein n is 1 to 4, R-groups are independently H, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_2-C_{18} alkynyl, C_6-C_{14} aryl, C_7-C_{18} arylalkyl, C_8-C_{18} arylalkenyl, C_8-C_{18} arylalkynyl, C_1-C_{18} alkoxy, C_6-C_{14} aryloxy, C_7-C_{18} arylalkyloxy, C_2-C_{18} alkenyloxy, C_2-C_{18} alkynyloxy, C_8-C_{18} arylalkenyloxy, C_8-C_{30} arylalkynyloxy, C_2-C_{30} alkylester, C_7-C_{15} arylester, C_8-C_{30} alkylarylester, C_3-C_{18} alkenylester, C_3-C_{18} alkynylester, C_3-C_{18} di- or poly-ether, C_3-C_{18} di- or poly-etherester, C_3-C_{18} di- or poly-ester, C_3-C_{18} di- or poly-amine, C_4-C_{18} di- or poly-ene and optionally substituted or multiply substituted with any of Cl, Br, I, F, OH, R'$_2$N, R'SO_2, R'SO, R'S, R'$_3$Si, R'O, NH_2, C(O)OH, N_3, C≡CH, vicinal disubstituted with C(O)OC(O), a cyclic conjugated diene, any salts derived therefrom or any condensation or addition derivative substituent therefrom, wherein R' can be C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_2-C_{18} alkynyl, C_6-C_{14} aryl, C_7-C_{18} arylalkyl, C_8-C_{18} arylalkenyl, C_8-C_{18} arylalkynyl, C_1-C_{18} alkoxy, C_6-C_{14} aryloxy, C_7-C_{18} arylalkyloxy, C_2-C_{18} alkenyloxy, C_2-C_{18} alkynyloxy, C_8-C_{18} arylalkenyloxy, C_8-C_{30} arylalkynyloxy, C_2-C_{30} alkylester, C_7-C_{15} arylester, C_8-C_{30} alkylarylester, C_3-C_{18} alkenylester, C_3-C_{18} alkynylester, C_3-C_{18} di- or poly-ether, C_3-C_{18} di- or poly-etherester, C_3-C_{18} di- or poly-ester, C_3-C_{18} di- or poly-amine, C_4-C_{18} di- or poly-ene, wherein one of the R groups of the *bis*-N-heterocyclic-carbene and framework comprises the condensation or addition derivative substituent comprising the coupling unit forming the stable bond between the N-heterocyclic-carbene metal complex and the aptamer, wherein the condensation or addition derivative substituent is derived from condensation or addition of a -NH_2 and -C(O)OH, -N_3, and -C≡CH; -NH_2 and vicinal disubstituted -C(O)OC(O)-, or homocyclic or heterocyclic conjugated diene and -C≡CH or –HC=CH_2, wherein R and R' groups are linear, branched, cyclic, polycyclic or any combination thereof, and wherein the N-heterocyclic-carbene is achiral, a racemic mixture, partially resolved enantiomer, resolved enantiomer, resolved diastereomers, or a mixture of diastereomers.

6. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 1, further comprising a fluorescent or phosphorescent dye attached to the at least one N-heterocyclic-carbene metal complex (NHCM) or *bis*-N-heterocyclic-carbene metal complex (*bis*-NHCM).

7. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 6, wherein the aptamer-NHCM conjugate is 1-(anthracen-9-ylmethyl)-3-(4-*N*-sgc8c-aptamer-carbamoylbenzyl)benzo[*d*]imidazolium gold(I) chloride or 1-(methyl)-3-(4-*N*-sgc8c-aptamer-carbamoylbenzyl)benzo[*d*]imidazolium gold(I) chloride.

8. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 1, wherein the aptamer binds to a cancer cell specific receptor.

9. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 8, wherein the cancer cell specific receptor is a G-protein coupled receptor, an epidermal growth factor receptor, an epidermal growth factor tyrosine kinase receptor mutation variant III, or a protein tyrosine kinase receptor 7.

10. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 1, wherein the aptamer-N-heterocyclic-carbene metal complex further comprises at least one bond between a metal of the N-heterocyclic-carbene metal complex and an ion or ligand, wherein the ions or ligands independently comprise Cl, Br, I, OH, Cl^-, Br^-, I^-, BF_4^-, PF_6^-, SbF_6^-, $^-OSO_2CF_3$, or $^-OSO_2C_6H_5$.

11. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 1, wherein a portion of the coupling unit comprises an amide -NHC(O)-, a 1,4-substituted triazole -N_3C_2H-; an imide $[-C(O)]_2N$-, a bicycle[2.2.1]heptane -C_7H_8-, a substituted bicycle[2.2.1]heptane, a 7-oxabicyclo[2.2.1]heptane -C_6H_6O-, a substituted 7-oxabicyclo[2.2.1]heptane, a 7-azabicyclo[2.2.1]heptane -C_6H_7N-, a substituted 7-azabicyclo[2.2.1]heptane, or a succinimide thioether.

12. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 1, wherein the aptamer is capable of binding to a specific cell surface receptor present on a target cell.

13. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 12, wherein the target cell is a cancer cell and the specific cell surface receptor is present on the surface of the cancer cell.

14. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 1, wherein the NHCM is conjugated to the aptamer in a covalent manner.

15. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 14, wherein the covalent bond between the NHCM and the aptamer is acid labile.

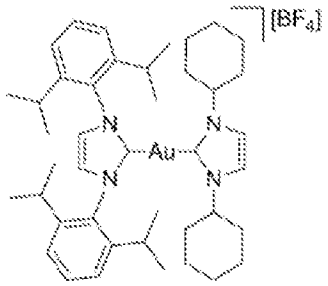
16. The aptamer-N-heterocyclic-carbene metal complex conjugate of claim 1, wherein the aptamer is conjugated to the NHCM in a non-covalent manner.

17. A method of treating a disease, comprising administering to a subject in need thereof, a pharmaceutically effective amount of the aptamer-N-heterocyclic-carbene metal complex conjugate of claim 1.

18. The method of claim 17, wherein the disease is a cancer or a microbial infection.

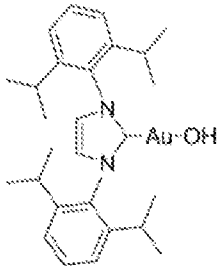
19. The method of claim 18, wherein the aptamer-N-heterocyclic-carbene metal complex conjugate binds to the disease causing cells via the specific cell surface receptor present on the disease causing cells.

20. The method of claim 17, wherein the subject is a mammal selected from the group consisting of a human, a porcine, a canine, a rodent, a feline, and a bovine.



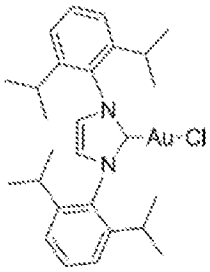
1

IC_{50}= 0.21 uM (MDA MB231)
Breast Carcinoma
IC_{50}= 0.75 uM (LNCaP)
Prostate Carcinoma



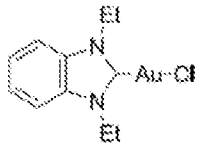
2

IC_{50}= 0.90 uM (MDA MB231)
Breast Carcinoma
IC_{50}= 1.40 uM (LNCaP)
Prostate Carcinoma



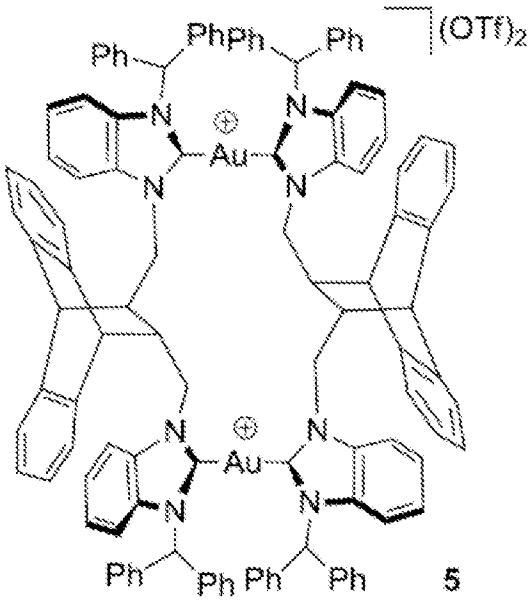
3

IC_{50}= 0.92 uM (MDA MB231)
Breast Carcinoma
IC_{50}= 1.90 uM (LNCaP)
Prostate Carcinoma



4

IC_{50}= 4.6 uM (MCF-7)
Breast Carcinoma
IC_{50}= 6.4 uM (HT-29)
Colon Carcinoma



5

IC_{50}= 8.7 uM (HeLa)
Cervical Carcinoma
IC_{50}= 4.6 uM (HEK)
Embryonic kidney

FIG. 1
(prior art)

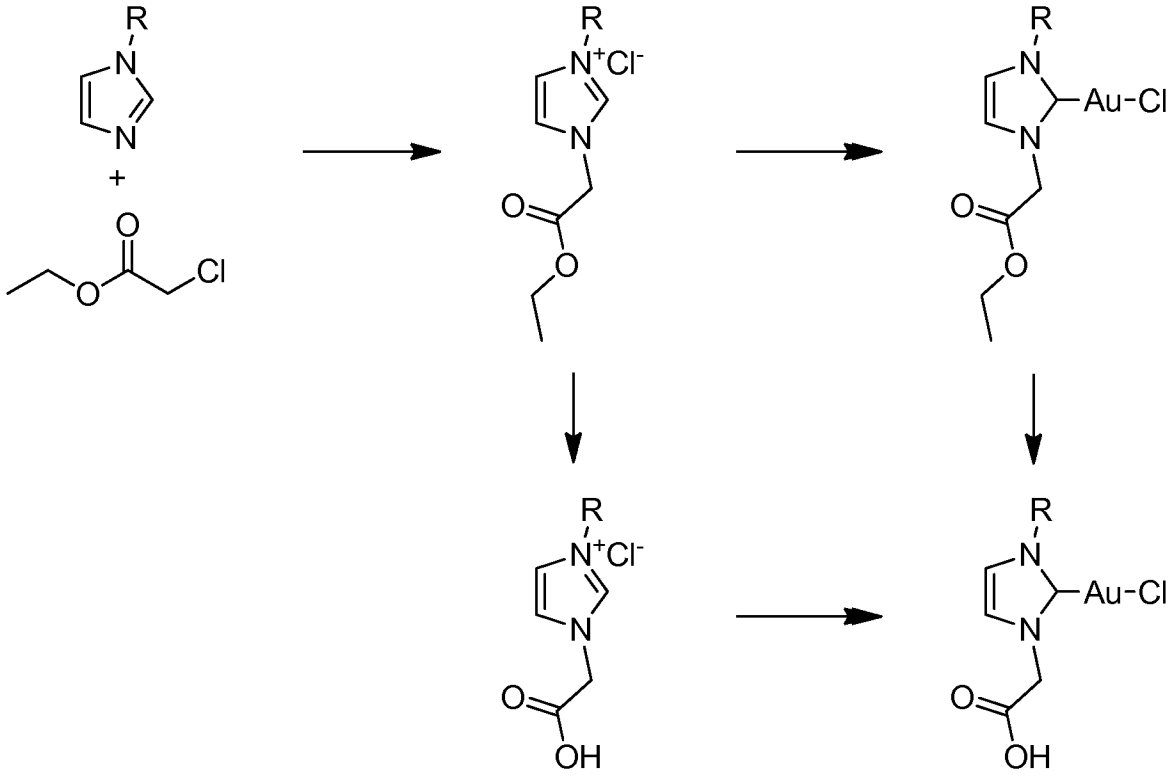

R
N
N
+
O
O
Cl
R
N+Cl-
N
O
O
R
N
Au-Cl
N
O
O
R
N+Cl-
N
O
OH
R
N
Au-Cl
N
O
OH

FIG. 2

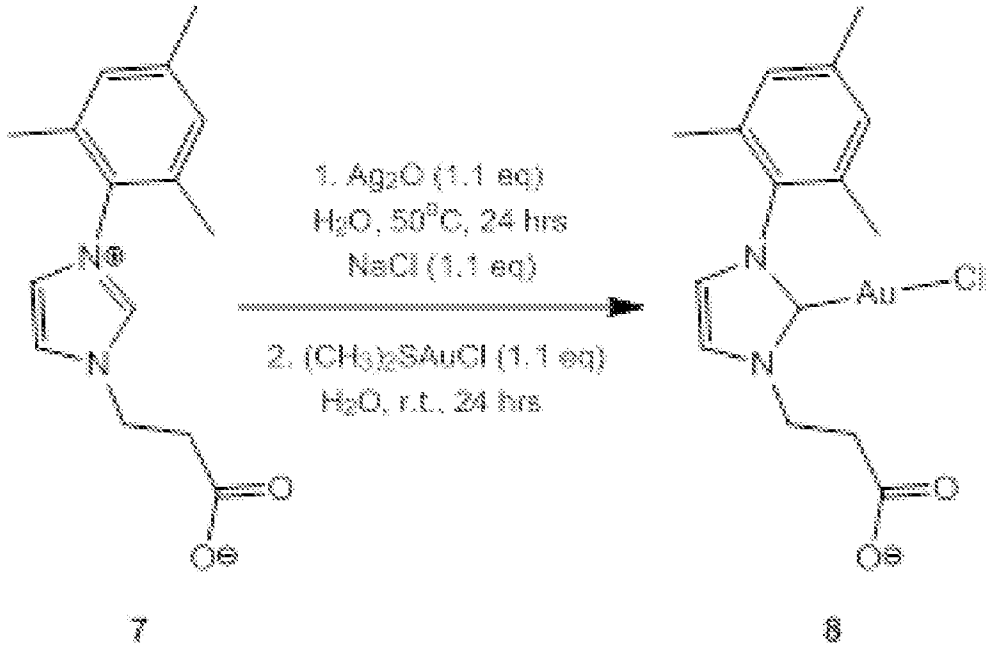

1. Ag2O (1.1 eq)
H2O, 50°C, 24 hrs
NaCl (1.1 eq)
2. (CH3)2SAuCl (1.1 eq)
H2O, r.t., 24 hrs
N
N
O
Oe
Au
Cl
7
8

FIG. 3

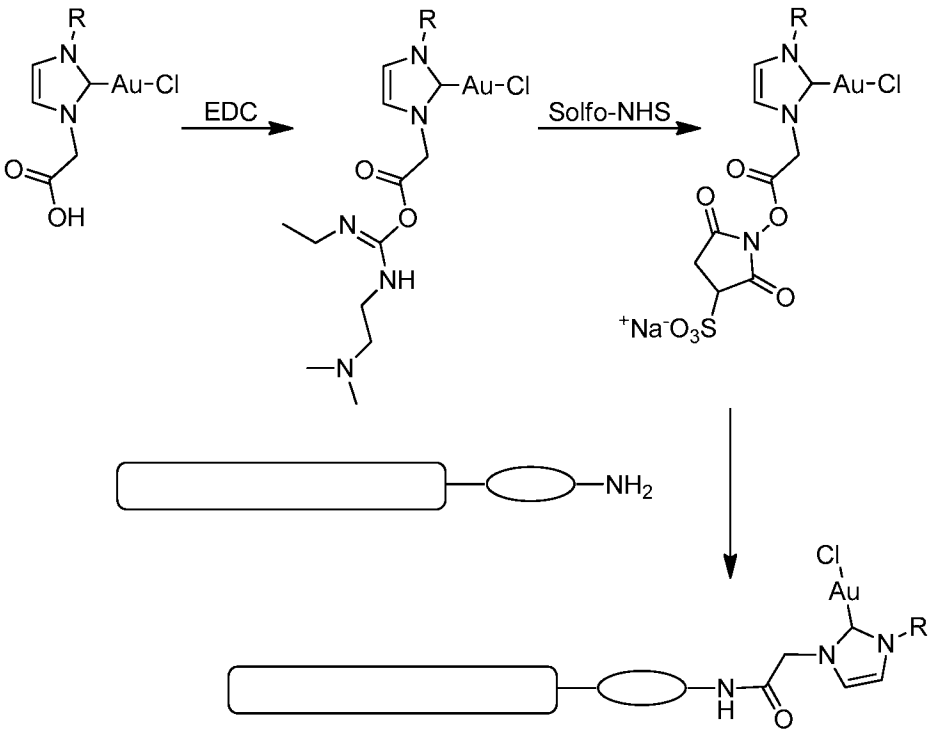

R
N
Au-Cl
N
O
OH
EDC
O
N
O
NH
N
Solfo-NHS
O
N
O
O
+Na-O3S
NH2
Cl
Au
N
R
N
N
H
O

FIG. 4

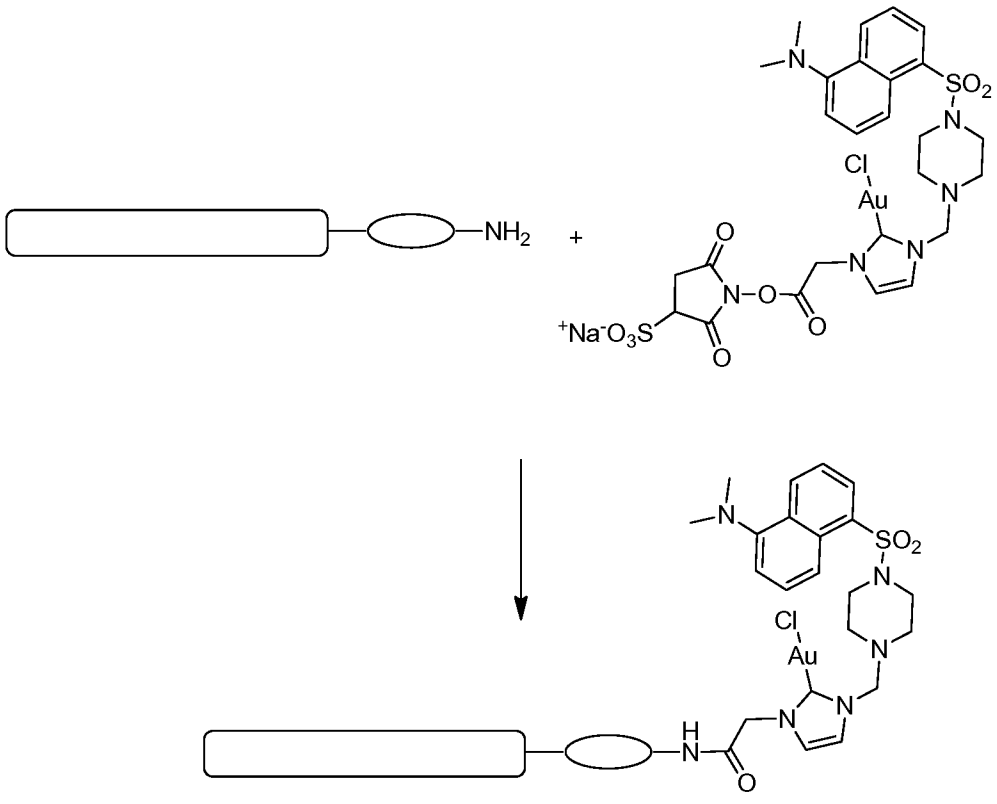

N
SO2
N
N
Cl
Au
N
N
NH2
+
O
N
O
O
+Na-O3S
O
O
N
SO2
N
N
Cl
Au
N
N
H
N
O

FIG. 5

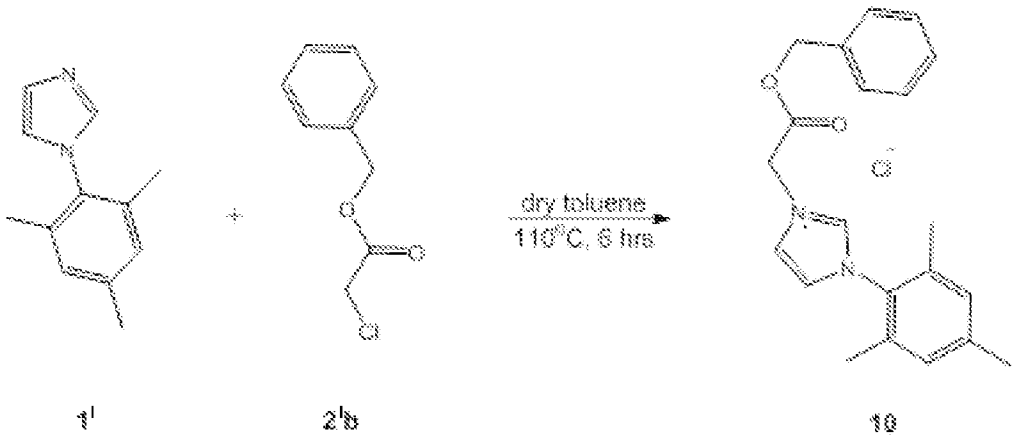

dry toluene
110°C, 6 hrs
1l
2b
10

FIG.6

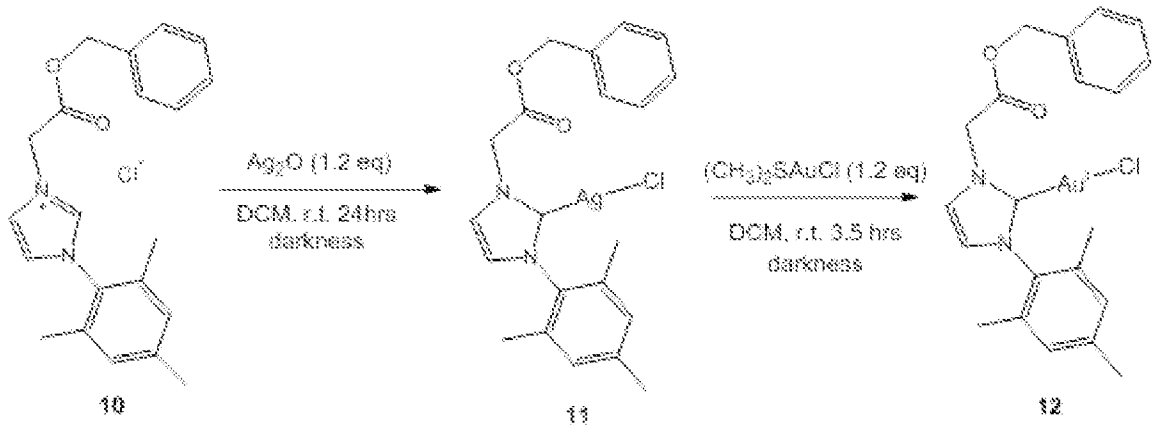

Ag2O (1.2 eq)
DCM, r.t. 24 hrs
darkness
(CH3)2SAuCl (1.2 eq)
DCM, r.t. 3.5 hrs
darkness
10
11
12

FIG. 7

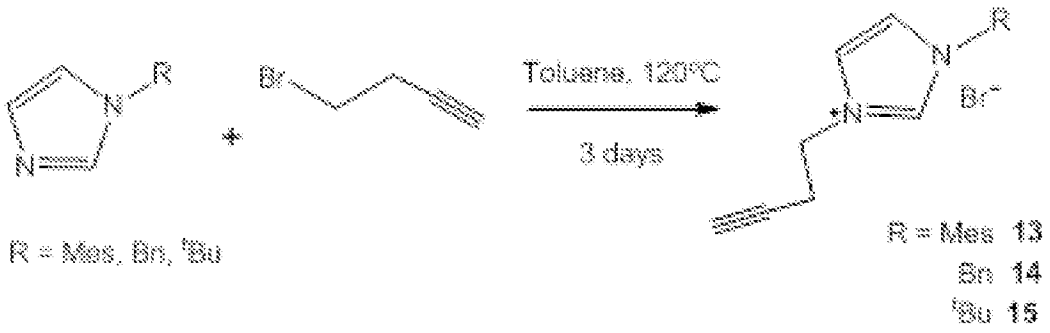

R
N
N
Br
+
Toluene, 120°C
3 days
R
N
+N
Br⁻
R = Mes, Bn, tBu
R = Mes 13
Bn 14
tBu 15

FIG. 8

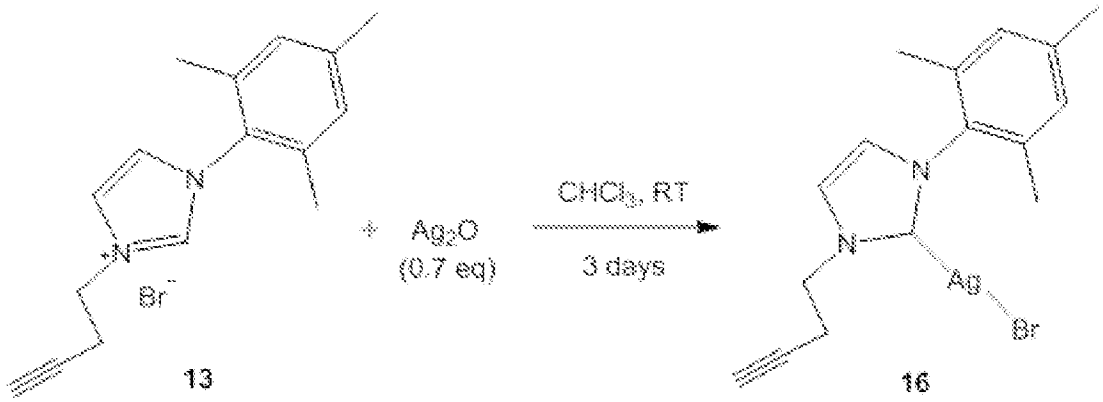

N
+N
Br⁻
13
+ Ag2O
(0.7 eq)
CHCl3, RT
3 days
N
N
Ag
Br
16

FIG. 9

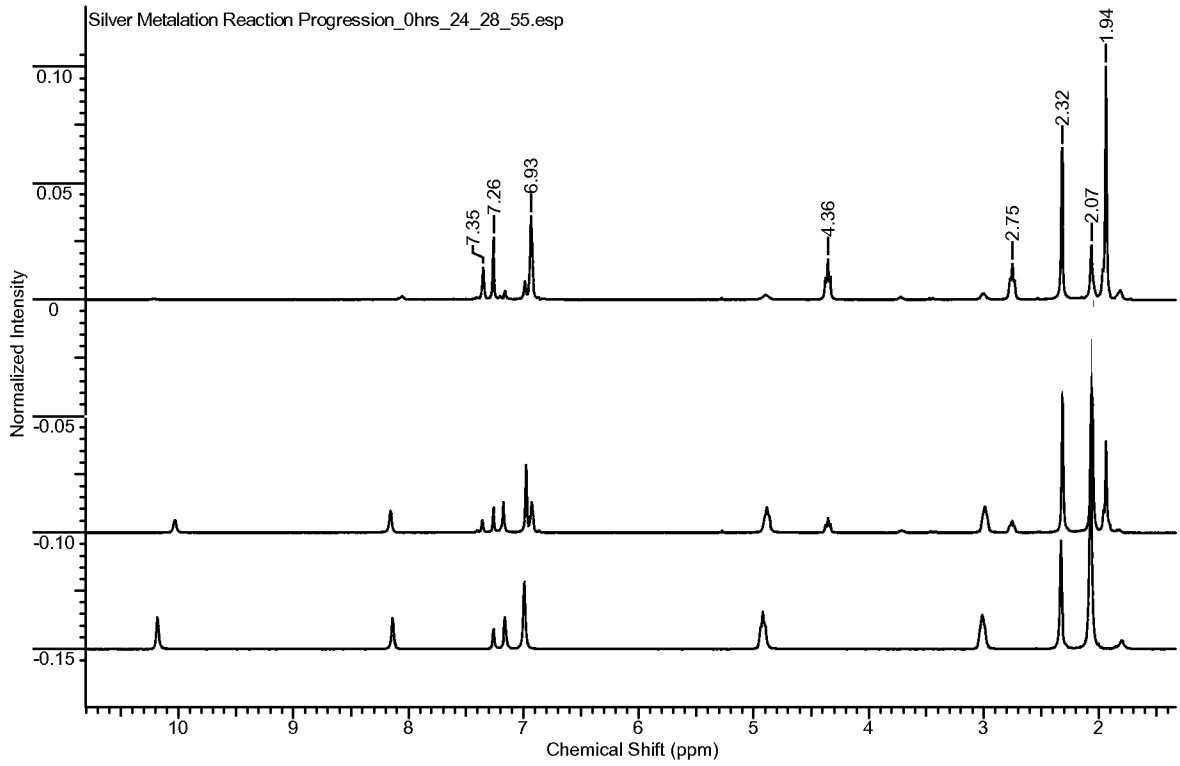

Silver Metalation Reaction Progression_0hrs_24_28_55.esp
Normalized Intensity
0.10
0.05
0
-0.05
-0.10
-0.15
7.35
7.26
6.93
4.36
2.75
2.32
2.07
1.94
10
9
8
7
6
5
4
3
2
Chemical Shift (ppm)

FIG. 10

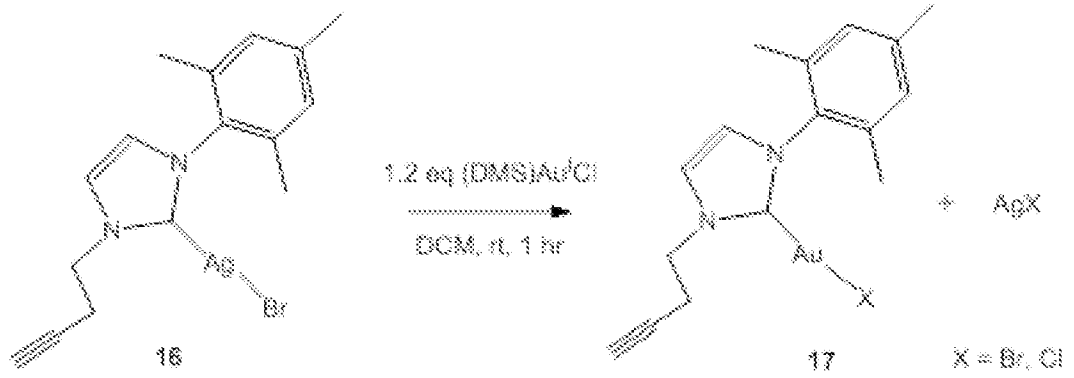

1.2 eq (DMS)AuCl
DCM, rt, 1 hr
+ AgX
16
17
X = Br, Cl

FIG. 11

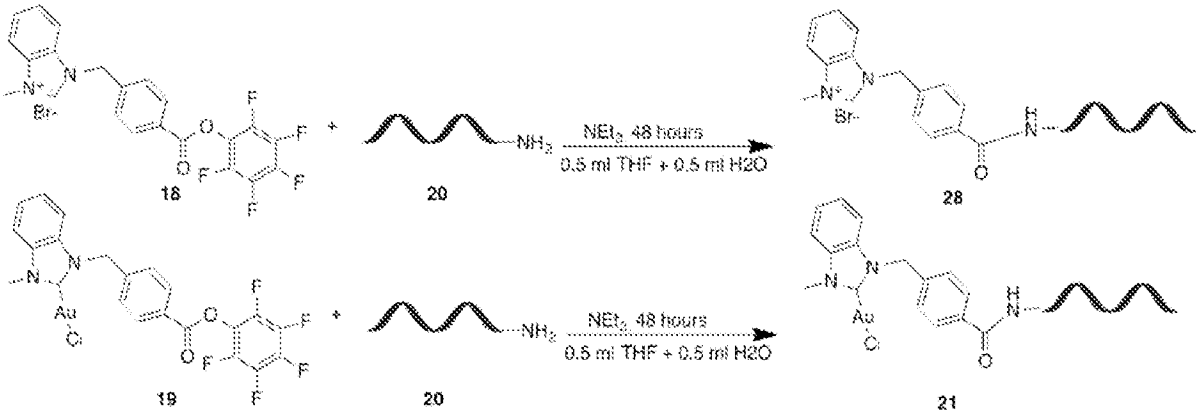

Br-
18
20
NEt3, 48 hours
0.5 ml THF + 0.5 ml H2O
28
Au
Cl
19
20
NEt3, 48 hours
0.5 ml THF + 0.5 ml H2O
21

FIG. 12

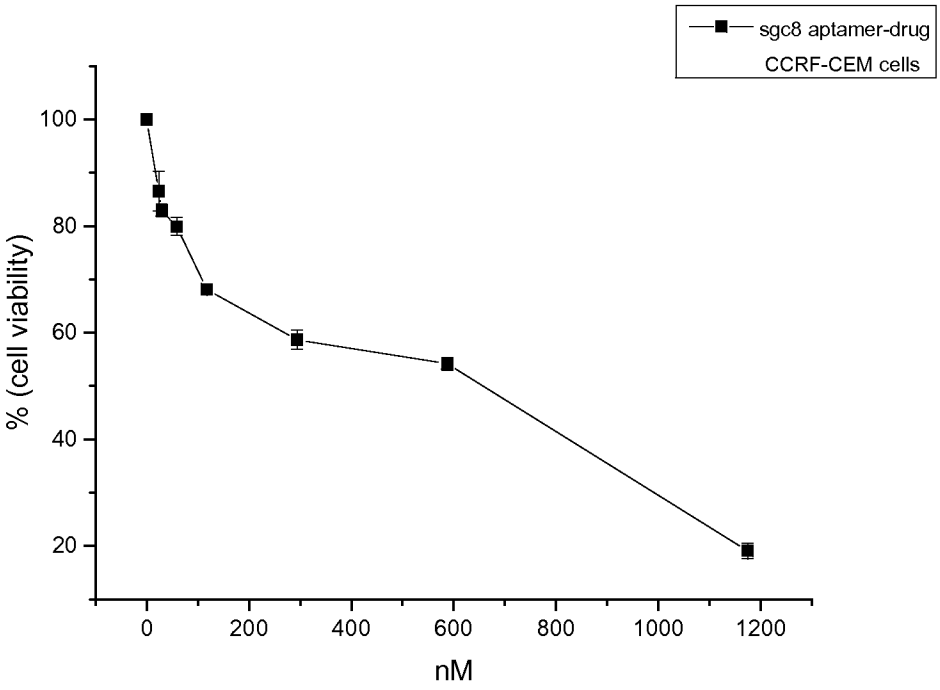


FIG. 13A

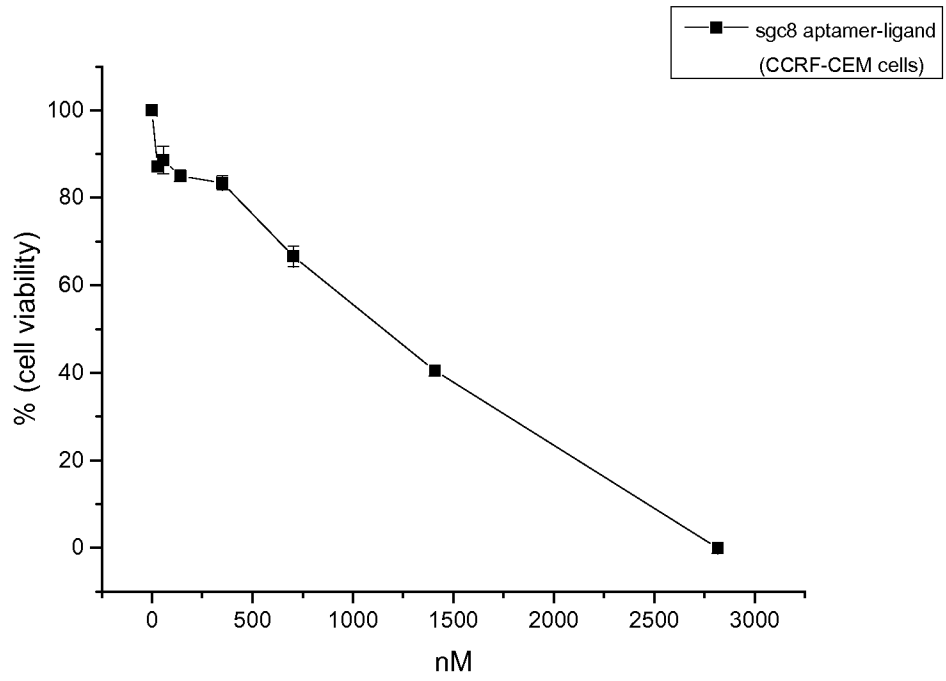


FIG. 13B

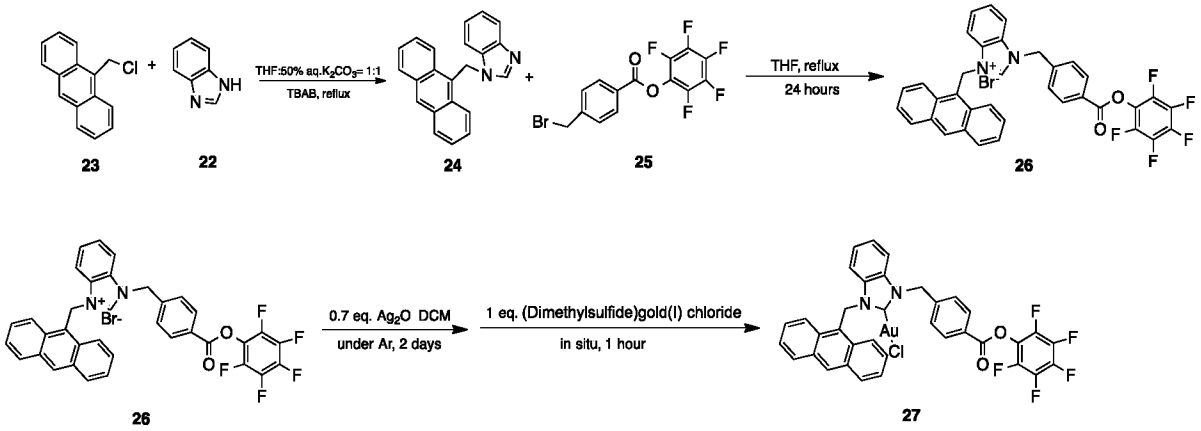

THF:50% aq.K2CO3= 1:1
TBAB, reflux
THF, reflux
24 hours
0.7 eq. Ag2O DCM
under Ar, 2 days
1 eq. (Dimethylsulfide)gold(I) chloride
in situ, 1 hour
23
22
24
25
26
26
27

FIG. 14

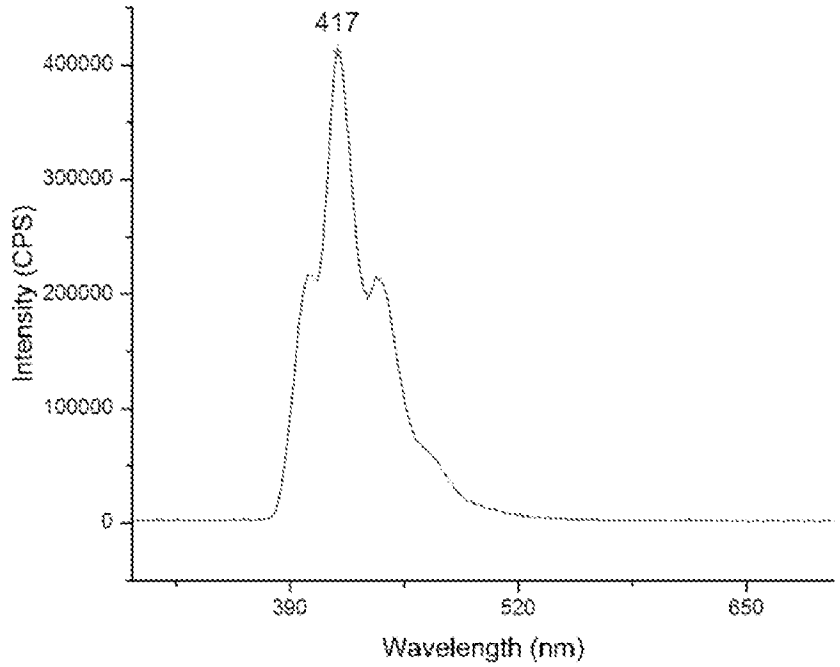

417
400000
300000
200000
100000
0
Intensity (CPS)
390
520
650
Wavelength (nm)

FIG. 15

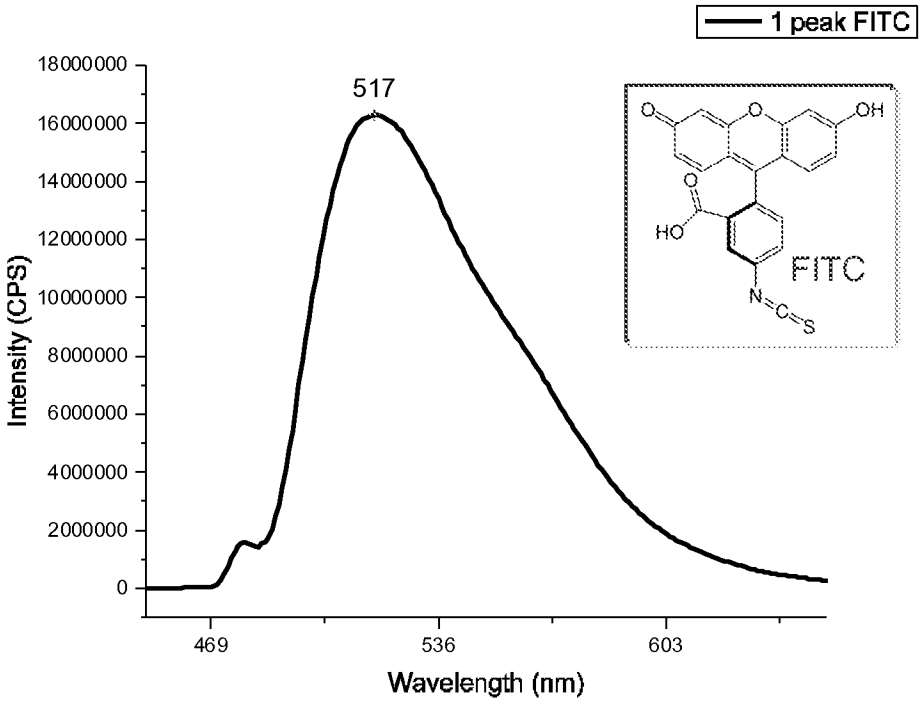

1 peak FITC
517
Intensity (CPS)
Wavelength (nm)
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
469
536
603
FITC

FIG. 16

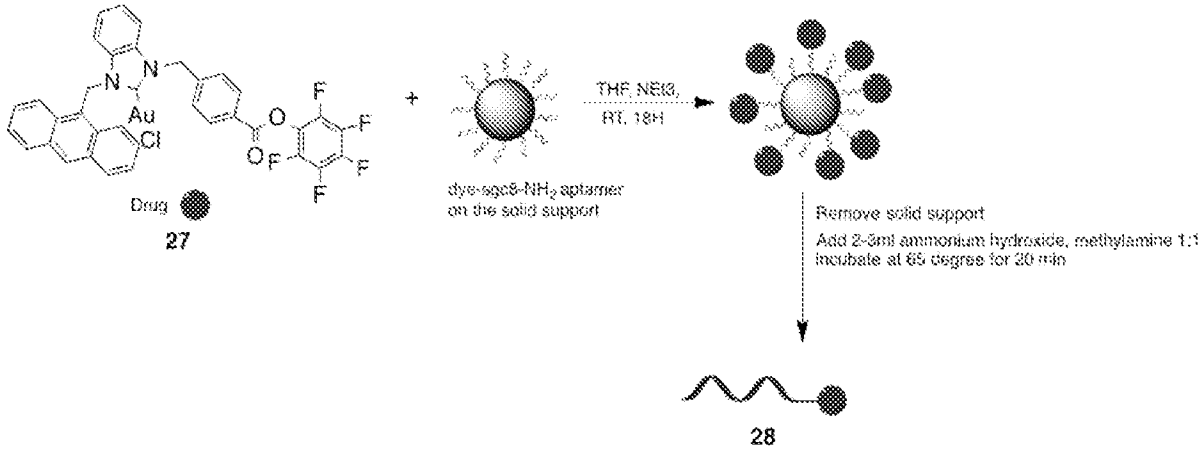

Drug
27
THF, NEt3,
RT, 18H
dye-sgc8-NH2 aptamer
on the solid support
Remove solid support
Add 2-3ml ammonium hydroxide, methylamine 1:1
incubate at 65 degree for 20 min
28

FIG. 17

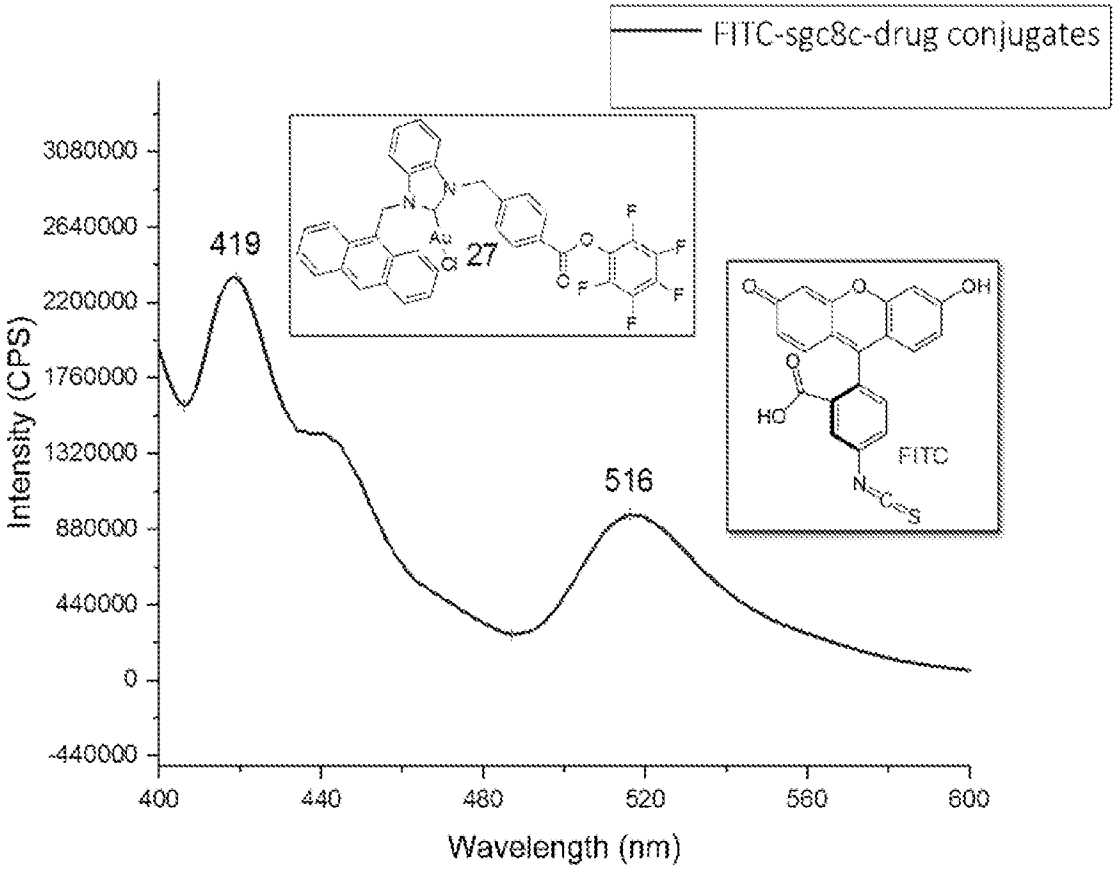

FITC-sgc8c-drug conjugates
Intensity (CPS)
3080000
2640000
2200000
1760000
1320000
880000
440000
0
-440000
400
440
480
520
560
600
Wavelength (nm)
419
516
27
FITC

FIG. 18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/039014

A. CLASSIFICATION OF SUBJECT MATTER

A61K 47/48(2006.01)i, A61K 33/24(2006.01)i, A61K 33/34(2006.01)i, A61K 33/38(2006.01)i, A61P 31/04(2006.01)i, A61P 35/00(2006.01)i

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)
A61K 47/48; A61K 31/555; C07F 15/00; C07F 1/10; A61P 13/02; C07F 1/00; G01N 33/50; A61K 33/24; A61K 33/34; A61K 33/38; A61P 31/04; A61P 35/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal), STN (Registry & Caplus) & Keywords: aptamer, N-heterocyclic, carbene, metal, cancer, microbial, infection, conjugate, complex, sgc8c aptamer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZHU, GUIZHI et al., Nucleic acid aptamers: an emerging frontier in cancer therapy. Chem. Commun., 2012, Vol. 48, pp. 10472-10480 See abstract; tables 1 and 2; figures 4 and 5; page 10477.	1-4,8-16
A		5-7
Y	US 2014-0142080 A1 (UNIVERSITY OF HONG KONG) 22 May 2014 See abstract; paragraphs [0008], [0067], [0199], [0223]; examples; claims 1 and 21-23.	1-4,8-16
Y	MCGINELY, NICOLA L. et al., DNA-based aptamer fails as a simultaneous cancer targeting agent and drug delivery vehicle for a phenanthroline-based platinum (II) complex. Journal of Inorganic Biochemistry, 2013, Vol. 128, pp. 124-130 See abstract; figures 1 and 4; section of "4. conclusions".	1-4,8-16
Y	US 2013-0324511 A1 (CHE, CHI MING et al.) 05 December 2013 See abstract; figures 1, 2; paragraphs [0047], [0048]; claims 1, 25-29.	1-4,8-16
Y	SUN, RAYMOND W.-Y. et al. Luminescent cyclometalated platinum(II) complexes containing N-heterocyclic carbene ligands with potent in vitro and in vivo anti-cancer properties accumulate in cytoplasmic structures of cancer cell. Chem. Sci., 2011, Vol. 2, pp. 728-736 See abstract; figure 1.	1-4,8-16

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 October 2015 (21.10.2015)	**21 October 2015 (21.10.2015)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
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Facsimile No. +82-42-472-7140

Authorized officer
LEE, Jeong A
Telephone No. +82-42-481-8740

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/039014

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011-0172199 A1 (MAILLIET, PATRICK et al.) 14 July 2011 See abstract; paragraphs [0044], [0046], [0105], [0112].	1-4,8-16
Y	WEAVER, JENNIFER et al., Cytotoxicity of Gold(I) N-Heterocyclic Carbene Complexes Assessed by Using Human Tumor Cell Lines. Chem. Eur. J., 2011, Vol. 17, pp. 6620-6624 See abstract; schemes 1 and 2.	1-4,8-16
A	US 2011-0306585 A1 (YOUNGS, WILEY J. et al.) 15 December 2011 See the whole document.	1-16
A	GUO, SHUAI et al., Gold and Palladium Hetero-Bis-NHC Complexes: Characterizations, Correlations, and Ligand Redistributions. Organometallics, 2013, Vol. 32, pp. 3685-3696 See the whole document.	1-16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/039014

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 17-20
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 17-20 pertain to methods for treatment of the human body by surgery or therapy, and thus relate to a subject matter which this International Searching Authority is not required to search (PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv)).

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2015/039014

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