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(C^N^{pz}^C)Au^{III} complexes of acyclic carbene ligands: synthesis and anticancer properties†

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Abstract: A series of cyclometallated gold(III) complexes supported by pyrazine-based (C^N^{pz}^C)-type pincer ligands were synthesized via two different pathways. Nucleophilic attack on the isocyanide complex [(C^N^{pz}^C)Au(C≡NC$_6$H$_3$Me$_2$-2,6)]SbF$_6$ (**2**) gave [(C^N^{pz}^C)Au(ACC)]SbF$_6$ complexes with aniline (**4·SbF$_6$**), adamantylamine (**5**), glycine ethyl ester (**6**), alanine methyl ester (**7**), valine methyl ester (**8**), phenylglycine methyl ester (**9**) and methionine methyl ester (**10**) substituents (ACC = acyclic carbene). The pathway via isocyanide insertion into gold-amide bonds was also investigated; e.g. the reaction of xylyl isocyanide with (C^N^{pz}^C)AuNHPh followed by protonation with HBF$_4$·OEt$_2$ gave the acyclic carbene complex **4·BF$_4$**. To the best of our knowledge compounds **6 - 10** represent the first examples of gold(III) acyclic carbene complexes bearing amino acid functions. The compounds provide a versatile platform for the study of anti-proliferative properties of gold(III) complexes. Tests against human adenoma-type lung cancer cells identified **5**, **6**, **7** and **10** as particularly promising and demonstrate the synthetic flexibility of acyclic carbene complexes and the potential of that class of compounds for anticancer applications. Compared to cisplatin, amino ester-containing ACC complexes showed improved selectivity for MCF-7 breast cancer cells over healthy fibroblasts.

Introduction

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Since the late 1970s *cis*-diamminedichloroplatinum(II) (cisplatin)[1] has become one of the most widely used compounds in the clinic for the treatment of various type of cancer including ovarian, testicular, bladder cancer, melanoma, non-small cell lung cancer, small cell lung cancer, lymphomas and myelomas.[2] Since the introduction of cisplatin only two other platinum-based compounds (carboplatin and oxaliplatin) have received worldwide approval as anticancer drugs.[3] However, despite their clinical success, platinum-based metallodrugs present major drawbacks such as a limited spectrum of action, acquired resistance to treatment, as well as severe side effects limiting administrable doses to patients.[4] For these reasons, alternatives are being sought, and organometallic gold(I/III) complexes have demonstrated promising anticancer properties.[5] Indeed, complexes presenting *N*-heterocyclic carbene (NHC) ligands, alkynyl ligands and cyclometallated arylpyridine ligands give rise to Au(I/III) complexes which are very stable in physiological environments. Special interest has focused on cyclometallated complexes in which the Au(III) centre is stabilized by (C^N), (C^N^N) or (C^N^C) pincer ligands.[5b,6] These complexes tolerate a broad range of ancillary ligands such as phosphines,[7,8] NHCs,[9] or N-donor ligands,[10,11] which enables optimization of the biological properties of these complexes. The cytotoxicity of some of these gold complexes has been demonstrated to be due to the inhibition of various enzymes such as thioredoxin reductase (TrxR), poly-(adenosine diphosphate (ADP)-ribose) polymerase 1 (PARP-1) and aquaporins.[12] Gold(I/III) complexes have also been shown to stabilize the G-quadruplex structure of DNA.[13] Post-metallation modifications have appeared as a very efficient and high-yielding method of introducing elaborated scaffolds into metal complexes, for example for derivatising metal gold complexes with peptides,[14] aptamers[15] or other metal complexes.[16]

The first gold(III) acyclic carbenes were reported in the 1970s by Balch *et al*. and Minghetti and co-workers.[17,18] The compounds were described to behave as oxidising agents. Further early work on gold(III) open-carbene systems by Usón and Villacampa *et al*. describes the synthesis of mononuclear, mono-carbene gold(III) complexes.[19] The authors utilised existing synthetic methodology, reacting primary amines or diamines with gold(III) isocyanide complexes to produce acyclic carbene complexes. Since then few investigations on these systems have been reported, except for a small number of papers describing cyclometallated gold(III) carbenes produced either by insertion of an isocyanide into an Au-N bond[20,21] or by nucleophilic attack of a gold-CNR complex by an amine.[22] We became interested in acyclic carbene complexes since their formation promised to present a simple

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way of functionalizing gold complexes with bio-inspired moieties bearing a amine functions such as amino acid derivatives.

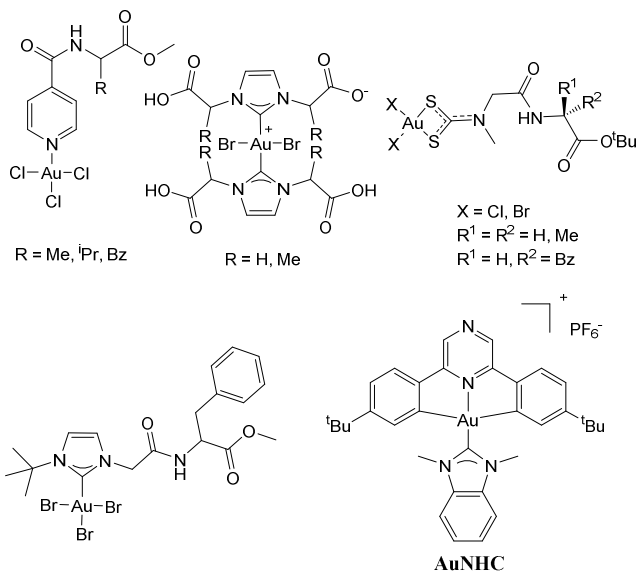

Fig. 1 Structures of previously reported Au(III) conjugates with amino acid derivatives and of the benzimidazolylidene complex **AuNHC**.

As we have recently reported, pyrazine-based pincer complexes[23] with benzimidazolylidene ligands $[(C\hat{}N^{pz}\hat{}C)Au(NHC)]^+$ (**AuNHC**, Fig. 1) possess promising anticancer properties against human leukemia, breast cancer and lung cancer cells.[24] However, although the compounds showed lower toxicity against healthy lung fibroblasts, the selectivity of the compound for cancer cells needs to be improved. Considering the high stability of the $[(C\hat{}N^{pz}\hat{}C)Au(carbene)]^+$ scaffold under physiological conditions, including their tolerance to increased concentrations of glutathione (GSH), the attachment of a potential vector via the carbene ligand seemed attractive in the search for improving target selectivity.

In recent years, coupling of organometallics to peptides has appeared as a promising way to increase their selectivity to cancer cells.[25] This concept has been applied predominantly to gold(I) complexes using mono- or polypeptide vectors.[26] Although some examples of Au(III) complexes conjugated to amino acid derivatives have been reported (Fig. 1),[26a,c,f,27] to the best of our knowledge no examples of cyclometallated Au(III) complexes bearing amino acid moieties have been synthesized so far. Within this context, we report here the synthesis and characterization of the first cyclometallated Au(III) complexes decorated

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with amino ester-based acyclic diamino carbene (ACC) ligands. The compounds were screened for their antiproliferative properties against a human lung cancer cell line (A549). The most promising ones were then tested against a broader panel of human cancer cells, including lung adenocarcinoma (A549), leukemia (HL60), colon cancer cells (HCT116), breast cancer cells (MCF-7 and MDA-MB-231), as well as healthy fibroblasts (MRC5) for comparison.

Results and Discussion

Synthesis and characterization. Following reported procedures,[17-19] we started by exploring the amine attack on cationic gold(III) isocyanide complexes, which seemed the most promising route to the expected amino-acid derivatised acyclic carbene complexes. Treatment of (C^N^{pz}^C)AuCl (**1**) with 2,6-$Me_2C_6H_3N{\equiv}C$ and $AgSbF_6$ under strictly anhydrous conditions gave the isocyanide complex **2** in good yield (Scheme 1). The synthesis of **2** was confirmed by NMR and IR spectroscopy and elemental analysis. The ^1H and ^{13}C NMR signals were assigned and confirmed with HMQC experiments. The IR spectrum showed a weak ν(C≡N) signal at 2267 cm^{-1}. This value is in good agreement with previously reported [(C^N^C)AuCNR]$^+$ complexes.[28] Complex **2** appeared to be very sensitive to hydrolysis and in the presence of moisture readily formed the formamide complex **3**, with characteristic IR bands of ν(NH) = 3296 cm^{-1} and ν(C=O) = 1643 cm^{-1}.

Complex **2** was reacted with dry, distilled aniline at room temperature to give the corresponding acyclic diamino carbene complex **4·SbF$_6$** in good yield (Scheme 1). The complex displays two NH peaks in the ^1H NMR spectrum, at δ 9.7 and 9.4 ppm. The ν(C=N) stretching frequency of **4·SbF$_6$** is found at 1586 cm^{-1}. The reaction of **2** with adamantylamine gave compound **5**, which shows an up-field shift of the characteristic H^2 peak to 8.9 ppm and the appearance of broad ^1H NMR singlets for the NH moieties at 8.3 and 6.6 ppm. The chemical shift difference is much larger than in **4·SbF$_6$**, illustrating the difference between the aryl and alkyl amine substituents of the carbene. The IR spectrum of **5** shows the characteristic $\nu_{C=N}$(carbene) band at 1585 cm^{-1}, together with two distinct ν(NH) bands of the carbene at 3312 cm^{-1} and 3210 cm^{-1}. The [(C^N^{pz}^C)Au(ACC)]$^+$ complexes are very stable even in solution, which contrasts with recent reports on (ACC)$AuCl_3$ complexes.[29]

The reaction of the amino esters Gly-OEt, Ala-OMe, Val-OMe, PhGly-OMe and Met-OMe with **2** afforded the corresponding amino ester complexes **6-10** in good yields. All

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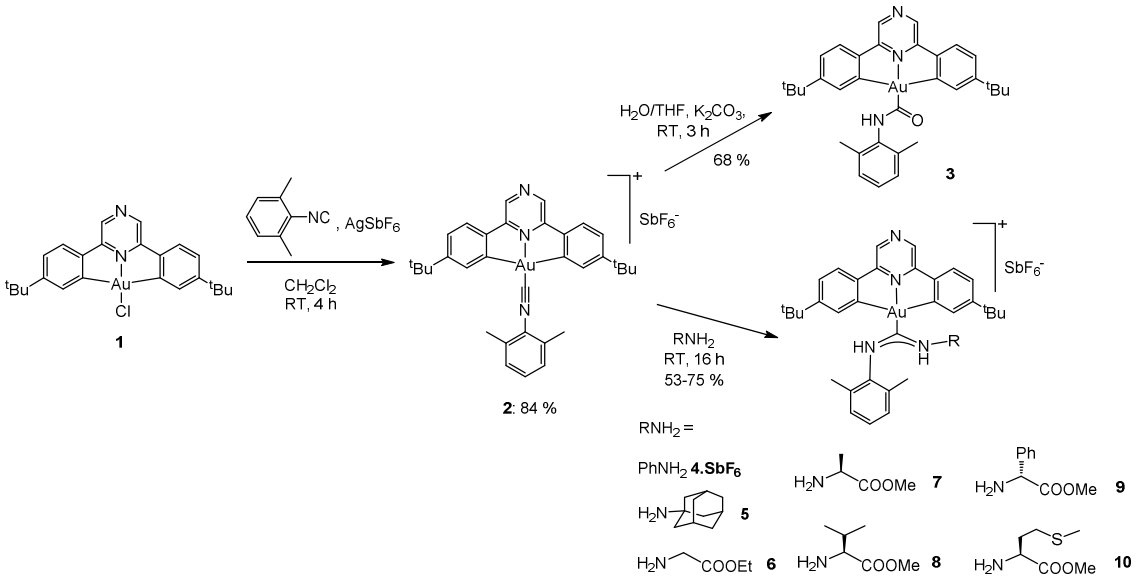
complexes feature the ν(carbene) band at 1585 cm^{-1} in their IR spectra, along with a ν(C=O) band at ~1740 cm^{-1}. While the ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of **6**, which contains a non-chiral glycine moiety, reflected the mirror symmetry of the (C^N^{pz}^C) pincer ligand, the ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of **7-10** showed the loss of lateral symmetry of the (C^N^{pz}^C) framework, indicated by a doubling of the *tert*-butyl, H^5, H^8 and CH_3(xylyl) signals. The effect is most prominent in the spectra of **8** and **9**, which contain the sterically most demanding side chains. The NMR spectra were fully assigned using HMQC and HMBC NMR experiments.



Scheme 1 Synthesis of (C^N^{pz}^C)Au complexes **2-10**.

The alternative pathway, the insertion of isocyanides into the gold-amide bond, was also briefly explored. The reaction of **1** with anilines in the presence of base afforded **11a**, **b** in high yields (Scheme 2). Both complexes **11a** and **11b** were reacted with 2,6-dimethylphenylisocyanide at room temperature overnight. **11a** gave the corresponding insertion product **12** in good yield, whereas **11b**, with the more bulky 2,6-diisopropylaniline ligand, did not react and returned the unreacted starting material. NMR analysis of compound **12** revealed the presence of a NH proton at 6.71 ppm in the ^{1}H NMR spectrum and a signal at 124.7 ppm in the $\{^{1}H\}^{13}C$ corresponding to the inserted carbon. The formation of **12** was confirmed by the IR spectrum which showed the NH vibration at 3340 cm^{-1} and the C=N stretch at 1607 cm^{-1}. Insertion of *tert*-butyl isocyanide and cyclohexyl isocyanide into **11a** was attempted in a variety of reaction conditions. *Tert*-butyl isocyanide inserted as expected

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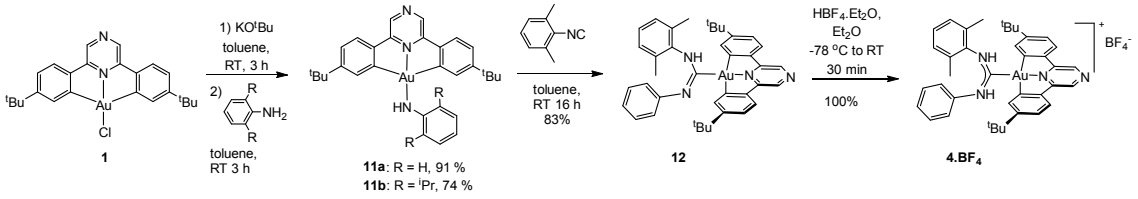
but promptly rearranged to produce the cyanide complex, as was previously noticed for pyridine-based complexes.[27] Cyclohexyl isocyanide generated a mixture of products which proved impossible to separate and purify. Protonation of **12** using $HBF_4{\cdot}OEt_2$ produced the air-stable carbene complex **4·BF$_4$**, with the same spectroscopic characteristics as its SbF_6 analogue **4·SbF$_6$**.



Scheme 2 Synthesis of acyclic carbene **4·BF$_4$** from gold(III) anilide.

Solid state structures. The crystal structures of **2**, **3**, **5**·1.5 toluene, **6**, **11a** and **12**·toluene were determined by X-ray diffraction. Selected views of the structures and distances and angles of **5**·1.5 C_7H_8 and **6** are shown in Fig. 2; views of the molecular and packing structures of all other compounds are given in the ESI. In all cases, the square-planar geometry of the Au^{III} centres is distorted due to the strain induced by the bis-cyclometallated C^N^C ligand.

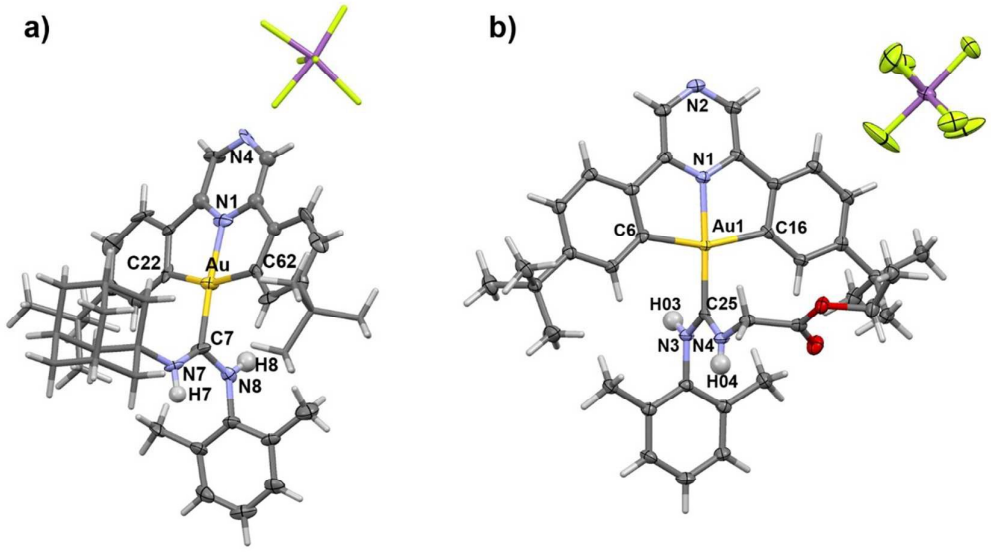


Fig. 2 (a) Crystal structure of complex **5**·1.5 C_7H_8. Ellipsoids set at 50% probability. Toluene molecules are omitted for clarity. Selected bond distances [Å] and angles [°]: Au-N1 2.01(1), Au-C7 2.03(1), Au-C22 2.10(1), Au-C62 2.09(1), N1-Au-C7 175.8(4), N1-Au-C22 80.4(4), N1-Au-C62 80.5(4), C22-Au-C7 101.6(5), C62-Au-C7 97.4(4), C22-Au-C62 160.9(4). (b)

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Structure of **6**. Ellipsoids set at 50% probability. Selected bond distances [Å] and angles [°]: Au-N1 2.012(2), Au-C25 2.010(3), Au-C6 2.098(3), Au-C16 2.102(3), N1-Au-C25 177.0(1), C6-Au-C16 160.8(1), N1-Au-C6 80.6(1), N1-Au-C16 80.3(1), C6-Au-C25 98.8(1), C16-Au-C25 100.4(4), N3-C25-N4 118.5(3).

The distances and angles in the C^N^{pz}^CAu moiety are comparable to those described previously.[23,29,30] In the neutral amidate complexes **3** and **12** the Au-C7/C25 distances of 2.0251(18) and 2.031(4) Å, respectively, are comparable to the Au-C σ-bond distances in gold alkyls, typically in the range 2.00-2.15 Å.[23,30,31]

In the cationic Au^{III}-carbene complexes the Au-C7/C25 distances (2.037(11) Å **5**, 2.010(3) Å **6**) are in the upper range of distances observed for complexes of cyclic carbenes [(C^N^C)Au(NHC)]$^+$ (1.967 to 2.017 Å),[23,32] possibly due to a combination of steric hindrance and lower donor strength of the acyclic carbenes. The carbene adopts an almost perfectly perpendicular orientation relative to the (C^N^{pz}^C)Au plane [torsion angles C22-Au-C7-N8 90.7(8) in **5**, and C6-Au-C25-N3 90.9(3) in **6**], in contrast with the value of 115-120º found in [(C^N^{pz}^N)Au(1,3-dimethylbenzimidazol-2-ylidene)]PF_6.[24]

This conformation of the carbene allows the formation of supramolecular interactions. As we showed recently, pyrazine-based pincer complexes have a tendency to aggregate through the formation of π···π interactions that involve the pyrazine rings.[33] In the present complexes, however, the presence of N-H protons in the ancillary ligand and the formation of intermolecular H···N^{pz} interactions are the main driving force for aggregation. Fig. 3 shows illustrative examples (see ESI for further details of intermolecular interactions of **2**, **3**, **5**, **11a** and **12** in the solid state).

As can be seen in Figs. 3a and 3b for complex **6** and Fig. S3 for **5**·1.5 C_7H_8, the carbene complexes crystallize as dimers held together by xylylNH···N^{pz} interactions (2.187 Å in **5**·1.5 C_7H_8; 2.443 Å in **6**). There are also C(aryl)H···F interactions with the SbF_6 anions. Rather than forming a π-stack of the arene rings, the two planar neighbouring (C^N^C)Au moieties show a head-to-head alignment of the pyrazine ring of one molecule with the Au-N bond of another, with an offset of about 1.15 Å. The formimidate complex **12**·C_7H_8 also forms dimers through xylylNH···N^{pz} interaction (2.407 Å). Here, too, the pyrazine ring of

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one molecule is aligned with the Au-N bond of the second, although in this case the off-set is minimal (Fig. 3c).

The neutral complexes **3** and **11a** form polymeric zig-zag chains. In the case of complex **3** (Fig. 3d) the intermolecular interactions involve again NH···N^{pz} contacts (2.280 Å), as well as additional O···H-C interactions between the oxygen atoms of the formamido ligand and protons of the pyrazine ring of a neighbouring molecule. In the case of **11a**, additional interactions contributing to chain formation involve the *ortho* protons of the phenyl ring of the aniline (see ESI, Fig. S4b).

In the absence of NH functional groups leading to NH···N(pz) interactions, π-stacking of the pyrazine rings seems to be preferred, as illustrated by the structure of the isocyanide complex **2** (see ESI. Fig. S1b). The presence or absence of such hydrogen bonding patterns is likely to be of importance not just for crystal packing arrangements but also for the interactions of pyrazine-based functionalised complexes with biomolecules and cells.

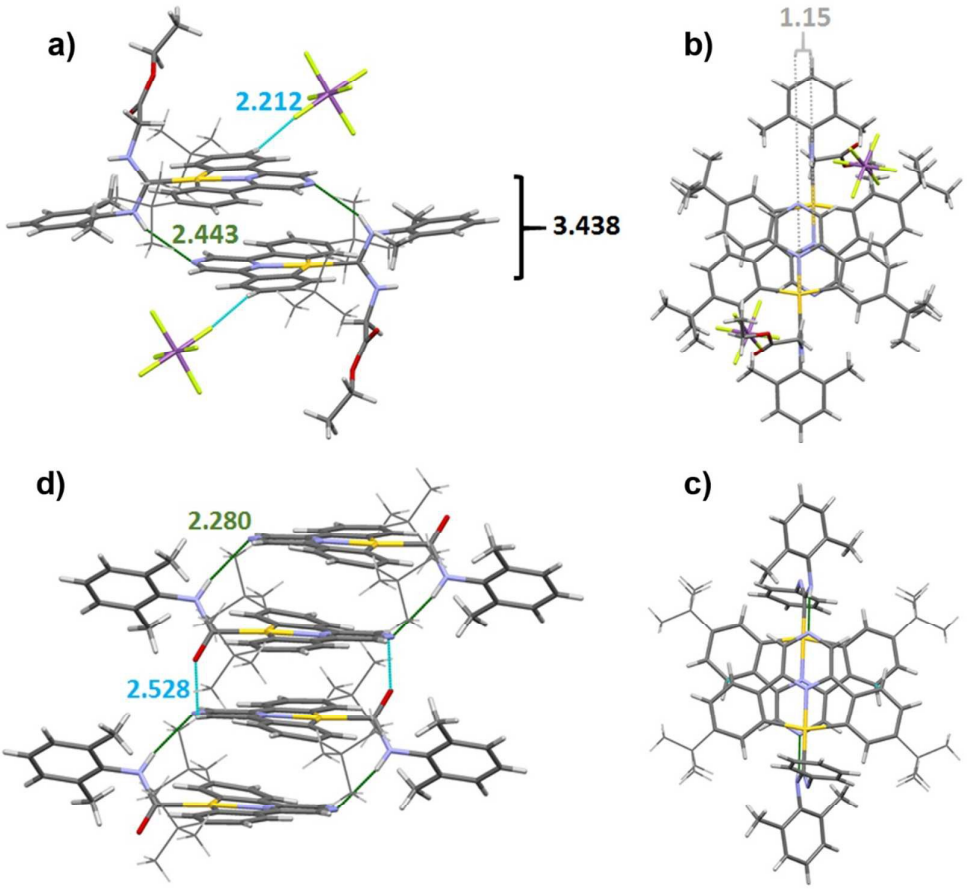

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Fig. 3 (a) Side view of the crystal packing of complex **6**, showing the carbene NH···N(pz) and CH···F interactions. (b) Top view of the dimer found in the crystal packing of complex **6**. (c) Top view of the aggregation in Complex **12**·C_7H_8. (d) Side view of the aggregation in complex **3**.

In vitro antiproliferative activity. Although the complexes proved to be only poorly soluble in purely aqueous media, all compounds are soluble enough in DMSO not to precipitate when diluted with aqueous buffer solution up to 100 μM with 1% DMSO. An initial screening of compounds **1**, **3 - 10** and **12** was carried out on human A549 lung adenocarcinoma cells. This cell line was chosen for its ability to discriminate efficiently between structurally very similar (C^N^{pz}^C)Au^{III} carbene complexes.[24] The inhibition of the proliferation of A549 cells was determined using the established MTS assay (see ESI) after 72 h of incubation with the starting chloride complex **1** and compounds **3 - 10** and **12** at concentrations of 100 μ*M* and 10 μ*M* (Fig. 4). Both neutral complexes **3** and **12** did not show any antiproliferative effects against A549 cells at both concentrations. This is consistent with our previous results using stable neutral (C^N^C)Au complexes bearing phenylacetylide and thiophenolate ligands.[24] The acyclic diamino carbene complexes **4**·$\mathbf{SbF_6}$ and **4**·$\mathbf{BF_4}$ (featuring an aniline substituent) demonstrated only poor antiproliferative effects on the tested cell line. Although the cell viability when treated with **4**·$\mathbf{SbF_6}$ was noticeably lower than when treated with **4**·$\mathbf{BF_4}$ at 100 μ*M* concentration (cell viability of 38 % and 65 % for **4**·$\mathbf{SbF_6}$ and **4**·$\mathbf{BF_4}$, respectively), at a concentration of 10 μ*M* no difference was observed, indicating that the SbF_6 anion has only a marginal effect on the antiproliferative properties of the compounds at low concentrations.

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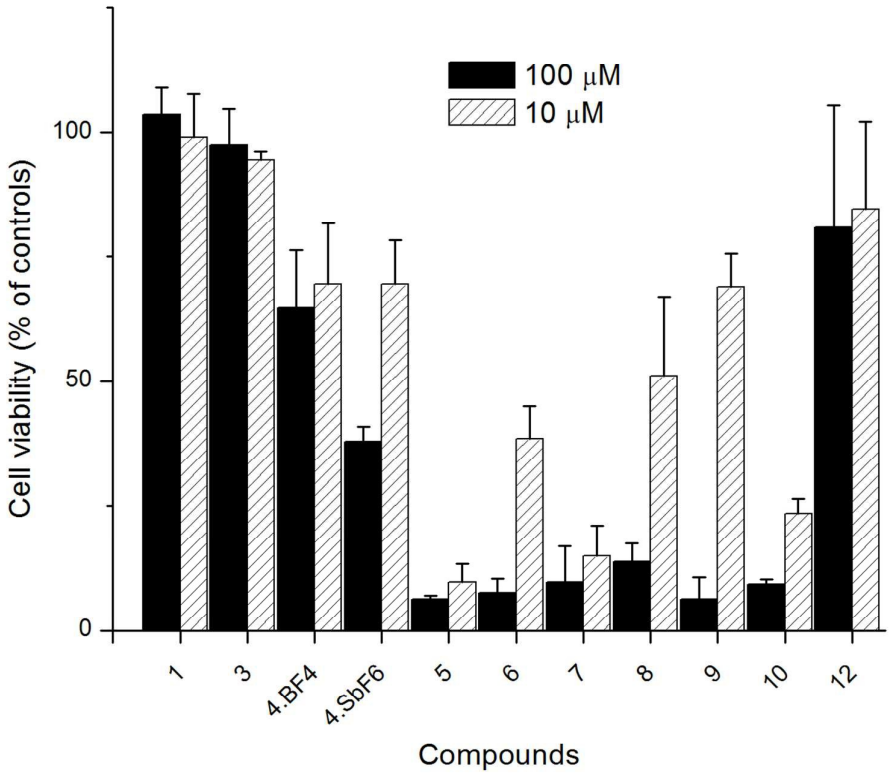

Fig. 4 Inhibition of A549 cells growth by complexes **1**, **3** - **10** and **12** in DMSO; data represent the average ± standard error of three experiments.

Complexes **5** - **10** all inhibited A549 cell proliferation at a concentration of 100 μ*M*. However, of those only **5 - 7** and **10** showed a cell viability of A549 cells below 50 % at 10 μ*M* (cell viability of 9.6 %, 38.4 %, 14.9 % and 23.5 % for **5**, **6**, **7** and **10**, respectively). The two complexes with the most lipophilic side-chains (isopropyl for **8,** and phenyl for **9**) turned out to have a reduced activity compared to the other amino ester decorated [(C^N^{pz}^C)Au(ACC)]X complexes.

The four complexes which inhibited cell proliferation by more than 50 % at 10 μ*M* concentration were selected for the determination of the IC_{50} values (i.e. the concentration required to inhibit 50 % cell growth) on a panel of human cell lines, including (*i*) solid tumour cells, that is human lung (A549), breast (MCF-7) and colon (HCT-116) adenocarcinomas; (*ii*) breast adenocarcinoma (MDA-MB-231) as an example of highly

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metastatic cancer cells; (*iii*) leukemia (HL60) as an example of suspension cells; and (*iv*) healthy fibroblasts (MRC-5). The results are reported in Table 1.

Table 1: Effects of compounds **5-7, 10, AuNHC** and cisplatin on cell viability of a panel of human cancer cell lines and of healthy fibroblast MRC-5 cells after 72 h incubation.

Complex	IC_{50} ± SD (μM)[a]						$S_{MCF7/MRC5}$
	A549	MCF-7	HL60	HCT-116	MDA-MB-231	MRC-5	
5	6.1 ± 1.1	5.2 ± 0.2	0.8 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	0.37 ± 0.03	0.07
6	13.0 ± 3.6	6.4 ± 1.6	16.7 ± 2.5	8.4 ± 0.5	5.1 ± 2.0	8.8 ± 1.0	1.4
7	7.9 ± 1.5	3.9 ± 0.6	8.1 ± 0.9	8.1 ± 0.4	4.7 ± 0.7	6.9 ± 1.1	1.8
10	7.7 ± 1.6	3.4 ± 0.2	6.9 ± 0.6	7.3 ± 0.4	11.1 ± 2.8	6.4 ± 0.7	1.9
AuNHC	7.8 ± 1.3[b]	0.56 ± 0.02[b]	0.3 ± 0.1[b]	11.2 ± 1.5	5.7 ± 0.4	1.4 ± 0.4[b]	2.5
Cisplatin	33.7 ± 3.7[b]	21.2 ± 3.9[b]	3.7 ± 0.3[b]	5.3 ± 0.2	28.4 ± 0.1	10.7 ± 3.0[b]	0.5

[a]Mean ± standard error of at least three independent experiments. [b] Values from ref. 24.

Complexes **5 - 7** and **10** all showed significantly higher cytotoxicity than cisplatin against A549, MCF-7 and MDA-MB-231 cells, while only the adamantyl derivative **5** was more toxic than cisplatin against HL60 and HCT-116 cells. In particular, the new compounds show promising antiproliferative effects against A549, MCF-7 and MDA-MB-231 cells, which are cell lines with the least sensitivity towards cisplatin, and which are the most difficult to treat. Overall the activity is similar to that of our benzimidazole-based compound **AuNHC**.[24]

The adamantyl compound **5** was over ten times more potent than **AuNHC** against HCT-116 and MBA-MB-231 cells. Among the series of amino ester derivatives, the glycine-based complex **6** appeared as the least toxic in the series. Overall, the low micromolar activities of the [(C^N^{pz}^C)Au(ACC)]X complexes, including against A549, MCF-7 and MDA-MB-231 cell lines which show only poor sensitivity towards cisplatin, render them promising for the development of anticancer drugs. Complexes **6**, **7** and **10** appeared to have a reduced activity against the healthy fibroblasts compared to the heterocyclic complex **AuNHC**. Indeed, all amino ester-containing complexes compounds **6**, **7**, **10** and **AuNHC** show improved selectivity for MCF-7 cells *versus* MRC-5 fibroblasts (selectivity factor

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$S_{MCF7/MRC5}$ >1) and proved significantly more selective than cisplatin ($S_{MCF7/MRC5}$ = 0.5). Only the adamantyl compound **5** is less selective).

Reactive oxygen species quantification. Inducing the formation of reactive oxygen species (ROS) is a well-known mechanism of action of metal-based drugs and especially of Au(I)-NHC complexes.[34] Our panel of acyclic carbene complexes was therefore tested in this respect. We measured the amount of intracellular ROS after treatment of A549 cells with 100 μ*M*, 50 μ*M* and 10 μ*M* concentrations of the four toxic complexes **5** - **7** and **10** and the non-toxic complex **4·SbF$_6$** as negative control (see Experimental for details). The results are summarized in Fig. 5. We observed no particular increase in ROS formation for any of the tested complexes, including the cytotoxic ones. These data suggest therefore that induction of ROS is unlikely to be the cause of cytotoxicity, and this mode of action can therefore be ruled out for this class of compounds.

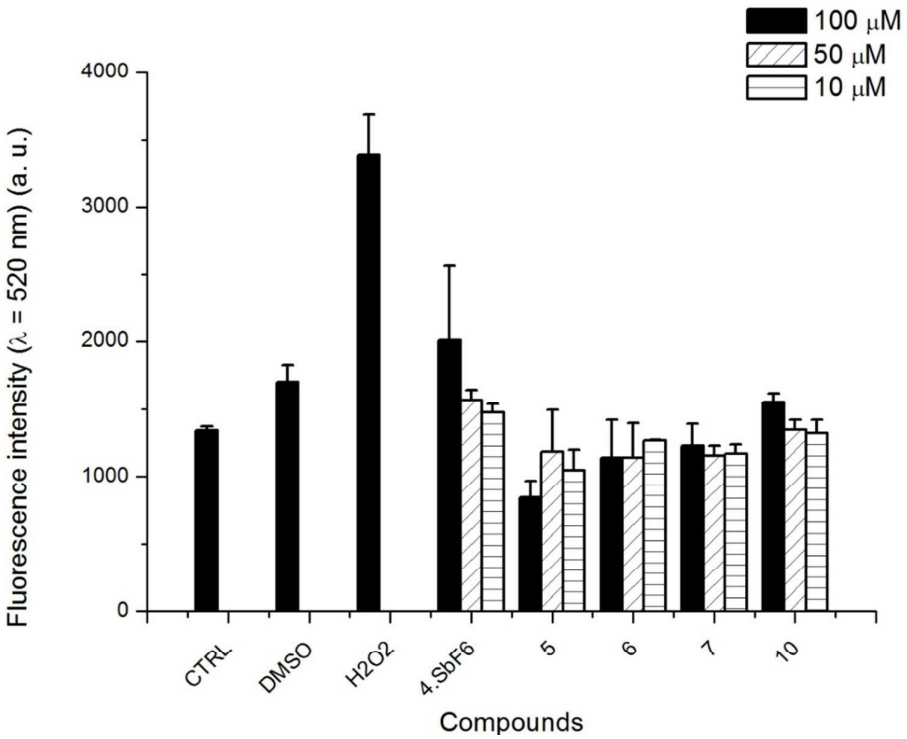

Fig. 5 ROS measurement in A549 cells after 24 h incubation with complexes **4·SbF$_6$**, **5** - **7** and **10**.

Reaction with glutathione. Glutathione (GSH) is a tripeptide present at millimolar levels inside cells and is found overexpressed in most cancer cells. GSH is involved in many

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different cellular functions such as xenobiotic detoxification and reactive oxygen species (ROS) scavenging.[35] Moreover, GSH has been related to resistance mechanism to cisplatin.[36] Although GSH is known to reduce Au(III) complexes bearing (N^N) or (N^N^N) chelating ligands,[37] we demonstrated the stability of bis-cyclometallated Au(III)-NHC complex toward reduction and substitution with GSH.[24] We have therefore explored the reactivity of GSH towards two representative complexes, **6** and **7**, by ^{1}H NMR spectroscopy. Both **6** and **7** were mixed at room temperature with GSH in DMSO-d_6/D_2O 1/1 over a period of time of 24 h (Fig. 6 and S6). Although neither **6** nor **7** showed any reaction with GSH over the first hour, over 24 h we observed the formation of oxidized glutathione (GSSG), the disappearance of the signals of the $[(C^N^C)Au^{(III)}(ACC)]^+$ cations and the formation of a pale yellow product, which we tentatively attribute to the reduction product $(C^N^CH)Au^{(I)}(ACC)$. This leads to the conclusion that acyclic carbene complexes are more sensitive to GSH than benzimidazole-type Au(III) carbene adducts.[24]

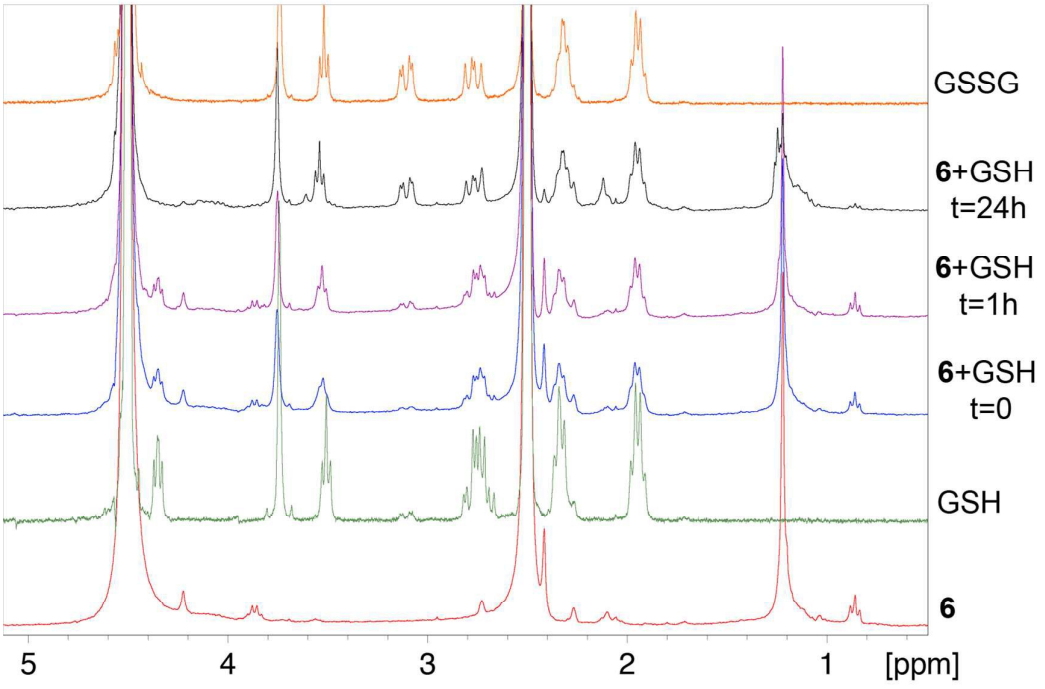

Figure 6: ^{1}H NMR spectra of a 1/1 mixture of **6** with GSH at different reaction times at room temperature, and comparison with the starting materials **6,** GSH and GSSG(DMSO-d_6/D_2O 1/1).

Conclusion

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We have reported the synthesis and characterization of thirteen new pyrazine-based cyclometallated (C^N^{pz}^C)Au^{III} complexes, including the first cyclometallated Au^{III} complexes decorated with amino acid derivatives. Gold(III) complexes of acyclic carbene ligands are readily synthesized and easily modified, including the introduction of biomolecules such as amino acids. The nucleophilic attack on gold(III) isocyanide complexes proved to be the most efficient pathway and is exemplified here by the synthesis of five amino ester decorated [(C^N^{pz}^C)Au(ACC)]X complexes. The crystal structures of these complexes showed the importance of hydrogen bonding involving the non-coordinated pyrazine-N atom as a major tool for organising intermolecular interactions. The antiproliferative screening on A549 cells of ten (C^N^{pz}^C)Au^{III} pincer complexes revealed compounds **5** - **7** and **10** as the most promising candidates, highlighting the usefulness of acyclic carbene ligands as well as the effect of the amino ester substituents. While the antiproliferative mode of action of these complexes remains largely a matter of conjecture, tests can be applied so that certain pathways can be ruled in or out. In the present case, it could be established that reactive oxygen species (ROS) are not enhanced by these compounds and are unlikely to play a role in inducing cell death. Studies are continuing to investigate possible modes of action of acyclic carbene complexes of gold in the search for enhanced cell selectivity.

Experimental Section

When required, manipulations were performed using standard Schlenk techniques under dry nitrogen or in an MBraun glove box. Nitrogen was purified by passing through columns of supported P_2O_5 with moisture indicator, and activated 4 Å molecular sieves. Anhydrous solvents were freshly distilled from appropriate drying agents. ^{1}H and ^{13}C{^{1}H} spectra were recorded using a Bruker Avance DPX-300 spectrometer. ^{1}H NMR spectra (300.13 MHz) were referenced to the residual protons of the deuterated solvent used. ^{13}C{^{1}H} NMR spectra (75.47 MHz) were referenced internally to the D-coupled ^{13}C resonances of the NMR solvent. Elemental analyses were carried out at London Metropolitan University. Compound **1** has been synthesized following reported procedures.[23]

Synthesis of [(C^N^{pz}^C)Au(2,6-dimethylphenyl isocyanide)]SbF$_6$ (**2**)

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Under an N_2 atmosphere, **1** (0.150 g, 0.261 mmol), 2,6-dimethylphenylisocyanide (0.041 g, 0.313 mmol), $AgSbF_6$ (0.107 g, 0.313 mmol) and a few pellets of 4A molecular sieves were placed in a flame-dried Schlenk flask and dichloromethane was added (15 mL). The mixture was left to stir at room temperature for 4 h. A white precipitate of AgCl was removed by filtration through celite, and the filtrate collected under an N_2 atmosphere. The solvent was evaporated to a minimum and the product precipitated with an excess of light petroleum (bp. 40-60 °C). The supernatant was removed and the residue dried under vacuum to yield a yellow solid (0.198 g, 84 %). Anal. Calcd. for $C_{33}H_{35}AuN_3SbF_6$ (906.4): C, 43.73; H, 3.89; N, 4.64. Found: C, 43.84; H, 3.69; N, 4.75. ^{1}H NMR (CD_2Cl_2, 300 MHz, 298 K): δ 8.93 (s, 2H, H^2), 7.89 (d, ^3J = 1.80 Hz, 2H, H^8), 7.77 (d, ^3J = 8.25 Hz, 2H, H^5), 7.59 (t, ^3J = 7.47 Hz, 1H, CH^{para}), 7.53 (dd, ^3J = 8.28 Hz, ^4J = 1.8 Hz, 2H, H^6), 7.42 (d, ^3J = 7.86 Hz, 2H, CH^{meta}), 2.73 (s, 6H, CH_3^{xylyl}), 1.36 (s, 18H, ^{t}Bu). $^{13}C\{^1H\}$ NMR (CD_2Cl_2, 75 MHz): δ 168.1 (s, C^9), 159.5 (s, C^4), 158.4 (s, C^7), 143.8 (s, C^3), 140.2 (s, C^2), 137.5 (s, C^{ortho}), 134.4 (s, C^8), 133.6 (s, CH^{meta}), 129.6 (s, CH^{para}), 127.6 (s, C^5), 126.7 (s, C^6), 36.2 (s, $C(CH_3)_3$), 31.1 (s, $C(CH_3)_3$), 19.4 (s, CH_3^{xylyl}). IR: ν_{max} (neat)/cm^{-1}: 2957 (CH), 2267 (C≡N).

Synthesis of [C^N^{pz}^C]Au(2,6-dimethylphenyl formamide) (3)

A mixture of **2** (0.055 g, 0.061 mmol) and K_2CO_3 (0.025 g, 0.182 mmol) dissolved in a mixture of THF (5 mL) and water (2 mL) was stirred at room temperature for 3 h. The THF was removed under vacuum and water (10 mL) was added. The product was extracted using dichloromethane (3 × 10 mL) and the organic layer washed with water (3 × 10 mL) and dried with anhydrous Na_2SO_4. The solution was evaporated and the solid residue washed twice with light petroleum (bp. 40-60 °C) and dried, to yield a bright yellow solid (0.038 g, 68 %). Anal. Calcd. for $C_{33}H_{36}AuN_3O$ (687.6): C, 57.64; H, 5.28; N, 6.11. Found: C, 57.42; H, 5.18; N, 6.19. ^{1}H NMR (CD_2Cl_2, 300 MHz, 298 K): δ 8.80 (s, 2H, H^2), 8.00 (d, ^3J = 2.01 Hz, 2H, H^8), 7.60 (d, ^3J = 8.16 Hz, 2H, H^5), 7.33 (dd, ^3J = 2.07 Hz, ^4J = 8.16 Hz, 2H, H^6), 7.16 (s, 3H, CH^{xylyl}), 7.07 (s, 1H, NH), 2.50 (s, 6H, CH^{xylyl}), 1.34 (s, 18H, ^{t}Bu). $^{13}C\{^1H\}$ NMR (CD_2Cl_2, 75 MHz): δ 168.8 (s, C^{10}), 167.5 (s, C^9), 156.6 (s, C^7), 156.4 (s, C^4), 144.8 (s, C^3), 138.4 (s, C^2), 136.7 (s, C^{ipso}), 135.8 (s, C^{ortho}), 134.9 (s, C^8), 128.5 (s, CH^{xylyl}), 127.2 (s, CH^{xylyl}), 126.0 (s, C^5), 124.3 (s, C^6), 35.7 (s, $C(CH_3)_3$), 31.4 (s, $C(CH_3)_3$), 19.7 (s, CH_3^{xylyl}). IR: ν_{max} (neat)/cm^{-1}: 3296 (NH), 2962 (CH), 1643 (C=O).

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Synthesis of [(C^N^{pz}^C)Au{C(NHPh)(NHC$_6$H$_3$Me$_2$-2,6)}]SbF$_6$ (4·SbF$_6$)

Complex **2** (0.066 g, 0.073 mmol) and aniline (0.014 g, 0.146 mmol) were combined in a flame-dried Schlenk flask, with molecular sieves, under N_2 atmosphere, dissolved in dichloromethane and filtered through a celite plug. The product was precipitated by addition of a 2/1 mixture of light petroleum (bp. 40-60 °C) and diethyl ether. The product was finally dried under vacuum to yield a bright yellow solid (0.060 g, 0.050 mmol, 58%). Calcd. for $C_{39}H_{42}AuF_6N_4Sb·3H_2O$ (1053.6): C, 44.46; H, 4.59; N, 5.32. Found: C, 44.02; H 4.94; N 5.25. ^{1}H NMR (CD_3CN, 300 MHz, 298 K): δ 9.68 (s, 1H, NH), 9.40 (s, 1H, NH), 8.96 (s, 2H, H^2), 7.80-7.71 (m, 4H, CH^{Ar}), 7.46-7.32 (m, 7H, CH^{Ar}), 7.24-7.14 (m, 3H, CH^{Ar}), 2.53 (s, 6H, CH^{xylyl}), 1.34 (s, 18H, ^{t}Bu). IR: ν_{max} (neat)/cm^{-1}: 3311 (NH) , 2958 (CH), 1586 (carbene).

Synthesis of [(C^N^{pz}^C)Au{C(NH-1-Ad)(NHC$_6$H$_3$Me$_2$-2,6)}]SbF$_6$ (5)

Complex **2** (0.050 g, 0.055 mmol) and 1-adamantylamine (0.017 g, 0.110 mmol) were combined in a flame-dried Schlenk flask, with molecular sieves, under N_2 atmosphere and dissolved in dichloromethane (5 mL). The solution was stirred at room temperature for 16 h. The product was precipitated with an excess of light petroleum (bp. 40-60 °C), filtered off and dried under vacuum to yield a bright yellow solid (0.031 g, 53%). Anal. Cald. for $C_{43}H_{52}AuN_4SbF_6·2H_2O$ (1093.66): C, 47.22; H, 5.16; N, 5.12. Found: C, 47.16; H, 4.71; N, 5.30. ^{1}H NMR (CD_2Cl_2, 300 MHz, 298 K): δ 8.89 (s, 2H, H^2), 8.31 (s, 1H, NH), 7.73 – 7.70 (m, 4H, $H^{5/8}$), 7.46 (dd, ^3J = 1.8 Hz, ^4J = 8.30 Hz, 2H, H^6), 7.40 – 7.34 (m, 3H, CH^{xylyl}), 6.61 (s, 1H, NH), 2.51 (s, 6H, CH^{xylyl}), 2.04 (s, 9H, $CH^{ad.}$ + $CH_2^{ad.}$), 1.54 – 1.51 (m, 6H, CH_2^{ad}), 1.36 (s, 18H, ^{t}Bu). ^{13}C{^{1}H} NMR (CD_2Cl_2, 75 MHz): δ 172.4 (s, C^{10}), 166.5 (s, C^9), 157.9 (s, C^7), 157.3 (s, C^4), 144.5 (s, C^3), 139.5 (s, C^2), 135.9 (s, C^{ortho}), 134.6 (s, C^8), 130.9 (s, CH^{xylyl}), 130.5 (s, CH^{xylyl}), 127.0 (s, C^5), 125.6 (s, C^6), 57.5 (s, $C^{ad.}$), 44.6 (s, $CH_2^{ad.}$), 36.0 (s, $C(CH_3)_3$), 35.6 (s, CH^{ad}), 31.3 (s, $C(CH_3)_3$), 29.8 (s, $CH_2^{ad.}$), 19.5 (s, CH_3^{xylyl}). IR: ν_{max} (neat)/cm^{-1}: 3311 (NH) , 2958 (CH), 1586 (carbene).

[(C^N^{pz}^C)Au{C(NHCH$_2$CO$_2$Et)(NHC$_6$H$_3$Me$_2$-2,6)}]SbF$_6$ (6)

Glycine ethyl ester HCl (0.020 g, 0.146 mmol) was added to a flame-dried Schlenk flask charged with K_2CO_3 (0.012 g, 0.088 mmol) and acetonitrile (10 mL). The solution was sonicated for 30 min and transferred to a separate flame-dried Schlenk flask containing **2**

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(0.066 g, 0.073 mmol). The reaction was then stirred for 16 h at room temperature and the precipitate removed from solution via filtration through a celite plug. Next, the solvent was removed under vacuum and the solid residue re-dissolved in dichloromethane (2 mL). The product was crashed out of solution using a 2:1 mixture of light petroleum (bp. 40-60 °C) / diethyl ether (5 mL), and after removing the solvent, dried under vacuum to yield a bright yellow solid (0.062 g, 0.061 mmol, 59%). Calcd. for $C_{37}H_{44}AuF_6N_4O_2Sb$ (1009.5): C, 44.02; H, 4.39; N, 5.55. Found: C, 43.91; H 4.45; N 5.48. ^{1}H NMR (CD_3CN, 300 MHz, 298 K): δ 9.66 (s, 1H, NH), 8.99 (s, 2H, H^2), 7.81 (d, $^3J_{H\text{-}H}$ = 8.3 Hz, 2H, H^5), 7.70 (d, $^4J_{H\text{-}H}$ = 2.0 Hz, 2H, H^8), 7.57 (s, 1H, NH), 7.45 (dd, $^3J_{H\text{-}H}$ = 8.3 Hz, $^4J_{H\text{-}H}$ = 2.0 Hz, 2H, H^6), 7.40 – 7.28 (m, 3H, $CH^{Ar.}$), 4.25 (d, $^3J_{H\text{-}H}$ = 4.3 Hz, 2H, NCH_2), 3.97 (q, $^3J_{H\text{-}H}$ = 7.2 Hz, 2H, OCH_2) 2.50 (s, 6H, CH_3^{xylyl}), 1.33 (s, 18H, ^{t}Bu), 1.01 (t, $^3J_{H\text{-}H}$ =7.2 Hz, 3H, CH_3^{Et}). ^{13}C{H} NMR (CD_3CN, 75 MHz): δ 177.7 (s, C^{10}), 168.2 (s, *C*=O), 165.2 (s, C^9), 156.8 (s, C^4), 156.4 (s, C^3), 144.9 (s, C^7), 139.6 (s, C^2), 135.8 (s, C^{ortho}), 133.3 (s, C^8), 132.4 (s, C^{ipso}), 129.8 (s, CH^{xylyl}), 129.4 (s, CH^{xylyl}), 126.8 (s, C^5), 125.0 (s, C^6), 61.6 (s, OCH_2), 50.0 (s, NCH_2), 35.3 (s, $C(CH_3)_3$), 30.4 (s, $C(CH_3)_3$), 18.3 (s, CH_3^{xylyl}), 13.3 (s, CH_2CH_3). IR: ν_{max} (neat)/cm^{-1}: 3317 (NH), 3171 (NH), 2963 (CH), 1740 (C=O), 1585 (carbene).

Synthesis of [(C^N^{pz}^C)Au{C(NHCH(Me)CO_2Me)($NHC_6H_3Me_2$-2,6)}]SbF_6 (7)

L-alanine methyl ester HCl (0.020 g, 0.146 mmol) was added to a flame-dried Schlenk flask charged with K_2CO_3 (0.012 g, 0.088 mmol) and acetonitrile (5 mL). The solution was sonicated for 30 min and transferred to a separate flame-dried Schlenk flask containing **2** (0.066 g, 0.073 mmol). The reaction was then stirred for 16 h at room temperature and the precipitate removed from solution via filtration through a celite plug. Next, the solvent was removed under vacuum and the solid residue re-dissolved in dichloromethane (2 mL). The product was crashed out of solution using a 2:1 mixture of light petroleum (bp. 40-60 °C) / diethyl ether (5 mL), and after removing the solvent, dried under vacuum to yield a bright yellow solid (0.070 g, 0.069 mmol, 67%). Calcd. for $C_{37}H_{44}AuF_6N_4O_2Sb$ (1009.50): C, 44.02; H, 4.39; N, 5.55. Found: C, 43.92; H 4.37; N 5.46. ^{1}H NMR (CD_3CN, 300 MHz, 298 K): δ 9.40 (s, 1H, NH), 9.00 (s, 2H, $H^{2/2'}$), 7.85 (d, $^3J_{H\text{-}H}$ = 8.3 Hz, 1H, $H^{5/5'}$), 7.81 (d, $^3J_{H\text{-}H}$ = 8.3 Hz, 1H, $H^{5/5'}$), 7.74 (d, $^4J_{H\text{-}H}$ = 2.0 Hz, 1H, $H^{8/8'}$), 7.70 (s, 1H, NH), 7.66 (d, $^4J_{H\text{-}H}$= 2.0 Hz, 1H, H $^{8/8'}$), 7.49 (dd, $^3J_{H\text{-}H}$ = 8.3 Hz, $^4J_{H\text{-}H}$ = 2.0 Hz, 1H, $H^{6/6'}$), 7.45 (dd, $^3J_{H\text{-}H}$ = 8.3 Hz, $^4J_{H\text{-}H}$ = 2 Hz, 1H, $H^{6/6'}$), 7.37-7.27 (m, 3H, CH^{xylyl}), 4.86 (m, 1H, NCH), 3.53 (s, 3H, OCH_3),

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2.58 (s, 3H, CH_3^{xylyl}), 2.44, (s, 3H, CH_3^{xylyl}), 1.39 (d, $^3J_{H\text{-}H}$ = 7.2 Hz, 3H, $CH_3^{ala.}$), 1.32 (s, 9H, ^{t}Bu), 1.32 (s, 9H, ^{t}Bu). $^{13}C\{H\}$ NMR (CD_3CN, 75 MHz): δ 176.2 (s, C^{10}), 170.9 (s, C=O), 165.2 (s, C^9), 164.9 (s, $C^{9'}$), 157.0 (s, C^7), 156.8 (s, $C^{7'}$), 156.4 (s, C^4), 156.3 (s, $C^{4'}$), 145.0 (s, C^3), 144.9 (s, $C^{3'}$), 139.8 (s, C^2), 139.7 (s, $C^{2'}$), 135.9 (s, C^{ortho}), 135.7 (s, $C^{ortho'}$), 133.9 (s, C^8), 132.9 (s, $C^{8'}$), 132.4 (s, C^{ipso}), 129.9 (s, CH^{xylyl}), 129.5 (s, CH^{xylyl}), 129.4 (s, CH^{xylyl}), 127.1 (s, C^5), 126.8 (s, $C^{5'}$), 125.3 (s, C^6), 125.1 (s, $C^{6'}$), 58.6 (s, NCH), 52.6 (s, OCH_3), 35.3 (s, $C(CH_3)_3$), 30.4 (s, $C(CH_3)_3$), 30.4 (s, $C(CH_3)_3$), 18.6 (s, CH_3^{xylyl}), 18.5 (s, CH_3^{xylyl}), 17.4 (s, $CH_3^{ala.}$). IR: ν_{max} (neat)/cm^{-1}: 3319 (NH), 3062 (NH), 2961 (CH), 1750 (C=O), 1582 (carbene).

Synthesis of [(C^N^{pz}^C)Au{C(NHCH(^{i}Pr)CO_2Me)($NHC_6H_3Me_2$-2,6)}]SbF_6 (8)

L-valine methyl ester HCl (0.029 g, 0.175 mmol) was added to a flame-dried Schlenk flask charged with K_2CO_3 (0.012 g, 0.088 mmol) and acetonitrile (5 mL). The solution was sonicated for 30 min and transferred to a separate flame-dried Schlenk flask containing **2** (0.079 g, 0.087 mmol). The mixture was stirred for 16 h at room temperature and the precipitate removed by filtration through a celite plug. Next, the solvent was removed under vacuum and the solid residue re-dissolved in dichloromethane (2 mL). The product was crashed out of solution using a 2:1 mixture of light petroleum (bp. 40-60 °C) / diethyl ether (5 mL), and after removing the solvent, dried under vacuum to yield a bright yellow solid (0.068 g, 75%). Anal. Calcd. for $C_{39}H_{48}AuN_4O_2SbF_6$ (1037.56): C, 45.15; H, 4.66; N, 5.40. Found: C, 44.86; H, 4.52; N, 5.52. ^{1}H NMR (CD_3CN, 300 MHz, 298 K): δ 9.26 (s, 1H, NH), 8.80 (s, 2H, H^2), 7.62 (2d, ^3J = 9.0 Hz, 2H, $H^{5+5'}$), 7.80 (d, ^4J = 1.89 Hz, 1H, H^8), 7.53 (s, 1H, H^8), 7.25-7.28 (m, 4H, NH + $H^{6+6'+8'}$), 7.13-7.24 (m, 3H, CH^{xylyl}), 4.26 (m, 1H, NCH), 3.15 (s, 3H, OCH_3), 2.33 (s, 3H, CH_3^{xylyl}), 2.30 (s, 3H, CH_3^{xylyl}), 1.88 (m, 1H, CH^{iPr}), 1.09 (s, 9H, ^{t}Bu), 1.08 (s, 9H, ^{t}Bu), 0.61 (d, $^3J_{H\text{-}H}$ = 6.9 Hz, 6H, CH_3^{iPr}). $^{13}C\{^1H\}$ NMR (CD_3CN, 75 MHz): δ 177.7 (s, C^{10}), 171.7 (s, C=O), 166.5 (s, C^9), 165.6 (s, $C^{9'}$), 157.9 (s, C^7), 157.8 (s, $C^{7'}$), 157.4 (s, C^4), 157.3 (s, $C^{4'}$), 145.9 (s, C^3), 145.8 (s, $C^{3'}$), 140.7 (s, C^2), 136.7 (s, C^{xylyl}), 136.6 (s, C^{xylyl}), 134.6 (s, C^8), 134.0 (s, $C^{8'}$), 133.1 (s, CH^{xylyl}), 130.7 (s, CH^{xylyl}), 130.6 (s, CH^{xylyl}), 127.9 (s, C^5), 127.8 (s, $C^{5'}$), 126.5 (s, C^6), 126.2 (s, $C^{6'}$), 69.5 (s, NCH), 53.2 (s, OCH_3), 36.3 (s, $C(CH_3)_3$), 36.3 (s $C(CH_3)_3$), 32.3 (s, CH^{iPr}), 31.4 (s, $C(CH_3)_3$), 31.3 (s, $C(CH_3)_3$), 19.9 (s, CH_3^{xylyl}), 19.7 (s, CH_3^{xylyl}), 19.5 (s, CH_3^{iPr}), 19.4 (s, CH_3^{iPr}), 0.9 (s, CH^{iPr}). IR: ν_{max} (neat)/cm^{-1}: 3300 (NH), 3189 (NH), 2965 (CH), 1742 (C=O), 1582 (carbene).

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Synthesis of [(C^N^{pz}^C)Au{C(NHCH(Ph)CO_2Me)($NHC_6H_3Me_2$-2,6)}]SbF_6 (9)

D-phenylglycine methyl ester HCl (0.030 g, 0.146 mmol) was added to a flame-dried Schlenk flask charged with K_2CO_3 (0.012 g, 0.087 mmol), molecular sieves and acetonitrile (5 mL). The solution was sonicated for 30 min and transferred to a separate flame-dried Schlenk flask containing **2** (0.066 g, 0.073 mmol). The reaction was stirred for 16 h at room temperature and the precipitate removed from solution via filtration through a celite plug. The solvent was removed under vacuum and the solid residue re-dissolved in dichloromethane (2 mL). The product was crashed out of solution using a 2:1 mixture of light petroleum (bp. 40-60 °C) / diethyl ether (5 mL), and after removing the solvent, dried under vacuum to yield an orange solid (0.059 g, 0.055 mmol, 53 %). Calcd. for $C_{42}H_{46}AuF_6N_4O_2Sb.2CH_2Cl_2$ (1241.4): C, 42.57; H, 4.06; N, 4.51. Found: C, 42.55; H 4.03; N 4.15. ^{1}H NMR (CD_3CN, 300 MHz, 298 K): δ 9.51 (s, 1H, NH), 9.02 (s, 1H, H^2), 8.97 (s, 1H, H$^{2'}$), 8.11 (d, $^3J_{H\text{-}H}$ = 6.0 Hz, 1H, NH), 7.87 (d, $^3J_{H\text{-}H}$ = 8.2 Hz, 1H, H^5), 7.74 (d, $^3J_{H\text{-}H}$ = 8.2 Hz, 1H, H$^{5'}$), 7.68 (d, $^4J_{H\text{-}H}$ = 1.6 Hz, 1H, H^8), 7.51 (dd, $^3J_{H\text{-}H}$ = 8.2 Hz, $^4J_{H\text{-}H}$ = 1.6 Hz, 1H, H^6), 7.41-7.30 (m, 4H, H$^{6'}$ + CH^{xylyl}), 7.28 (d, $^4J_{H\text{-}H}$ = 1.6 Hz, 1H, H$^{8'}$), 7.21-7.06 (m, 5H, CH^{PhGly}), 5.68 (d, $^3J_{H\text{-}H}$ = 6.0 Hz, 1H, NCH), 3.54 (s, 3H, OCH_3), 2.51 (s, 3H, CH_3^{xylyl}), 2.43 (s, 3H, CH_3^{xylyl}), 1.35 (s, 9H, ^{t}Bu), 1.17 (s, 9H, ^{t}Bu). ^{13}C{H} NMR (CD_3CN, 75 MHz): δ 176.5 (s, *C*^{10}), 169.4 (s, *C*=O), 165.3 (s, *C*^{9}), 164.3 (s, *C*$^{9'}$), 157.1 (s, *C*^{7}), 156.5 (s, *C*$^{7'}$), 156.4 (s, *C*^{4}), 156.4 (s, *C*$^{4'}$), 145.0 (s, *C*^{3}), 144.4 (s, *C*$^{3'}$), 139.6 (s, *C*^{2}), 139.6 (s, *C*$^{2'}$), 135.7 (s, *C*$^{Ar.}$), 135.6 (s, *C*$^{Ar.}$), 134.7 (s, *C*$^{Ar.}$), 133.4 (s, *C*^{8}), 133.0 (s, *C*$^{8'}$), 132.1 (s, *C*$^{Ar.}$), 130.0 (s, *C*H$^{Ar.}$), 129.7 (s, *C*H$^{Ar.}$), 129.6 (s, *C*H$^{Ar.}$), 129.4 (s, *C*H$^{Ar.}$), 129.0 (s, *C*H$^{Ar.}$), 128.3, (s, *C*H$^{Ar.}$), 127.1 (s, *C*^{5}), 126.5 (s, *C*$^{5'}$), 125.3 (s, *C*^{6}), 125.0 (s, *C*$^{6'}$), 65.5 (s, N*C*H), 53.2 (s, O*C*H_3), 35.3 (s, *C*$(CH_3)_3$), 35.1 (s, *C*$(CH_3)_3$), 30.4 (s, C(*C*$H_3)_3$), 30.4 (s, C(*C*$H_3)_3$), 18.6 (s, *C*H_3^{xylyl}), 18.5 (s, *C*H_3^{xylyl}). IR: ν_{max} (neat)/cm^{-1}: 3295 (NH), 3173 (NH), 2963 (CH), 1744 (C=O), 1586 (carbene).

Synthesis of [(C^N^{pz}^C)Au{C(NHCH(CH_2CH_2SMe)CO_2Me)($NHC_6H_3Me_2$-2,6)}]SbF_6 (10)

L-methionine methyl ester HCl (0.035 g, 0.175 mmol) was added to a flame-dried Schlenk flask charged with K_2CO_3 (0.012 g, 0.087 mmol), molecular sieves and acetonitrile (5 mL). The solution was sonicated for 30 min and transferred to a separate flame-dried Schlenk flask containing **2** (0.079 g, 0.087 mmol). The reaction was then stirred for 16 h at room

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temperature and the precipitate removed from solution via filtration through a celite plug. Next, the solvent was removed under vacuum and the solid residue re-dissolved in dichloromethane (2 mL). The product was crashed out of solution using a 2:1 mixture of light petroleum (bp. 40-60 °C) / diethyl ether (5 mL), and after removing the solvent, dried under vacuum to yield an orange solid (0.069 g, 74%). Anal. Calcd. for $C_{39}H_{48}AuN_4O_2SSbF_6$ (1069.6): C, 43.79; H, 4.52; N, 5.24. Found: C, 43.58; H, 4.37; N, 5.08. ^{1}H NMR (CD_2Cl_2, 300 MHz, 298 K): δ 8.85 (s, 2H, H^2), 8.71 (s, 1H, NH), 8.29 (d, $^3J_{H\text{-}H}$ = 9.0 Hz, 1H, NH), 7.74 – 7.62 (m, 4H, $H^{5/8}$), 7.48 – 7.35 (m, 5H, H^6 + H^{xylyl}), 5.00 (m, 1H, NCH), 3.60 (s, 3H, OCH_3), 2.63 (s, 3H, CH_3^{xylyl}), 2.52 (s, 3H, CH_3^{xylyl}), 2.14 (m, 2H, SCH_2), 1.66 (s, 3H, SCH_3), 1.36 (s, 9H, ^{t}Bu), 1.33 (s, 9H, ^{t}Bu), 1.14 (s, 2H, CH$\underline{CH_2}$). ^{13}C{^{1}H} NMR (CD_2Cl_2, 75 MHz): δ 178.2 (s, C^{10}), 170.6 (s, *C*=O), 165.8 (s, C^9), 165.1 (s, $C^{9'}$), 158.2 (s, C^7), 158.0 (s, $C^{7'}$), 157.0 (s, C^4), 156.9 (s, $C^{4'}$), 144.7 (s, C^3), 144.6 (s, $C^{3'}$), 139.5 (s, C^2), 139.4 (s, $C^{2'}$), 136.3 (s, $C^{Ar.}$), 135.7 (s, $C^{Ar.}$), 134.0 (s, C^8), 132.9 (s, $C^{8'}$), 131.7 (s, $CH^{Ar.}$), 130.9 (s, $CH^{Ar.}$), 130.5 (s, $CH^{Ar.}$), 130.2 (s, $C^{Ar.}$), 127.3 (s, C^5), 127.1 (s, $C^{5'}$), 125.9 (s, C^6), 125.8 (s, $C^{6'}$), 61.6 (s, OCH_3), 36.0 (s, N*C*H), 31.9 (s, CH*C*H_2), 31.3 (s, C(CH_3)$_3$), 31.2 (s, C(CH_3)$_3$),30.4 (s, *C*(CH_3)$_3$), 30.1 (s, *C*(CH_3)$_3$), 19.4 (s, CH_3^{xylyl}), 19.3 (s, CH_3^{xylyl}), 18.9 (s, S*C*H_2), 14.8 (s, S*C*H_3). IR: ν_{max} (neat)/cm^{-1}: 3304 (NH), 3169 (NH), 2960 (CH), 1744 (C=O), 1585 (carbene).

Synthesis of (C^N^{pz}^C)Au(NHPh) (11a)

A mixture of **1** (0.050 g, 0.087 mmol) and KO^{t}Bu (0.012 g, 0.104 mmol) in a flame-dried Schlenk flask with molecular sieves under N_2 atmosphere was dissolved in toluene (5 mL) and stirred at room temperature for 3 h. Distilled aniline (8.26 µL, 0.087 mmol) was added and the mixture stirred for a further 3 h. The solvent was removed under vacuum and the product dissolved in dichloromethane. The solution was filtered through celite and the solvent removed under vacuum. The solid residue was washed twice with light petroleum and dried under vacuum to yield a black solid (0.050 g, 91 %). Anal. Calcd. for $C_{30}H_{32}AuN_3$ (631.6): C, 57.05; H, 5.11; N, 6.65. Found: C, 57.17; H, 5.26; N, 6.55. ^{1}H NMR (CD_2Cl_2, 300 MHz, 298 K): δ 8.69 (s, 2H, H^2), 7.60 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 2H, H^5), 7.41 (d, $^3J_{H\text{-}H}$ = 1.8 Hz, 2H, H^8), 7.30 (dd, $^3J_{H\text{-}H}$ = 8.4 Hz, $^4J_{H\text{-}H}$ = 2.1 Hz, 2H, H^6), 7.01 (dd, $^3J_{H\text{-}H}$ = 6.75, 2H, CH^{meta}), 6.80 (dd, $^3J_{H\text{-}H}$ = 7.8 Hz, $^4J_{H\text{-}H}$ = 1.2 Hz, 2H, CH^{ortho}), 6.58 (t, $^3J_{H\text{-}H}$ = 7.2, 1H, CH^{para}), 3.84 (s, 1H, NH), 1.23 (s, 18H, ^{t}Bu). ^{13}C{^{1}H} NMR (CD_2Cl_2, 75 MHz): δ 170.7 (s, C^9), 156.3 (s, C^7 + C^4), 144.2 (s, C^3), 138.4 (s, C^2), 133.9 (s, C^{10}), 131.5 (s, C^8) 129.2 (s, C^{ipso}), 125.6 (s, C^5),

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124.7 (s, C^6), 120.0 (s, $C^{meta,para}$), 117.1 (s, C^{ortho}), 36.6 (s, $C(CH_3)_3$), 31.3 (s, $C(CH_3)_3$). IR: ν_{max} (neat)/cm^{-1}: 3315 (NH), 2951 (CH).

Synthesis of (C^N^{pz}^C)Au(NHC$_6$H$_3$^{i}Pr$_2$-2,6)}] (11b)

The procedure for **11a** was followed to make **11b** from **1** (0.050 g, 0.087 mmol), KO^{t}Bu (0.024 g, 0.218 mmol) and distilled 2,6-diisopropylaniline (0.015 g, 0.087 mmol). The solvent was removed under vacuum and the product dissolved in dichloromethane, filtered through celite and the solvent removed under vacuum to give an oily precipitate which was recrystallized from dichloromethane/ light petroleum at -20 °C to yield a brown solid (0.046 g, 74 %). Anal. Calcd. for $C_{36}H_{44}AuN_3$ (715.7): C, 60.41; H, 6.20; N, 5.87. Found: C, 60.29; H, 6.27; N, 5.74. ^{1}H NMR (CD_2Cl_2, 300 MHz, 298 K): δ 8.81 (s, 2H, H^2), 7.65 (d, $^3J_{H-H}$ = 8.1 Hz, 2H, H^5), 7.28 (dd, $^3J_{H-H}$ = 8.1 Hz, J = 2.0 Hz, 2H, H^6), 7.13 (d, J = 7.6 Hz 2H, CH^{meta}), 7.02 (m, 3H, H^8 CH^{para}), 4.12 (s, 1H, NH), 3.69 (h, $^3J_{H-H}$ = 6.9 Hz, 2H, CH^{iPr}) 1.23 (s, 18H, ^{t}Bu), 1.15 (d, $^3J_{H-H}$ = 6.9 Hz, 12H, CH_3^{iPr}). ^{13}C{^{1}H} NMR (CD_2Cl_2, 75 MHz): δ 169.6 (s, C^9), 156.4 (s, C^7), 155.8 (s, C^4), 148.4 (s, C^{ipso}), 144.4 (s, C^3), 141.8 (s, C^{ortho}), 138.4 (s, C^2), 129.3 (s, CH^{meta}), 125.3 (s, C^5), 124.2 (s, C^8), 123.7 (s, C^6), 121.9 (s, CH^{para}), 35.5 (s, $C(CH_3)_3$), 31.1 (s, $C(CH_3)_3$), 28.2 (s, CH^{iPr}), 23.0 (s, CH_3^{iPr}). IR: ν_{max} (neat)/cm^{-1}: 3322 (NH), 2958 (CH).

Synthesis of (C^N^{pz}^C)Au{C(NPh)(NHC$_6$H$_3$Me$_2$-2,6)} (12)

Product **11a** (0.174 mmol) was made in-situ as described and, without prior purification, was treated with 2,6-dimethylphenyl isocyanide (0.023 g, 0.174 mmol). The mixture was stirred for 16 h. The solvent was removed under vacuum and the product dissolved in dichloromethane and filtered through Celite. The solvent removed again under vacuum to give a yellow oil which was washed with ice-cold hexane. The residue was dried under vacuum to yield a yellow solid (0.108 g, 83 %). Anal. Calcd. for $C_{39}H_{41}AuN_4$ (762.8): C, 61.41; H, 5.42; N, 7.35. Found: C, 61.44; H, 5.36; N, 7.25. ^{1}H NMR (CD_3CN, 300 MHz, 298 K): δ 8.87 (s, 2H, H^2), 8.05 (s, 2H, H^8), 7.67 (d, $^3J_{H-H}$ = 8.4 Hz, 2H, H^5), 7.34 (dd, $^3J_{H-H}$ = 8.1 Hz, $^4J_{H-H}$ = 2.1 Hz, 2H, H^6), 7.14-7.12 (m, 4H, $CH^{Ar.}$), 6.96-6.94 (m, 4H, $CH^{Ar.}$), 6.71 (s, 1H, NH), 2.52 (s, 6H, CH_3^{xylyl}), 1.36 (s, 18H, ^{t}Bu). ^{13}C{^{1}H} NMR (CD_3CN, 75 MHz): δ 167.3 (s, C^9), 156.7 (s, C^7), 156.5 (s, C^4), 146.0 (s, C^3), 139.8 (s, C^2), 135.9 (s, C^8), 130.7 (s, $C^{Ar.}$),

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129.4 (s, *C*H$^{Ar.}$), 128.9 (s, *C*$^{Ar.}$), 127.0 (s, *C*^{5}), 124.8 (s, *C*^{6}), 124.7 (s, *C*^{10}), 123.4 (s, *C*H$^{Ar.}$), 36.0 (s, *C*(CH$_3$)$_3$), 31.5 (s, C(*C*H$_3$)$_3$), 20.0 (s, *C*H$_3^{xylyl}$). IR: ν_{max} (neat)/cm^{-1}: 3340 (NH), 2957 (CH), 1607 (C=N).

Synthesis of [(C^N^{pz}^C)Au{C(NHPh)(NHC$_6$H$_3$Me$_2$-2,6)}]BF$_4$ (4·BF$_4$)

A solution of **12** (0.050 g, 0.067 mmol) in diethyl ether at -78 ºC was treated with HBF$_4$·OEt$_2$ (9 μL, 0.067 mmol). There was an immediate colour change from yellow to orange. The mixture was allowed to warm to room temperature and left to stir for 30 min. The solvent was removed under vacuum to yield an orange powder (0.057 g, 99 %). Anal. Calcd. for C$_{39}$H$_{42}$AuN$_4$BF$_4$•3H$_2$O (904.6): C, 51.78; H, 5.35; N, 6.19. Found: C, 51.23; H, 4.05; N, 6.25. ^{1}H NMR (CD$_3$CN, 300 MHz, 298 K): δ 9.71 (s, 1H, NH), 9.47 (s, 1H, NH), 8.98 (s, 2H, H^2), 7.82-7.79 (m, 4H, CH$^{Ar.}$), 7.48-7.37 (m, 7H, CH$^{Ar.}$), 7.24-7.17 (m, 3H, CH$^{Ar.}$), 2.55 (s, 6H, CH$_3^{xylyl}$), 1.36 (s, 18H, ^{t}Bu). ^{13}C{^{1}H} NMR (CD$_3$CN, 75 MHz): δ 176.2 (s, *C*^{10}), 165.8 (s, *C*^{9}), 157.8 (s, *C*^{7}), 157.4 (s, *C*^{4}), 145.7 (s, *C*^{3}), 140.6 (s, C^2), 139.9 (s, *C*$^{Ar.}$), 136.6 (s, *C*$^{Ar.}$), 134.1 (s, *C*^{8}), 133.5 (s, *C*$^{Ar.}$), 130.9 (s, *C*H$^{Ar.}$), 130.6 (s, *C*H$^{Ar.}$), 130.5 (s, *C*H$^{Ar.}$), 128.8 (s, *C*H$^{Ar.}$), 127.7 (s, *C*^{5}), 126.1 (s, *C*^{6}), 124.9 (s, *C*H$^{Ar.}$), 36.3 (s, *C*(CH$_3$)$_3$), 31.4 (s, C(*C*H$_3$)$_3$), 19.4 (s, *C*H$_3^{xylyl}$). IR: ν_{max} (neat)/cm^{-1}: 3215 (NH), 2956 (CH), 1586 (carbene).

X-ray crystallography

Pale-yellow square plates of **2** were grown by slow evaporation of a saturated solution of the crude product in CH_2Cl_2. Complex **3** was crystallized by slow diffusion of light petroleum in a saturated solution of the complex in CH_2Cl_2. Yellow square plates with stoichiometry **5**·1.5 toluene were grown by diffusion of petroleum into a saturated solution of the complex in toluene. Crystals of **6** and **11a** were grown by the slow evaporation of saturated dichloromethane solutions. Slow diffusion of light petroleum into a saturated solution of **12** produced yellow needles with stoichiometry **12**·toluene. The crystals were mounted on a MiTeGen MicroMesh and fixed in a cold nitrogen stream. Diffraction intensities were recorded at 298, 173 or 140 K on a Rigaku HG Saturn724+ (2×2 bin mode) diffractometer or an Oxford Diffraction Xcalibur-3 instrument, both equipped with Mo-Kα radiation. Data collection, refinement and reduction were performed using the CrystalClear-SM Expert 3.1 b27 or CrysAlisPro software and the absorption correction was applied at this stage.[38] All structures were solved using SHELXS/T and refined by full-matrix least-squares methods on

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F^2 with SHELXL.[39] Non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were included in idealised positions. No missed symmetry was reported by PLATON.[40] Computer programs used in this analysis were run through WinGX.[41] Scattering factors for neutral atoms were taken from reference.[42]

Antiproliferation assay

The human A549 and HL60 cancer cell lines (from ECACC) were cultured in RPMI 1640 medium with 10% fetal calf serum, 2m*M* L-glutamine, 100U/mL penicillin and 100 µg/mL streptomycin (Invitrogen). Cells were maintained in a humidified atmosphere at 37°C and 5% CO_2. The human MCF-7, HCT116 and MDA-MB-231 cancer cell lines (from ECACC) and the healthy fibroblasts MRC-5 cells were cultured in DMEM medium with 10% fetal calf serum, 2m*M* L-glutamine, 100U/mL penicillin and 100 µg/mL streptomycin (Invitrogen). Cells were maintained in a humidified atmosphere at 37 °C and 5% CO_2. Inhibition of cancer cell proliferation was measured by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega) and following the manufacturer's instructions. Briefly, cells (3×10^4/100 µL for HL-60, 8×10^3/100 µL for A549, MCF-7, HCT116, MDA-MB-231 and 2×10^3/100 µL for MRC-5) were seeded in 96-well plates and left untreated or treated with 1 µL of DMSO (vehicle control) or 1 µL of complexes diluted in DMSO at different concentrations, in triplicate for 72 h at 37 °C with 5% CO_2. Following this, MTS assay reagent was added for 4 h and absorbance measured at 490nm using a Polarstar Optima microplate reader (BMG Labtech). IC_{50} values were calculated using GraphPad Prism Version 5.0 software.

ROS assay

100 µL of A549 cells were seeded at a density of 1×10^5 cells/mL in a 96-well black plate with a transparent bottom. The cells were incubated at 37 °C for 24 h. The medium was removed, and replaced with 50 µM H_2DCFDA (from Life Technologies) solution in PBS for 40 min. H_2DCFDA was removed and replaced with fresh medium. The cells were left for recovery for 20 min at 37°C. Basal fluorescence was measured at 485/520 nm on a POLARstar Optima. Cells were incubated with 10 µ*M*, 50µ*M*, or 100 µ*M* of compounds, 1% DMSO (negative control) and 100 µ*M* of H_2O_2 (positive control) for 24 h. Fluorescence was read at 485/520 nm. Basal fluorescence was subtracted from the fluorescence in treated cells to calculate the amount of fluorescence caused by the compounds.

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†Electronic supplementary information (ESI) available: Crystal structure diagrams, NMR spectra. See DOI: 10.1039/xxxxxx. CCDC 1561647 (**2**), 1561651 (**3**), 1561652 (**5**·1.5C_7H_8), 1561648 (**6**), 1561649 (**11a**) and 1561650 (**12**·C_7H_8) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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