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Original article

Synthesis, structures, and antimalarial activities of some silver(I), gold(I) and gold(III) complexes involving *N*-heterocyclic carbene ligands

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ABSTRACT

A series of mono-and dinuclear silver(I) and mononuclear gold(I) complexes containing bis(*N*-heterocyclic carbene) (NHC) or *N*-functionalized NHC ligands were synthesized and fully characterized by spectroscopic methods and, in some cases, by single crystal X-ray diffraction. The *in vitro* antiplasmodial and antifungal activities of a previously described family of *N*-functionalized bis(imidazolium) proligands and their corresponding silver(I), gold(I) and gold(III) complexes but also the new here described compounds were investigated in a chloroquine-resistant strain of *Plasmodium falciparum*, and against two *Candida* strains, respectively. For the first family, interesting antiplasmodial and antifungal activities were found for the dinuclear silver(I) species but they also showed strong hemolytic properties. Pharmaco-modulations leading to the second series of complexes allowed notably increase in the antiplasmodial activity, in particular of the mononuclear gold(I) complexes with IC_{50} values up to 330 nM, without any hemolysis.

1. Introduction

Coinage metal-NHCs (NHC = *N*-heterocyclic carbene) are widely studied for their intriguing structural properties and numerous applications; in particular, a newly emerging interest in medicinal applications of stable NHC(s) complexes has recently expanded. Due to their easy preparation in mild conditions via the Ag_2O route and their ability as transmetalating agents, Ag^I–NHCs have become the most synthesized and studied among coinage metal-NHCs [1]. Furthermore, Youngs and co-workers first demonstrated the exceptional antimicrobial efficacy of Ag^I–NHCs against a broad spectrum of both gram-positive and gram-negative bacteria as well as fungi [2–4]. The same research group recently investigated the anticancer activity of Ag^I–NHCs stable to light and water [5]. The interest in Au^I-and Au^{III}–NHC complexes has surged in the past decade because of their easy preparation via the Ag carbene transfer route and the variety in properties and applications [1]. Many new Au^I-or Au^{III}–NHC(s)-catalyzed reactions were recently devised [6]. Au^I-and Au^{III}–NHC compounds are generally readily prepared, stable to air and moisture, and some of them, especially dinuclear gold(I) and polynuclear gold(I)-heterometal species display long-lived intense photoluminescence at room temperature [1]. Biomedical applications of gold complexes based on NHCs are beginning to unfold. In particular, given that these ligands are extremely good σ–donors, they form strong Au-carbene bonds, giving stable Au^I–NHC complexes that are insensitive to biologically important thiol groups. Au^I–NHCs have shown potential medical applications [7], especially as anticancer [8–12], antiarthritis [13], and antimicrobial agents [14]. It has to be mentioned that gold-based compounds, including some gold(I)-NHCs, show anti-mitochondrial activity, a promising mode of action to fight cancer. Their antitumor activity may stem from the lipophilic and cationic properties, allowing their accumulation in mitochondria of tumor cells with great specificity [7].

In biomedical research, one of the most important fields concerns infectious disease. Candidemia are opportunistic fungal infections causing alarming problems with serious infections in immunologically compromised people [15]. *Candida albicans* and *Candida glabrata* are the most frequent *Candida* isolates [15]b.

Malaria remains the most important parasitic disease affecting humans. Recent reports estimate more than one million deaths in

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2010 [16]. The major antimalarial drug namely chloroquine (CQ) is today almost completely ineffective, due to parasite resistance. In the approach to find new compounds against infective diseases, Navarro reviewed many gold complexes showing potential antiparasitic properties [17]. Gold is among the most ancient of all metals used in medicine and its current use has allowed information regarding toxicological and clinical administration to become available along with valuable studies concerning its metabolism and molecular targets. Due to the urgent need to develop new drugs, gold compounds appear as good candidates in the design of novel metal-based anti-malaria drugs. For example, the CQ complex of triphenylphosphine gold(I) was found to be more active *in vitro* than CQ against two CQ-resistant strains of *Plasmodium falciparum* (IC_{50} (50% inhibitory doses) = 5.1–23 nM) and also *in vivo* against *Plasmodium berghei* [17].

In recent years, we focused our research on a series of heteroditopic polydentate bis(NHC) ligands designed for the complexation of transition metals [18–21]. We developed short and modulable synthetic routes for the preparation of functionalized bis(NHC) precursor ligands and their Ag^{I}-, Au^{I}-and Au^{III}–bis(NHC) complexes (see Scheme 1 [22] for some examples). Such ligands were found to allow the stabilization of bimetallic silver and gold complexes, exhibiting sometimes particularly short metal–metal contacts, thus favoring the occurrence of metallophilic interactions [17,18]. In the case of silver and gold complexes, we studied the influence of the non-coordinating functionalized side arms on the luminescent properties [19,21].

In this work, we aim to test a first series of air and moisture stable dinuclear silver and gold *N*-functionalized NHC complexes (Scheme 1) against *P. falciparum*, the parasite responsible of malaria. In addition, we report the preparation and characterization of a second series of mono-and dinuclear silver(I) and mononuclear gold(I) complexes, in order to examine how modifications of the different building blocks of the ligands can influence the biological activity of the corresponding complexes. The pharmacomodulation of these complexes permitted a notably increase in their antiplasmodial activity and to remove their hemolytic characteristics.

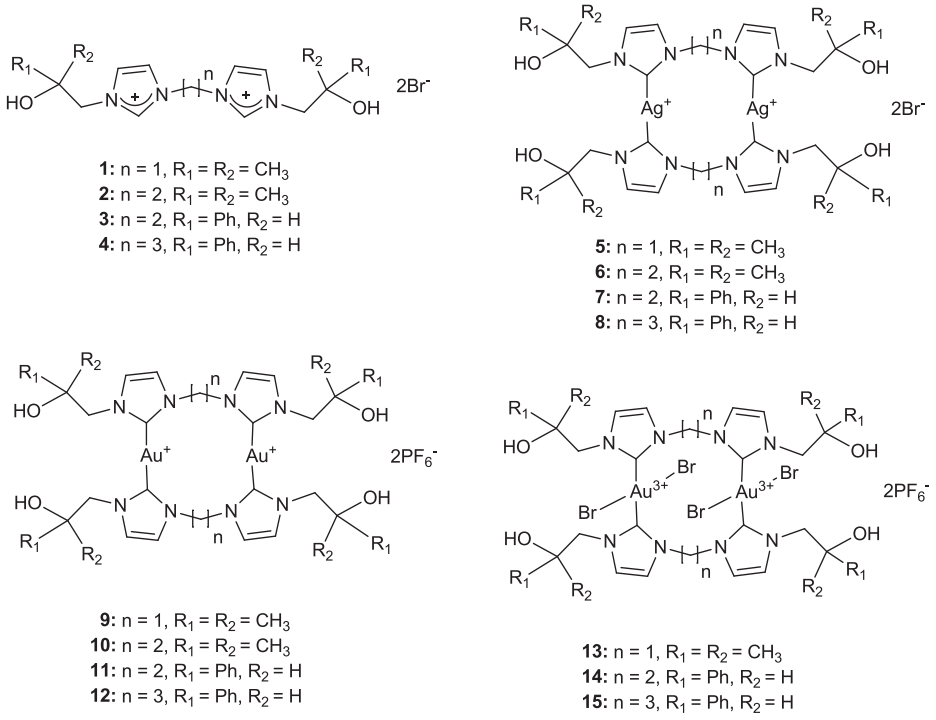
2. Results and discussion

2.1. Chemistry

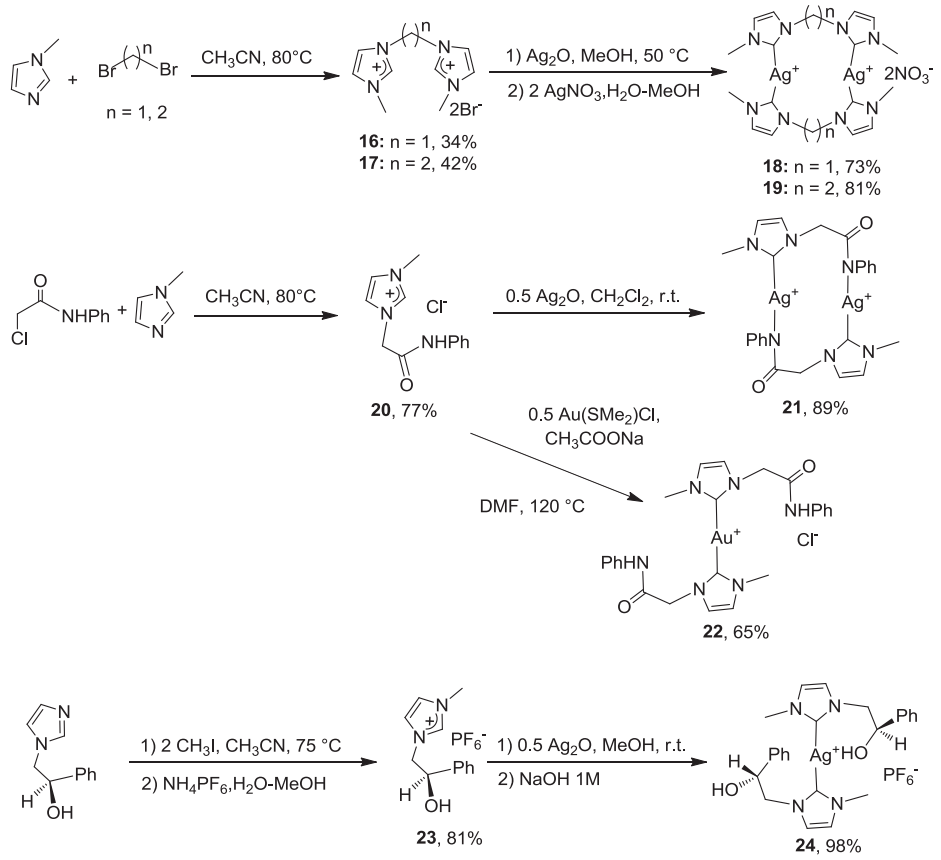
2.1.1. Synthesis of N-functionalized mono and bis(imidazolium) salts

The bis(imidazolium) salts **16** and **17** and the imidazolium salt **20** were obtained from commercially available reagents, after a quaternization step of 1-methylimidazole by reaction with half an equivalent of dibromoalkane (dibromomethane for **16** and 1,2-dibromoethane for **17**), or with one equivalent of 2-chloro-*N*-phenylacetamide for **20** (Scheme 2) [23]. The proligand **23** was prepared by alkylation of (*1R*)-2-(1*H*-imidazol-1-yl)-1-phenylethanol [18] with methyliodide, followed by an ion-exchange with ammonium hexafluorophosphate (Scheme 2). The generation of the quinoline derivative **25** requires more drastic reaction conditions to activate the 2-chloroquinoline (excess of 1-methylimidazole, toluene, 130 °C), followed by an anionic metathesis (Scheme 3). 5-(Bromomethyl)-2,2′-bipyridine was synthetized in three steps according literature procedures [24,25]. 2-Acetylpyridine and iodine were heated in pyridine to give the corresponding pyridinium salt, which was then reacted with metacrolein in formamide in the presence of NH_4OAc to afford 5-methyl-2,2′-bipyridine. The last step involves the conversion of the methyl group to the corresponding bromoethyl by treatment with *N*-bromosuccinimide and azoisobutyronitrile. The carbene precursor **28** was prepared under the same conditions as **25**, starting from 1-methylimidazole and 5-(bromomethyl)-2,2′-bipyridine (Scheme 4).

The most notable features in the ^{1}H and ^{13}C spectra of the mono-and bis-imidazolium salts are the resonances for imidazolium protons (H_2) located between 9.07 and 10.48 ppm and the corresponding imidazolium carbons (C_2) in the range of 134.9–139.0 ppm. The FAB-MS spectra of all compounds exhibit



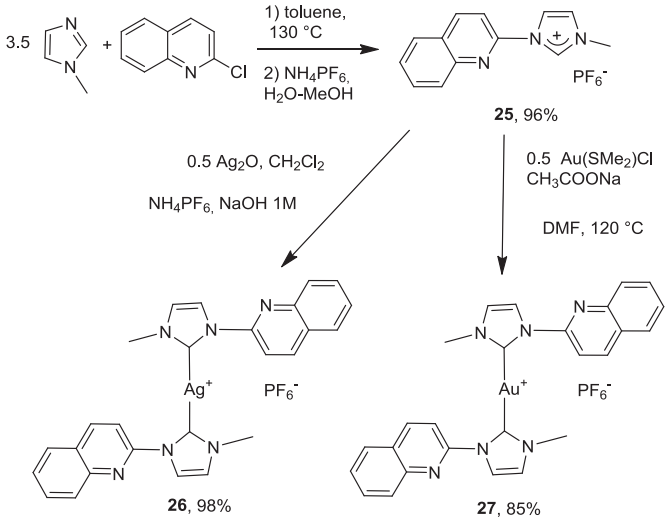
Scheme 1. First series of dinuclear complexes.



Scheme 2. Synthesis of proligands **16**, **17**, **20** and **23**, silver(I) complexes **18**, **19**, **21** and **24** and gold(I) complex **22**.

the classical peak corresponding to $[M - X]^+$ cations (X = Cl, Br or PF_6).

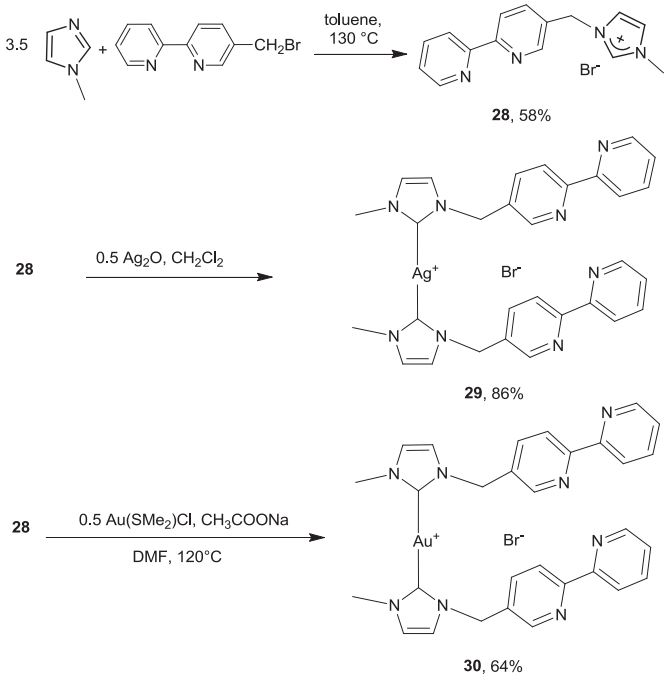
In the bis(imidazolium) salts **16** and **17**, the two azolium rings are held together by an aliphatic bridge and contain methyl groups. The other imidazolium precursors are functionalized by various groups, namely amide (**20**), alcohol (**23**) and nitrogen containing heterocyles (quinoline for **25** and bipyridine for **28**).



Scheme 3. Synthesis of proligand **25**, silver(I) complex **26** and gold(I) complex **27**.

2.1.2. Synthesis and characterization of mono-and dinuclear silver(I) and gold(I) bis(carbene) complexes

All silver complexes were prepared by deprotonation of mono- or bis(imidazolium) salts **16**, **17**, **20**, **23**, **25** and **28**, with the mild



Scheme 4. Synthesis of proligand **28**, silver(I) complex **29** and gold(I) complex **30**.

base Ag_2O in a suitable dry solvent (dichloromethane or methanol) at room temperature, except for the dinuclear complexes **18** and **19** (50 °C) (Schemes 2–4). The complexation of the bis(imidazolium) precursors **16** and **17** to silver(I) in equimolar amounts led probably to coordination polymers of the general formula $[Ag^{I}_2\{bis(NHC)\}_2][AgBr_2]_2$ (formula proposed from the elemental analysis) with interactions between the cationic silver ions and the $AgBr_2$ anions, which are totally insoluble; an ion exchange with silver(I) nitrate was thus necessary to obtain **18** and **19** as soluble compounds in protic solvents.

For complexes **21**, **24**, **26** and **29**, a stoichiometry of one half equivalent of Ag_2O for one equivalent of ligand precursor was used. Silver(I) complexes **24**, **26** and **29** were obtained as the desired mononuclear cationic species $[Ag^{I}(NHC)_2][X]$ (X = Cl, Br or PF_6). However, under our reaction conditions (ratio silver salt/ligand precursor), we obtained the neutral dinuclear dimeric complex **21**, involving amido-NHC ligands. Ghosh and coll. previously reported a series of structurally diverse gold and silver complexes ranging from mononuclear ionic $(NHC\text{-}amide)_2M^{+}Cl^{-}$ type complexes to large 12-membered macrometallacyclic $(NHC\text{-}amido)_2M_2$ species, prepared by varying the *N*-substituent of the amido-functionalized side arm from a *t*-butyl to a 2,6-di*i*-propylphenyl moiety; they obtained neutral dinuclear complexes when starting from stoichiometric quantities of Ag_2O and imidazolium salts whereas cationic mononuclear complexes were isolated with a ratio of one half Ag_2O for one proligand. In their case, the authors explain the observed structural diversity as a direct consequence of the variation of the *N*-substituent of the amido-functionalized side arm, despite a common synthetic route [26]. Thus, we can state that it is difficult to predict the coordination patterns of Ag(I) compounds involving NHC(s) ligands. The solid state structure depends on the substituents of the imidazolium ring, the anion, the solvent and the temperature used and is not directly correlated to the stoichiometry of the starting materials.

The formation of the silver(I) complexes was confirmed by the absence of the ^{1}H NMR resonance of the acidic imidazolium H_2, and for complex **21**, the lack of the amide protons at 11.14 ppm. The ^{13}C NMR spectra exhibit a resonance signal located between 180.3 and 182.7 ppm, ascribed to the carbenic carbon atoms C_2, which is consistent with the reported values for other cationic mononuclear and dinuclear silver(I) NHC complexes [1]. Except for compound **24**, in which the carbene resonance appears as one doublet with a coupling constant of $J^{107,109}_{Ag-C}$ = 180.1 Hz, all the other complexes show only a singlet for the C_2 carbons. There is no evidence of coordination of the metal by a nitrogen atom of the quinoline or bipyridine moieties in complexes **26** and **29**. The FAB-MS spectra showed mass peaks corresponding to the cations of dinuclear species $[Ag^{I}_2L_2NO_3]^{+}$ for **18** and **19**, mononuclear species $[Ag^{I}L_2]^{+}$ for **24**, **26** and **29** and also a mononuclear fragment $[Ag^{I}(NHC\text{-}amide)_2]^{+}$ for **21**, the parent peak $[Ag^{I}_2(NHC\text{-}amido)_2 + H^{+}]^{+}$ being not detected by FAB spectrometry. The elemental analyses are consistent with the proposed structures depicted in Schemes 2–4.

The three cationic mononuclear gold(I) complexes **22**, **27** and **30** were prepared according a classical way, from the *N*-functionalized imidazolium salts **20**, **25** or **28** and one half equivalent of $Au(SMe_2)Cl$ with sodium acetate as a mild base in hot *N*,*N*-dimethylformamide (120 °C). NMR spectroscopy unequivocally demonstrates the formation of the gold(I) complexes; the ^{13}C spectra show the resonance for the carbene carbon atoms at 188.1, 182.9 and 183.2 ppm, for **22**, **27** and **30**, respectively. These values are in the range of reported values for Au^{I}–NHC complexes having C–Au–X (X = halide) or C–Au–C motifs [1]. It has to be pointed out that in contrast to the silver(I) complex **21**, the corresponding gold(I) analog **22** is a cationic mononuclear species as expected from the reaction conditions with the presence of the amide protons at 11.58 ppm in the ^{1}H NMR spectrum. The elemental analysis of the gold(I) complexes correspond to the general $[AuL_2][X]$ formula (X = Cl for **22**, PF_6 for **27** and Br for **30**) and the FAB-MS spectra exhibit the classical peak corresponding to the cationic fragment $[M - X]^{+}$.

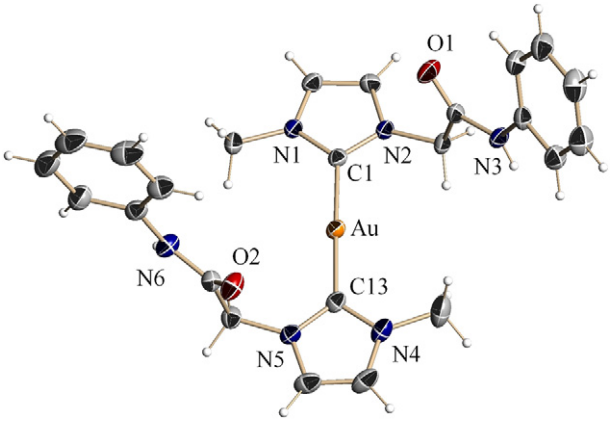


Fig. 1. Molecular structure of **22** (50% probability level for the thermal ellipsoids). Only the cationic part is shown for clarity. Selected bond lengths [Å] and angles [°]: Au C1 2.016(2), Au C13 2.018(2), C1 Au C13 178.40(5).

2.1.3. *Crystal structures of **22**, **26**, **27** and **30***

Crystals of the gold complex **22** involving the amide functionalized carbene ligands were obtained by slow evaporation of dichloromethane. The asymmetric unit contains one gold cation coordinated by two of the ligands, one chlorine anion and one molecule of water. The cationic part (see Fig. 1) shows a classical linear coordination of the gold atom. It is remarkable, that the amide functions of the ligands are involved in hydrogen bonds formed either with the anions or with water molecules (see Fig. 2). The ligands form hydrogen bonds exclusively by the NH function and not at all by the C=O. In this network each chlorine atom is linked to one NH and to two water molecules, whereas each water molecule is linked to two chlorine atoms and one NH from the ligands.

Crystals of the silver complex **26** were obtained by slow evaporation from an acetonitrile solution. As shown in Fig. 3 the silver cation is coordinated in a linear manner by two carbene units.

In the case of **26** neither classical hydrogen-bonds nor short metal–metal interactions are present in the crystalline solid state. Short contacts between C–H groups and fluorine atoms with C···F

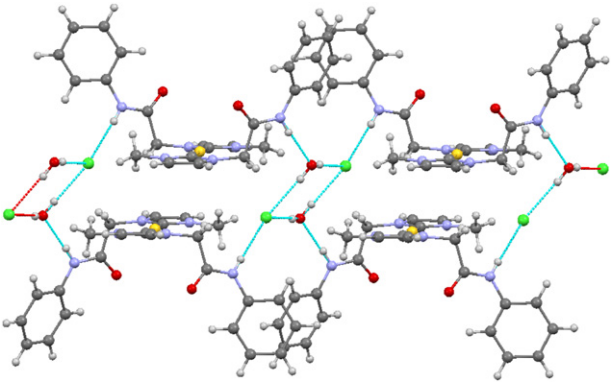


Fig. 2. Hydrogen-bond-network in the solid state of **22**.

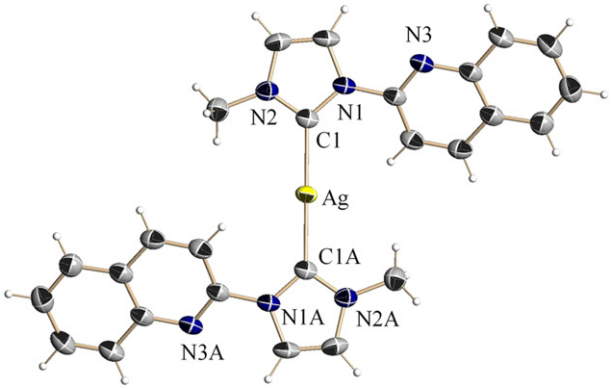


Fig. 3. Molecular structure of **26** (50% probability level for the thermal ellipsoids). Only the cationic part is shown for clarity. Selected bond lengths [Å] and angles [°]: Ag C1 2.093(2), C1 Au C1A 180.0.

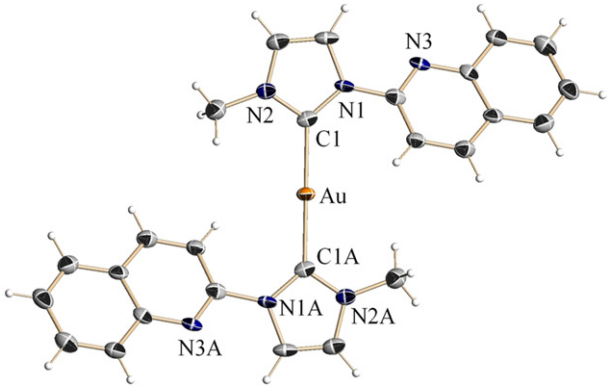


Fig. 5. Solid state structure of **27** (50% probability level for the thermal ellipsoids). Only the cationic part is shown for clarity. Selected bond lengths [Å] and angles [°]: Au C1 2.035(6), C1 Au C1A 179.999(1).

distances between 3.015 and 3.058 Å can be observed. As shown in Fig. 4 the system is arranged in layers of cations and anions, respectively.

Crystals of the corresponding gold complex **27** were also obtained by slow evaporation from an acetonitrile solution. As in the case of the silver complex **26** the gold cation is coordinated in a linear manner by two NHC ligands (see Fig. 5) and neither classical hydrogen-bonds nor short metal–metal interactions are present in the crystalline solid state of **27**. Very short contacts between C–H groups and fluorine atoms with C···F distances between 2.992 and 3.012 Å can be observed. As shown in Fig. 6 the system is arranged in layers of cations and anions, respectively.

In the case of compound **30** slow evaporation from a chloroform solution gave suitable crystals for an X-ray analysis. As in the other compounds, the metal cation is linearly coordinated by two ligands. Apart from the cationic unit shown in Fig. 7, only the bromine anion is present in the asymmetric unit.

In complexes **22**, **26** and **27** a trans-arrangement of the functionalized side arms related to the coordination-axis can be observed. Only in **30** the functionalized side arms are on the same side, an arrangement which is not supported by interactions between the two bipyridyl groups. The perturbation of the C–Au–C angle, 174.9° instead of 180°, may be due to steric repulsion resulting from this arrangement.

As in the case of complexes **26** and **27** neither classical hydrogen-bonds nor short metal–metal interactions are present in the crystalline solid state of **30**. Very short contacts between C–H groups and bromine anions with C···Br distances between 2.811 and 3.030 Å, reflecting the higher charge density of bromine in comparison to PF_6, can be observed. As shown in Fig. 8 also this system is arranged in layers of cations and anions, respectively.

2.2. Antimalarial and antifungal activities

The results of the preliminary antiplasmodial tests of the first series of molecules (Scheme 1) are presented in the Table 1. Fifteen molecules, involving bis(imidazolium) salts and their corresponding dinuclear silver(I), gold(I) and gold(III) complexes were tested against the CQ-resistant *Plasmodium falciparum* strain FcM29-Cameroon (IC_{50} of CQ = 445 nM) for doses ranging from 0.05 μg/mL to 50 μg/mL. All the $[Ag^{I}$–bis(NHC)$]_2$ complexes **5**–**8** showed a very promising antiplasmodial activity with IC_{50} values from 1.2 to 2.6 μM when tested immediately. Interestingly, these molecules appeared stable since they kept their antiplasmodial activity with similar IC_{50} values (1.5–2 μM) after storage several days at 4 °C. Three compounds, namely the $[Au^{I}$–bis(NHC)$]_2$ complex **11** and two of the three $[Au^{III}$–bis(NHC)$]_2$ complexes **14**–**15** showed moderate activity with IC_{50} values between 9 and 15 μM. We can note the weak stability of compound **11** which totally lost its activity after conservation of the stock solution in DMSO at 4 °C for 6 days. Finally, eight species including the bis(imidazolium) salts (**1**–**4**) and most of the $[Au^{I}$–bis(NHC)$]_2$ complexes (**9**, **10** and **12**) and one gold(III) complex (**13**) showed no activity with IC_{50} values ranging from 30 to higher than 110 μM.

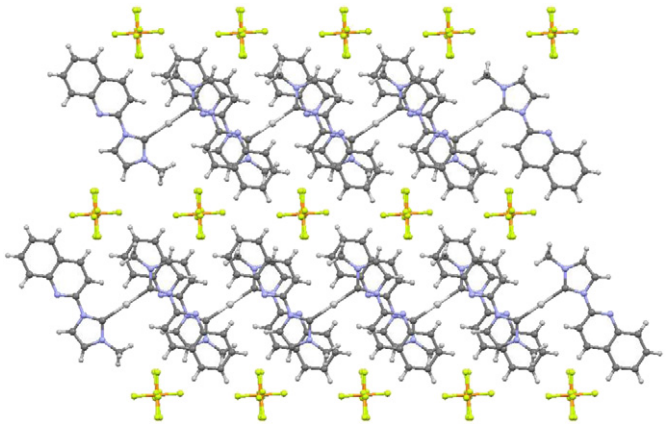


Fig. 4. Crystal packing of **26** with a view in direction of *b*.

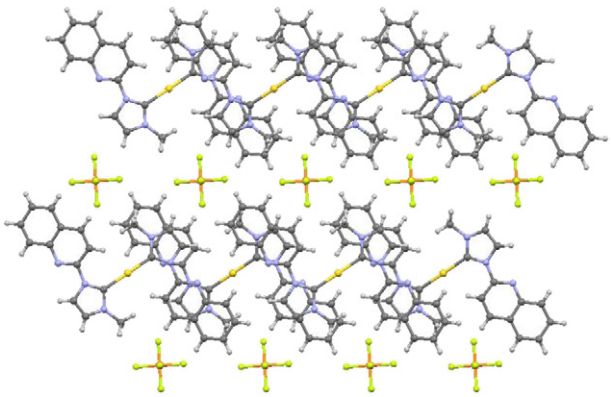


Fig. 6. Crystal packing of **27** with a view in direction of *b*.

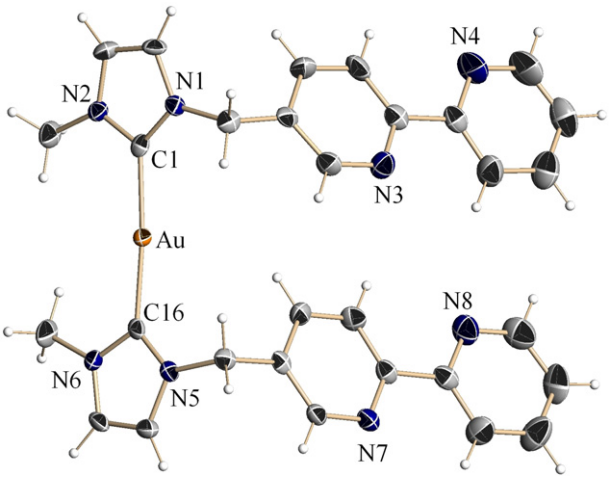


Fig. 7. Solid state structure of **30** (50% probability level for the thermal ellipsoids). Only the cationic part is shown for clarity. Selected bond lengths [Å] and angles [°]: Au C1 2.020(4), Au C16 2.019(4), C1 Au C16 174.91(17).

The antifungal assay showed that the same **5**–**8** compounds with the best antiplasmodial activity presented also the best antifungal activities against both *Candida* strains with MIC ranging from 0.8 μM to 2 μM whereas all the eleven other compounds tested here had no activity against neither *Candida albicans* nor *Candida glabrata* (MIC > 50 μg/ml corresponding to values ranging from >27 to >107 μM, according to molar weight of compounds). The MIC values of compounds **5**–**8** appeared as very interesting since in the same range values that the antifungal control drug 5-Fluorocytosine (MIC: 0.7–1.6 μM). Unfortunately these four best antiplasmodial and antifungal silver(I) compounds **5**–**8** also showed strong hemolytic properties on the parasite culture and even at the weakest doses tested (0.5 μg/ml). The absence of specific activity of the compounds **5**–**8** against pathogens is representative of a toxic activity of these compounds. This is confirmed by the high hemolysis reported on parasite cultures with these same four compounds. These preliminary results do not allow the extraction of structure–activity relations based on the key groups of the *N*-functionalized bis(NHC) used in this first series of compounds. Nevertheless, some interesting experimental facts deserve further attention and a general trend appears from the IC_{50} values. First, the control tests with the proligands are indicative of the role of the metallic centre in the biological activity of the resulting complexes. For the dinuclear gold complexes, the oxidation state of the gold cation seems to have an effect because the higher-valent gold(III) complexes **14**–**15** exhibit a moderate but better activity than their gold(I) counterparts, which are ineffective. These results are probably due to the high stability of the dinuclear gold(I) complexes, which could be attributed both to the protective cage effect of the ligands and to aurophilic interactions. Finally, the highest activity for this first series was found for the dinuclear silver(I) complexes **5**–**8** but they are also toxic. The examples of silver compounds used against malaria are scarce. In 2003, the *in vitro* antimalarial activity against a CQ-sensitive FCR-3 strain was evaluated for a series of metalloporphyrins, among which a silver(I) protoporphyrin IX with a moderate IC_{50} value of 15.5 μM (IC_{50} = 0.022 μM for CQ) was described [27]. In an old patent, it was reported that silver sulfadiazine in doses of 1.050 mg/kg/day, when administrated orally or subcutaneously, once a day for five days cured CF-1 mice of *Plasmodium berghei* with a minimal toxicity but at higher doses death occurred within 24 h [28]. Even if the issue of silver toxicity is still under debate, silver is known to be one of the least toxic metals [2].

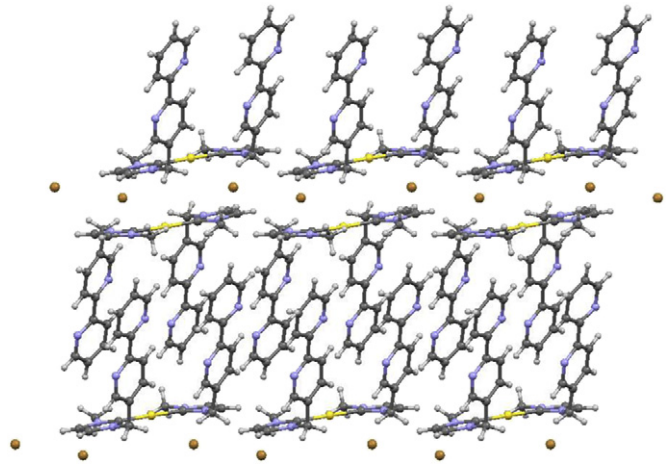


Fig. 8. Crystal packing of **30** with a view in direction of *a*.

According to these first results, pharmaco-modulations of our compounds were carried out in order to increase the antiplasmodial activity and to decrease their cytotoxic effect by decreasing hemolysis. First, we have prepared dinuclear [Ag^{I}–bis(NHC)]$_2$ complexes bearing simple methyl groups on the nitrogens of the rings (**18**, **19**), in order to see if the functionalized arms in the first series could have an influence on the antiplasmodial activity. Furthermore, we were interested in the synthesis of various mononuclear $Ag^{I}(NHC)_2$ and $Au^{I}(NHC)_2$ which have a less protected environment around the metal and in particular a better delivery of the metallic cation. The results of the antiplasmodial tests of the second series of molecules are presented in the Table 2. Nine new silver(I) and gold(I) complexes were tested against the same CQ-resistant strain FcM29-Cameroon for doses ranging from 0.01 μg/mL to 10 μg/mL. The antimalarial activity of this second series of complexes was highly improved with IC_{50} values ranging from 0.33 to 4.1 μM except for the complex Au^{I}–bis(NHC) (**22**) showing no activity even at 15 μM. The molecules with the best antiplasmodial activity are the two dinuclear [Ag^{I}–bis(NHC)]$_2$ complexes **18** and **19** with IC_{50} values of 1.2 and 1.5 μM and the two mononuclear Au^{I}–bis(NHC) **27** and **30** with IC_{50} values of 1.1 and 0.33 μM, respectively.

Except the silver(I) complex **24**, which decomposes in the solid state after six months and more rapidly in solution, all the other Ag^{I} and Au^{I} complexes had the same activity whatever the test in extemporaneous conditions or after conservation of the stock solution in DMSO at 4 °C for 4–6 days. Very interestingly, this new series of complexes had lost their hemolytic properties. No hemolysis was observed for any compound of this new series and even at the highest doses tested (10 μg/ml). Concerning the dinuclear silver(I) compounds, it seems that the aliphatic side arms have no real influence on the activity because the IC_{50} values are very close, the main difference being the hemolysis for the first series, when compared to the second one, which cannot be explained at this stage of the work. The neutral complex **21**, in which the silver cations are coordinated by one NHC and one amido group and the mononuclear silver(I) complexes **24** and **26** are slightly less effective than **18** and **19**. This could be attributed to the fact that first, two NHCs stabilize the silver better than a heteroditopic NHC-amido ligand and second, that bis(NHC) ligands can stabilize dinuclear species with metallophilic interactions, thereby controlling a slower release of the Ag^{+} in the culture medium. Taking into account that silver is more labile than gold

Table 1
Antiplasmodial and antifungal activities screening of the first series of compounds **1**–**15**.

Compounds	Antiplasmodial activity[a]				Antifungal activity[b]		Hemolysis[c]
	Extempore		Stored		*C. albicans*	*C. glabrata*	
	μg/ml	μM	μg/ml	μM	μM	μM	
1	>50	>92	>50	>110	>92	>92	–
2	>50	>107	>50	>107	>107	>107	–
3	>50	>89	>50	>89	>89	>89	–
4	25	43	30	52	>86	>86	–
5	1.1	1.2	2	2.1	1.6	1	+
6	1.8	1.8	2	2	1.3	0.8	+
7	1.7	1.4	2	1.7	2	1.5	+
8	1.8	1.5	1.8	1.5	1.4	1.5	+
9	>50	>39	>50	>39	>39	>39	–
10	>50	>39	>50	>39	>39	>39	–
11	22	15	>50	>34	>34	>34	–
12	>50	>33	>50	>33	>33	>33	–
13	48	30	>50	>32	>32	>32	–
14	17	9	25	14	>28	>28	–
15	23	13	33	18	>27	>27	–
5-Fluorocytosine[d]	ND				1.6	0.7	ND
Artemisinin[d]	$3.10^{-3} \pm 0.01$ (10.10^{-3} μM)				ND		–
CQ[d]	$230.10^{-3} \pm 0.4$ (445.10^{-3} μM)				ND		–

[a] IC_{50} values against the *P. falciparum* strain FcM29 are reported in μg/ml and in μM. Data represented the IC_{50} values obtained in one preliminary experiment and for each one both kinds of conservation (extemporaneously or after conservation of the stock solution in DMSO at 4 °C for 4–6 days).
[b] Antifungal activity on both *C. albicans* and *C. glabrata* are expressed in μM.
[c] +Hemolysis on *P. falciparum* parasite culture. –No hemolysis on *P. falciparum* parasite culture.
[d] 5-Fluorocytosine and, artemisinin and CQ were routinely tested as antifungal and antiplasmodial control drugs, respectively.

(silver–NHC complexes are often used for transmetallation reactions), a good protection of the silver cation is needed to ensure the slow release of silver ions, in order to avoid AgCl precipitation in the culture medium. This is in strong contrast with the gold(I) compounds, for which a drastic increase of the parasite growth inhibition is obtained for the mononuclear complexes **27** and **30**, both containing nitrogen heterocyclic functionalized arms, namely quinoline and bipyridine moieties. It has to be noted that CQ contains a quinoline entity. The improved potency of **27** and **30** against the CQ-resistant strain FcM29-Cameroon confirmed our hypothesis of a better drug-delivery in less protected gold containing species. It should be mentioned that the potential of gold derivatives as drugs against parasitic diseases has been very little explored until very recently. Messori and coll. have demonstrated that the anti-arthritic drug auranofin displays very pronounced antiplasmodial effects, which are probably mediated by severe oxidative stress originating from *Plasmodium falciparum* thioredoxin reductase (TrxR) inhibition [29]. Interestingly, several Au^{I}–NHC complexes tested as anticancer agents inhibit the activity of TrxR, leading to cell death through a mitochondrial apoptotic pathway [10]. Gold(I) complexes involving functionalized alkynes have shown low *in vitro* activity against the malaria parasite strains 3D7 (CQ-sensitive, IC_{50} of CQ = 0.01 μM) and K1 (CQ-resistant, IC_{50} of CQ = 0.3 μM) with IC_{50} values between 7.2 and 23 μM [30]. The same authors described a series of mono-and dinuclear gold(I) phosphine complexes containing mono-anionic seleno-and thiosemicarbazones ligands; the IC_{50} results showed that the sulfur containing compounds exhibit activity similar to CQ against the 3D7 strain (IC_{50} = 7.06 and 10.7 nM, IC_{50} of CQ = 8.84 nM) [31]. Similarly, Chibale and coll. reported gold(I) and gold(III) thiosemicarbazone complexes, which exhibit moderate *in vitro* activity against two strains D10 (CQ-sensitive, IC_{50} of CQ = 0.0173 μM) and W2 (CQ-resistant, IC_{50} of CQ = 0.095 μM) with IC_{50} ranging from 1.36 to 6.92 μM for the gold(I) compounds and IC_{50} ranging from 3.04 to >20 μM for the gold(III) complexes [32]. Very recently, Cronje and coll. reported preliminary *in vitro* antimalarial activities of *N*-heterocyclic ylideneamine gold(I) against *P. falciparum*; the coordination of ancillary phosphine or NHC ligands to these complexes results in better activities with an IC_{50} value of 5.1 μg/ml for the NHC derivative [33].

Table 2
Antiplasmodial activity of the second series of Ag^{I} and Au^{I} complexes **18**, **19**, **21**, **22**, **24**, **26**, **27**, **29** and **30** against the *P. falciparum* strain FcM29-Cameroon.

Compounds	Antiplasmodial activity				Hemolysis[c]
	Extempore[a]		Stored[a]		
	μg/ml[b]	μM[b]	μg/ml[b]	μM[b]	
18	0.87 ± 0.6	1.2	1.05 ± 0.2	1.4	–
19	1.08 ± 0.3	1.5	1.9 ± 0.8	2.6	–
21	2.5 ± 0.5	4.0	3.8 ± 0.5	6.1	–
22	>10	>15	>10	>15	–
24	2.7 ± 0.6	3.9	–	–	–
26	2.8 ± 0.9	4.1	3.1 ± 0.8	4.6	–
27	0.9 ± 0.1	1.1	1.4 ± 0.6	1.8	–
29	1.45 ± 0.8	2.8	2.2 ± 0.9	3.3	–
30	0.25 ± 0.03	0.33	0.37 ± 0.2	0.49	–
Artemisinin[d]	$4.10^{-3} \pm 0.2$ (14.10^{-3} μM)				–
CQ[d]	$260.10^{-3} \pm 0.5$ (503.10^{-3} μM)				–

[a] For each compound, data were obtained both extemporaneously or after conservation of the stock solution in DMSO at 4 °C for 4–6 days.
[b] IC_{50} values are reported in μg/ml and in μM. Data represented the mean of 4–8 independent experiments.
[c] +Hemolysis on *P. falciparum* parasite culture. –No hemolysis on *P. falciparum* parasite culture.
[d] Both antiplasmodial control drugs artemisinin and CQ were routinely tested.

3. Conclusion

In summary, a series of mono-and dinuclear silver(I) and mononuclear gold(I) complexes based on NHC ligands were prepared and characterized. The *in vitro* anti-malaria activity of two different families of compounds has been assessed against the chloroquine-resistant *P. falciparum* strain FcM29-Cameroon. For the first tested family, including *N*-functionalized bis(imidazolium) proligands and their corresponding silver(I), gold(I) and gold(III) complexes, the dinuclear silver(I) compounds have shown the best antiplasmodial and antifungal activities but also strong hemolytic

properties. From these preliminary results, pharmaco-modulations have been realized to obtain the second series of silver(I) and gold(I) complexes. Their antiplasmodial activity was significantly increased and no hemolysis was observed. It seems that the presence of nitrogen containing heterocycles in the series of mononuclear gold(I) complexes has a great importance on the antiplasmodial activity, probably related to the lipophilicity, the basicity, and structural features of these compounds. The activities of metal NHCs as antimalarials have not been extensively explored and this work is also among the first to investigate silver complexes as potential antimalarials. Further studies for the design of metallodrugs combining gold(I)-NHC covalently bound to well-known antimalarial drugs or analogs are ongoing in our laboratories.

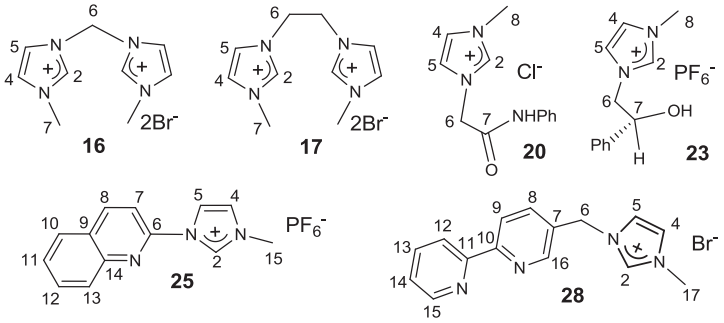
4. Experimental section

4.1. General information

Unless otherwise stated, all reactions were performed in air. CH_2Cl_2, CH_3CN and DMF were dried over 4 Å molecular sieves. All other reagents were used as received from commercial suppliers. All reactions involving silver compounds were performed with the exclusion of light. 2-Chloro-*N*-phenylacetamide [23], 2-(1*H*-imidazol-1-yl)-1-phenylethanol [18], 5-(bromomethyl)-2,2′-bipyridine [24,25] were prepared according literature procedures. ^{1}H (250, 300 or 400 MHz) and ^{13}C spectra (63 or 75 MHz) were recorded at 298 K on Bruker ARX250, Bruker DPX300 or Bruker AV400 spectrometers in D_2O, $CDCl_3$, CD_3CN, CD_3OD or DMSO-d_6 as solvents. Elemental analyses were carried out by the "Service de Microanalyse du Laboratoire de Chimie de Coordination" (Toulouse). Mass spectrometry analysis were performed on a Nermag R1010 (FAB$^+$/*meta-nitrobenzylalcohol* (MNBA)) spectrometer, by the "Service de Spectrométrie de Masse de Chimie UPS-CNRS" (Toulouse).

4.1.1. Preparation of imidazolium salts

The following picture describes the numbering of H (^{1}H NMR) and C (^{13}C NMR). These notations are used in the following experimental section.



4.1.1.1. 3-Methyl-1-[(3-methyl-1H-imidazol-3-ium-1-yl)methyl]-1H-imidazol-3-ium dibromide ***(16)***. An excess of dibromomethane (3.5 mL, 50.2 mmol) was added to an acetonitrile solution (10 mL) of 1-methylimidazole (2 mL, 25.1 mmol) and the mixture was stirred at 80 °C for 4 h. After cooling to room temperature, the desired white solid was filtered off and dried under vacuum (1.443 g, 34% yield). Anal. Calcd. For $C_9H_{14}N_4Br_2$: C, 31.98; H, 4.17; N, 16.57. Found: C, 31.96; H, 3.92; N, 16.56. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm) = 9.52 (s, 2H, H_2), 8.07 (s, 2H, H_5), 7.83 (bs, 2H, H_4), 6.76 (s, 2H, H_6), 3.92 (s, 6H, H_7). ^{13}C NMR (63 MHz, DMSO-d_6) δ (ppm) = 138.0 (2C, C_2), 124.8 (2C, C_4), 122.1 (2C, C_5), 58.4 (1C, C_6), 36.7 (2C, C_7). MS (FAB$^+$): *m/z* 257 [M − Br$^-$]$^+$.

4.1.1.2. 3-Methyl-1-[(3-methyl-1H-imidazol-3-ium-1-yl)ethyl]-1H-imidazol-3-ium dibromide ***(17)***. Dibromoethane (1.08 mL, 12.6 mmol) was added to an acetonitrile solution (10 mL) of 1-methylimidazole (2 mL, 25.1 mmol) and the mixture was stirred at 80 °C for 4 h. After cooling to room temperature, the desired white solid was filtered off and dried under vacuum (1.864 g, 42% yield). Anal. Calcd. For $C_{10}H_{16}N_4Br_2$: C, 34.11; H, 4.58; N, 15.91. Found: C, 33.99; H, 4.37; N, 15.82. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm) = 9.21 (s, 2H, H_2), 7.76 (s, 2H, H_5), 7.71 (bs, 2H, H_4), 4.75 (s, 4H, H_6), 3.87 (s, 6H, H_7). ^{13}C NMR (63 MHz, D_2O) δ (ppm) = 136.8 (2C, C_2), 124.8 (2C, C_4), 122.4 (2C, C_5), 49.0 (2C, C_6), 36.5 (2C, C_7). MS (FAB$^+$): *m/z* 271 [M − Br$^-$]$^+$.

4.1.1.3. 3-Methyl-1-[(phenylcarbamoyl)methyl]-1H-imidazol-3-ium chloride ***(20)***. 1-Methylimidazole (0.735 mL, 9.22 mmol) and 2-chloro-*N*-phenylacetamide (0.782 g, 4.61 mmol) were stirred in acetonitrile (20 mL) at 80 °C for 12 h. After cooling to room temperature, the desired white solid was filtered off and dried under vacuum (0.889 g, 77% yield). Anal. Calcd. For $C_{12}H_{14}N_3OCl$: C, 57.26; H, 5.61; N, 16.69. Found: C, 57.28; H, 5.71; N, 16.71. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm) = 11.14 (bs, 1H, N*H*), 9.22 (s, 1H, H_2), 7.88 (d, 2H, H_4, H_5), 7.66 (d, 2H, H_{Ar}, ^3J = 7.7 Hz), 7.34 (t, 2H, H_{Ar}, ^3J = 7.6 Hz), 7.09 (t, 1H, H_{Ar}, ^3J = 7.1 Hz), 5.31 (s, 2H, H_6), 3.93 (s, 3H, H_8). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) = 164.2 (1C, C_7), 139.0 (1C, C_2), 138.4 (1C, C_{Ar}), 129.3.2 (2C, C_{Ar}), 124.3 (1C, C_5), 124.2 (1C, C_{Ar}), 123.5 (1C, C_4), 119.6 (2C, C_{Ar}), 51.7 (1C, C_6), 36.3 (1C, C_8). MS (FAB$^+$): *m/z* 216 [M − Cl$^-$]$^+$.

4.1.1.4. 1-[(2R)-Hydroxy-2-phenylethyl]-3-methyl-1H-imidazol-3-ium hexafluorophosphate ***(23)***. An excess of iodomethane (0.288 mL, 4.64 mmol) was added to an acetonitrile solution (40 mL) of (*1R*)-2-(1*H*-imidazol-1-yl)-1-phenylethanol (0.437 g, 2.32 mmol) and the mixture was stirred at 75 °C overnight. After cooling to room temperature, the desired white solid was filtered off and dried under vacuum (0.529 g, 69% yield). The solid was dissolved in a mixture of H_2O–MeOH (20 mL–10 mL) and the subsequent addition of an aqueous solution (10 mL) of NH_4PF_6 (0.261 g, 1.60 mmol) afforded a white precipitate, which was collected by filtration and dried under vacuum (0.522 g, 94% yield). Anal. Calcd. For $C_{12}H_{15}N_2OPF_6$: C, 41.39; H, 4.34; N, 8.04. Found: C, 41.18; H, 4.19; N, 8.11. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm) = 9.07 (s, 1H, H_2), 7.67 (bs, 2H, H_4, H_5), 7.39 (m, 5H, H_{Ar}), 6.00 (bs, 1H, O*H*), 4.94 (m 1H, H_7), 4.41 (d, 1H, H_6, ^2J = 14.7 Hz), 4.23 (dd, 1H, H_6, ^2J = 13.2 Hz, ^3J = 9.1 Hz), 3.87 (s, 3H, H_8). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 141.7 (1C, C_{Ar}), 137.4 (1C, C_2), 128.8 (3C, C_{Ar}), 128.3 (1C, C_5), 126.4 (2C, C_{Ar}), 123.5 (1C, C_4), 71.1 (1C, C_7), 56.1 (1C, C_6), 36.3 (1C, C_8). MS (FAB$^+$): *m/z* 155 [M − PF_6^-]$^+$.

*4.1.1.5. 3-Methyl-1-(quinolin-2-yl)-1H-imidazol-3-ium hexafluorophosphate **(25)**.* 1-Methylimidazole (4 mL, 47.3 mmol) and 2-chloroquinoline (2.23 g, 13.63 mmol) were stirred in toluene (20 mL) at 130 °C for 24 h. After cooling to room temperature, the desired brown solid was filtered and dried under vacuum (1.106 g, 28% yield). The solid was dissolved in a mixture of H_2O–MeOH (5 mL–10 mL) and the subsequent addition of an aqueous solution (10 mL) of NH_4PF_6 (0.746 g, 4.574 mmol) afforded a white precipitate, which was collected by filtration and dried under vacuum (1.30 g, 96% yield). Anal. Calcd. For $C_{13}H_{12}N_3PF_6$: C, 43.96; H, 3.41; N, 11.83. Found: C, 43.91; H, 3.28; N, 11.79. ^{1}H NMR (300 MHz, CD_3CN) δ (ppm) = 9.47 (s, 1H, H_2), 8.69 (d, 1H, H_8, ^3J = 8.8 Hz), 8.28 (*pseudo*-t, 1H, H_5, $^{3,4}J$ = 1.7 Hz), 8.12 (d, 2H, H_{10}, H_{13}, ^3J = 9.2 Hz), 7.96 (t, 1H, H_{12}, ^3J = 7.0 Hz), 7.86 (d, 1H, H_7, ^3J = 8.8 Hz), 7.80 (t, 1H, H_{11}, ^3J = 7.9 Hz), 7.62 (*pseudo*-t, 1H, H_4, $^{3,4}J$ = 1.7 Hz), 4.03 (s, 3H, H_{15}). ^{13}C NMR (63 MHz, CD_3CN) δ (ppm) = 146.1 (1C, C_6), 145.0 (1C, C_{14}), 141.4 (1C, C_8), 134.9 (1C, C_2), 131.8 (1C, C_{12}), 128.5 (1C, C_{13}), 128.2 (1C, C_{10}), 128.1 (2C, C_9, C_{11}), 124.9 (1C, C_7), 119.4 (1C, C_5), 111.8 (1C, C_4), 36.6 (1C, C_{15}). MS (FAB$^+$): *m/z* 210 [M − PF_6^-]$^+$.

*4.1.1.6. 3-Methyl-1-{[6-(pyridin-2-yl)pyridin-3-yl]methyl}-1H-imidazol-3-ium bromide **(28)**.* 1-Methylimidazole (2.02 mL, 25.32 mmol) and 5-(bromomethyl)-2,2′-bipyridine (1.802 g, 7.234 mmol) were stirred in toluene (40 mL) at 130 °C for 24 h. After cooling to room temperature, the desired beige precipitate was filtered off and dried under vacuum (1.385 g, 58% yield). Anal. Calcd. For $C_{15}H_{15}N_4Br0.3H_2O$: C, 53.52; H, 4.67; N, 16.64. Found: C, 53.40; H, 4.62; N, 16.61. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 10.76 (s, 1H, H_2), 8.85 (d, 1H, H_{16}, ^3J = 1.8 Hz), 8.67 (m, 1H, H_{15}), 8.42 (d, 1H, H_{12}, ^3J = 8.3 Hz), 8.35 (d, 1H, H_9, ^3J = 7.8 Hz), 8.15 (dd, 1H, H_{13}, ^3J = 8.3 Hz, ^4J = 2.3 Hz), 7.83 (td, 1H, H_8, ^3J = 7.8 Hz, ^4J = 1.8 Hz), 7.50 (*pseudo*-t, 1H, H_5, $^{3,4}J$ = 1.7 Hz), 7.37 (*pseudo*-t, 1H, H_4, $^{3,4}J$ = 1.7 Hz), 7.34 (m, 1H, H_{14}), 5.85 (s, 2H, H_6), 4.08 (s, 3H, H_{17}). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 156.7 (1C, C_{10}), 155.0 (1C, C_{11}), 149.5 (1C, C_{15}), 149.2 (1C, C_{16}), 137.8 (1C, C_2), 137.0 (2C, C_8, C_{13}), 129.4 (1C, C_7), 124.1 (1C, C_{14}), 122.5 (1C, C_5), 121.2 (1C, C_4), 111.8 (2C, C_9, C_{12}), 50.1 (1C, C_6), 36.8 (1C, C_{17}). MS (FAB$^+$): *m/z* 251 [M − Br^-]$^+$.

4.1.2. Preparation of silver(I) complexes

*4.1.2.1. Complex **18**.* A Schlenk tube was charged with silver(I) oxide (0.206 g, 0.888 mmol), **16** (0.338 g, 0.888 mmol) and dry MeOH (5 mL). The resulting mixture was stirred under a nitrogen atmosphere at 50 °C for 8 h. A white solid precipitated. After filtration, the solid was suspended in a mixture of H_2O–MeOH (15 mL–15 mL) and $Ag(NO_3)$ (0.115 g, 0.339 mmol) was added. After stirring for 2 h at room temperature, the solution was filtered through a pad of celite and the solvents were evaporated under vacuum, to afford the desired white complex (0.196 g, 73% yield). Anal. Calcd. For $C_{18}H_{24}N_{10}Ag_2O_6$: C, 31.23; H, 3.49; N, 20.24. Found: C, 31.16; H, 3.62; N, 20.46. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) = 7.89 (s, 4H, H_5), 7.55 (s, 4H, H_4), 6.67 (bs, 4H, H_6), 3.86 (s, 12H, H_7). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) = 181.6 (4C, C_2), 124.8 (4C, C_5), 122.2 (4C, C_4), 63.5 (2C, C_6), 39.0 (4C, C_7). MS (FAB$^+$): *m/z* 630 [M − NO_3^-]$^+$, 567 [M − 2NO_3^- − H$^+$]$^+$.

*4.1.2.2. Complex **19**.* A Schlenk tube was charged with silver(I) oxide (0.197 g, 0.852 mmol), **17** (0.300 g, 0.852 mmol) and dry MeOH (5 mL). The resulting mixture was stirred under a nitrogen atmosphere at 50 °C for 8 h. A white solid precipitated. After filtration, the solid was suspended in a mixture of H_2O–MeOH (20 mL–20 mL) and $Ag(NO_3)$ (0.119 g, 0.703 mmol) was added. After stirring for 2 h at room temperature, the solution was filtered through a pad of celite and the solvents were evaporated under vacuum, to afford the desired white complex (0.248 g, 81% yield). Anal. Calcd. For $C_{20}H_{28}N_8Ag_2N_2O_6$: C, 33.35; H, 3.92; N, 19.45. Found: C, 33.57; H, 3.75; N, 19.06. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm) = 7.40 (s, 4H, H_5), 7.36 (s, 4H, H_4), 4.68 (s, 8H, H_6), 3.77 (s, 12H, H_7). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) = 180.3 (4C, C_2), 123.5 (4C, C_5), 122.9 (4C, C_4), 51.4 (4C, C_6), 38.8 (4C, C_7). MS (FAB$^+$): *m/z* 658 [M − NO_3^-]$^+$.

*4.1.2.3. Complex **21**.* A Schlenk tube was charged with silver(I) oxide (0.046 g, 0.199 mmol), **20** (0.100 g, 0.399 mmol) and dry CH_2Cl_2 (25 mL). The resulting mixture was stirred under a nitrogen atmosphere at room temperature overnight. A solid precipitated. The solvent was evaporated under vacuum, to afford the desired white complex (0.115 g, 89% yield). Anal. Calcd. For $C_{24}H_{24}N_6O_2Ag_2$: C, 44.74; H, 3.75; N, 13.05. Found: C, 44.57; H, 3.57; N, 13.48. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.79 (d, 4H, H_{Ar}, ^3J = 7.5 Hz), 7.29 (d, 2H, H_5, $^{3,4}J$ = 1.7 Hz), 7.24 (t, 4H, H_{Ar}, ^3J = 7.9 Hz), 7.09 (t, 2H, H_{Ar}, ^3J = 7.4 Hz), 6.93 (d, 2H, H_4, $^{3,4}J$ = 1.7 Hz), 5.32 (s, 4H, H_6), 3.74 (s, 6H, H_8). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 182.4 (2C, C_2), 165.4 (2C, C_7), 138.4 (2C, C_{Ar}), 128.7 (4C, C_{Ar}), 124.1 (2C, C_5), 123.4 (2C, C_4), 121.5 (2C, C_{Ar}), 119.9 (4C, C_{Ar}), 54.7 (2C, C_6), 38.7 (2C, C_8). MS (FAB$^+$): *m/z* 537 [Ag(LH)$_2$]$^+$.

*4.1.2.4. Complex **24**.* A Schlenk tube was charged with silver(I) oxide (0.100 g, 0.431 mmol), **23** (0.300 g, 0.862 mmol) and dry MeOH (15 mL). A solution of NaOH (1 N, 5 mL) in H_2O was added and the resulting mixture was stirred under a nitrogen atmosphere at room temperature overnight. After filtration through a pad of celite, the solvents were evaporated to dryness, and the brown solid obtained was dried under vacuum (0.252 g, 89% yield). Anal. Calcd. For $C_{24}H_{28}N_4O_2AgPF_6$: C, 43.85; H, 4.29; N, 8.52. Found: C, 43.57; H, 4.64; N, 8.35. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) = 7.42–7.24 (m, 14H, H_4, H_5, H_{Ar}), 4.94 (d, 2H, H_7, ^3J = 4.5 Hz), 4.29 (m, 4H, H_6), 3.78 (s, 6H, H_8). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) = 182.4 (2C, C_2, $J^{107,109}_{Ag-C}$ = 180.1 Hz), 147.3 (2C, C_{Ar}), 127.9 (4C, C_{Ar}), 126.8 (4C, C_{Ar}), 126.5 (2C, C_{Ar}), 122.7 (2C, C_5), 121.5 (2C, C_4), 78.8 (2C, C_7), 60.8 (2C, C_6), 38.4 (2C, C_8). MS (FAB$^+$): *m/z* 511 [M − PF_6^-]$^+$.

*4.1.2.5. Complex **26**.* A Schlenk tube was charged with silver(I) oxide (0.098 g, 0.422 mmol), **25** (0.300 g, 0.845 mmol), dry CH_2Cl_2 (25 mL) and NH_4PF_6 (0.035 mg, 0.211 mmol). A solution of NaOH (1 N, 15 mL) in H_2O was added and the resulting mixture was stirred under a nitrogen atmosphere at room temperature for 2 h. After filtration through a pad of celite, the organic layer was extracted, dried over Na_2SO_4, filtered and evaporated to dryness, affording a pink solid (0.279 g, 98% yield). Crystals suitable for X-ray diffraction analysis were obtained by slow evaporation from an acetonitrile solution of **26**. Anal. Calcd. For $C_{26}H_{22}N_6AgPF_6$: C, 46.52; H, 3.30; N, 12.52. Found: C, 46.57; H, 3.64; N, 12.35. ^{1}H NMR (400 MHz, CD_3CN) δ (ppm) = 8.22 (d, 2H, H_8, ^3J = 8.8 Hz), 7.99 (d, 2H, H_5, ^3J = 1.7 Hz), 7.84 (d, 2H, H_7, ^3J = 8.8 Hz), 7.71 (m, 2H, H_{11}), 7.50 (d, 2H, H_4, ^3J = 1.7 Hz), 7.44–7.40 (m, 6H, H_{10}, H_{12}, H_{13}), 4.05 (s, 6H, H_{15}). ^{13}C NMR (63 MHz, DMSO-d_6) δ (ppm) = 182.7 (2C, C_2), 149.6 (2C, C_6), 145.6 (2C, C_{14}), 140.8. (2C, C_8), 131.2 (2C, C_{12}), 128.2 (2C, C_{13}), 127.7 (2C, C_{10}), 127.5 (2C, C_9), 127.1 (2C, C_{11}), 125.1 (2C, C_7), 120.6 (2C, C_5), 113.9 (2C, C_4), 39.9 (2C, C_{15}). MS (FAB$^+$): *m/z* 525 [M − PF_6^-]$^+$, 316 [LAg]$^+$.

*4.1.2.6. Complex **29**.* A Schlenk tube was charged with silver(I) oxide (0.071 g, 0.305 mmol), **28** (0.202 g, 0.610 mmol) and dry CH_2Cl_2 (8 mL). The resulting mixture was stirred under a nitrogen atmosphere at room temperature overnight. After filtration through a pad of celite, the solvent was evaporated under vacuum, to afford the desired beige complex (0.181 g, 86% yield). Anal. Calcd. For $C_{30}H_{28}N_8AgBr$: C, 52.34; H, 4.10; N, 16.28. Found: C, 52.57; H, 4.34; N, 16.35. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 8.70 (m, 2H, H_{15}), 8.62 (m, 2H, H_{16}), 8.42 (m, 4H, H_9, H_{12}), 7.85 (m, 2H, H_{13}), 7.74

(td, 2H, H_8, $^3J = 7.8$ Hz, $^4J = 1.8$ Hz), 7.35 (m, 2H, H_{14}), 7.04 (d, 2H, H_5, $^3J = 1.7$ Hz), 6.97 (d, 2H, H_4, $^3J = 1.7$ Hz), 5.41 (s, 4H, H_6), 3.90 (s, 6H, H_{17}). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) = 181.0 (2C, C_2), 155.5 (2C, C_{10}), 155.2 (2C, C_{11}), 149.8 (2C, C_{15}), 149.1 (2C, C_{16}), 137.8 (2C, C_{13}), 137.1 (2C, C_8), 133.8 (2C, C_7), 124.8 (2C, C_{14}), 123.9 (2C, C_5), 122.6 (2C, C_4), 121.0 (2C, C_{12}), 120.9 (2C, C_9), 51.8 (2C, C_6), 38.7 (2C, C_{17}). MS (FAB$^+$): *m/z* 607 [M − Br$^-$]$^+$, 357 [AgL]$^+$.

4.1.3. Preparation of gold(I) complexes

*4.1.3.1. Complex **22**.* Under a nitrogen atmosphere, sodium acetate (0.079 g, 0.958 mmol) was added to a mixture of **20** (0.200 g, 0.798 mmol) and Au(SMe$_2$)Cl (0.118 g, 0.399 mmol) in dry DMF (4 mL) at 100 °C. The mixture was then heated to 120 °C and this temperature was maintained for 2 h. After cooling to room temperature, a white solid precipitated. After filtration, complex **22** was dried under vacuum (0.171 g, 65% yield). Crystals suitable for X-ray diffraction analysis were obtained by slow evaporation from a dichloromethane solution of **22**. Anal. Calcd. For $C_{24}H_{26}N_6O_2AuCl$: C, 43.48; H, 3.95; N, 12.68. Found: C, 43.39; H, 3.88; N, 12.61. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 11.58 (s, 2H, N*H*), 7.85 (d, 4H, H_{Ar}, $^3J = 8.0$ Hz), 7.26–7.22 (m, 6H, H_5, H_{Ar}), 7.05 (t, 2H, H_{Ar}, $^3J = 7.94$ Hz), 6.99 (d, 2H, H_4, $^3J = 1.1$ Hz), 5.47 (s, 4H, H_6), 3.87 (s, 6H, H_8). ^{13}C NMR (75 MHz, $CDCl_3$–CD_3OD/0.4–0.6) δ (ppm) = 188.1 (2C, C_2), 168.1 (2C, C_7), 140.4 (2C, C_{Ar}), 131.5 (4C, C_{Ar}), 127.3 (2C, C_5), 126.0 (2C, C_{Ar}), 125.1 (2C, C_4), 122.5 (4C, C_{Ar}), 56.1 (2C, C_6), 40.3 (2C, C_8). MS (FAB$^+$): *m/z* 627 [M − Cl$^-$]$^+$.

*4.1.3.2. Complex **27**.* Under a nitrogen atmosphere, sodium acetate (0.106 g, 1.29 mmol) was added to a mixture of **25** (0.204 g, 0.575 mmol) and Au(SMe$_2$)Cl (0.169 g, 0.575 mmol) in dry DMF (6 mL) at 100 °C. The mixture was then heated to 120 °C and this temperature was maintained for 3 h. After cooling to room temperature, the solvent was evaporated and the white solid obtained was dried under vacuum (0.218 g, 85% yield). Crystals suitable for X-ray diffraction analysis were obtained by slow evaporation from an acetonitrile solution of **27**. Anal. Calcd. For $C_{26}H_{22}N_6AuPF_6$: C, 41.07; H, 2.92; N, 11.05. Found: C, 40.80; H, 2.79; N, 11.24. ^{1}H NMR (250 MHz, CD_3CN) δ (ppm) = 8.14 (d, 2H, H_8, $^3J = 8.7$ Hz), 8.06 (d, 2H, H_9, $^3J = 8.7$ Hz), 7.95 (d, 2H, H_5, $^3J = 2.0$ Hz), 7.82 (m, 4H, H_7, H_{12}), 7.70 (td, 2H, H_{11}, $^3J = 7.1$ Hz, $^4J = 1.3$ Hz), 7.56 (td, 2H, H_{10}, $^3J = 6.9$ Hz, $^4J = 1.2$ Hz), 7.46 (d, 2H, H_4, $^3J = 2.0$ Hz), 4.04 (s, 6H, H_{15}). ^{13}C NMR (75 MHz, CD_3CN-DMSO-d_6/0.9–0.1) δ (ppm) = 182.9 (2C, C_2), 149.3 (2C, C_6), 146.2 (2C, C_{14}), 139.5 (4C, C_8), 130.9 (2C, C_{12}), 128.3 (2C, C_{13}), 127.9 (2C, C_{10}), 127.7 (2C, C_9), 127.4 (2C, C_{11}), 123.9 (2C, C_7), 121.0 (2C, C_5), 115.7 (2C, C_4), 38.7 (2C, C_{15}). MS (FAB$^+$): *m/z* 615 [M − PF_6^-].

*4.1.3.3. Complex **30**.* Under a nitrogen atmosphere, sodium acetate (0.089 g, 0.453 mmol) was added to a mixture of **28** (0.300 g, 0.906 mmol) and Au(SMe$_2$)Cl (0.133 g, 0.453 mmol) in dry DMF (6 mL) at 100 °C. The mixture was then heated to 120 °C and this temperature was maintained for 2 h. After cooling to room temperature, a white solid precipitated. After filtration, complex **30** was dried under vacuum (0.218 g, 64% yield). Crystals suitable for X-ray diffraction analysis were obtained by slow evaporation from a chloroform solution of **30**. Anal. Calcd. For $C_{30}H_{28}N_8AuBr$: C, 46.35; H, 3.63; N, 14.41. Found: C, 46.69; H, 3.23; N, 14.42. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 8.64 (m, 4H, H_{15}, H_{16}), 8.34 (d, 2H, H_{12}, $^3J = 8.2$ Hz), 8.30 (d, 2H, H_9, $^3J = 8.0$ Hz), 7.82–7.75 (m, 4H, H_8, H_{13}), 7.34 (d, 2H, H_5, $^3J = 1.8$ Hz), 7.32–7.28 (m, 2H, H_{14}), 7.18 (d, 2H, H_4, $^3J = 1.8$ Hz), 5.60 (s, 4H, H_6), 3.90 (s, 6H, H_{17}). ^{13}C NMR (75 MHz, CD_3CN-DMSO-d_6) δ (ppm) = 183.2 (2C, C_2), 154.7 (2C, C_{10}), 154.0 (2C, C_{11}), 148.8 (2C, C_{15}), 147.6 (2C, C_{16}), 137.4 (2C, C_{13}), 136.1 (2C, C_8), 132.7 (2C, C_7), 124.2 (2C, C_{14}), 123.4 (2C, C_5), 122.1 (2C, C_4), 120.7 (2C, C_{12}), 120.5 (2C, C_9), 50.6 (2C, C_6), 37.3 (2C, C_{17}). MS (FAB$^+$): *m/z* 697 [M − Br$^-$].

*4.1.4. Crystallographic data for **22**, **26**, **27** and **30***

All data were collected at low temperature using oil-coated shock-cooled crystals on a Bruker-AXS APEX II diffractometer with MoKα radiation ($\lambda = 0.71073$ Å). The structures were solved by direct methods [34] and all non-hydrogen atoms were refined anisotropically using the least-squares method on F^2 [35]. The data are summarized in Table 3. CCDC-884301 (**22**), CCDC-884302 (2**6**),

Table 3
Crystal data. Parameters for data collection and structure refinements of **22**, **26**, **27** and **30**.

Complex	**22**	**26**	**27**	**30**
Empirical formula	$C_{24}H_{28}AuClN_6O_3$	$C_{52}H_{44}Ag_2F_{12}N_{12}P_2$	$C_{26}H_{22}AuF_6N_6P$	$C_{30}H_{28}AuBrN_8$
Formula weight	680.94	1342.67	760.43	777.48
T/K	173(2)	173(2)	173(2)	173(2)
Crystal system	Triclinic	Monoclinic	Monoclinic	Triclinic
Space group	*P*$\bar{1}$	*C2/c*	*C2/c*	*P*$\bar{1}$
a/Å	10.3446(7)	25.400(11)	25.459(4)	9.9307(4)
b/Å	11.4826(8)	7.2786(4)	7.242(2)	10.7149(5)
c/Å	11.6938(8)	14.2569(6)	14.154(3)	14.8413(8)
α/°	96.711(3)	–	–	93.436(2)
β/°	107.935(3)	103.078(2)	102.591(5)	102.189(2)
γ/°	96.914(3)	–	–	111.718(2)
V/Å^3	1294.4(2)	2591.7(2)	2546.9(7)	1417.4(2)
Z	2	2	4	2
ρ_{calcd}/Mg m^{-3}	1.747	1.721	1.983	1.822
μ/mm^{-1}	5.823	0.912	5.913	6.335
F(000)	668	1344	1472	756
Crystal size/mm^3	0.40 × 0.30 × 0.20	0.40 × 0.20 × 0.20	0.40 × 0.10 × 0.10	0.40 × 0.10 × 0.10
θ Range for data collection/°	5.12–30.51	5.11–28.28	5.16–26.37	2.33–26.48
Reflections collected	52295	15221	9367	42250
Independent reflections	7865	3196	2579	5829
R (int)	0.0242	0.0261	0.0792	0.0774
Restraints/parameters	0/334	0/202	0/185	0/363
Goodness-of-fit on F^2	1.027	1.018	0.931	1.000
R1 ($I > 2\sigma(I)$)	0.0156	0.0270	0.0324	0.0320
wR2 ($I > 2\sigma(I)$)	0.0354	0.0588	0.0517	0.0459
R1 (all data)	0.0188	0.0422	0.0747	0.0565
wR2 (all data)	0.0364	0.0636	0.0599	0.0506
$\Delta\rho_{max}$/e Å^{-3}	0.821	0.302	1.009	0.783

CCDC-884303 (**27**) and CCDC-884304 (**30**) contain the Supplementary crystallographic data. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

4.2. Antiplasmodial tests

The CQ-resistant FcM29-Cameroon strain of *Plasmodium falciparum* was cultured continuously according to the modified Trager and Jensen's method [36]. The antiplasmodial activity was evaluated by the radioactive micro-method described by Desjardins et al. [37] with modifications [36]b. The antiplasmodial tests of molecules were performed from one to eight different times in triplicate in 96-well culture plates (TPP) on synchronous cultures at ring stages.

The antiplasmodial drug controls, CQ and artemisinin, were routinely tested on this strain and the IC_{50} values were ranged from 445 nM to 503 nM and from 10 nM to 14 nM, respectively. CQ was dissolved directly in the culture medium. The compounds and artemisinin were dissolved in dimethyl sulfoxide (DMSO, Sigma) (stock solution: 1 mg/mL) and further diluted in the culture medium so that the final DMSO concentration never exceeded 1%. For each experiment, we verified that this 1% concentration of DMSO did not affect parasite growth. Parasite growth was estimated by [^{3}H]-hypoxanthine incorporation (Perkin Elmer). The control parasite culture (RPMI with 5% of human serum alone or with 1% DMSO) was referred to as 100% growth.

The ranges of concentrations of the molecules tested were for the first preliminary series from 0.05 μg/mL to 50 μg/mL and for the second series from 0.01 μg/mL to 10 μg/mL. Each solution was tested both in extemporaneous conditions and after a conservation of the stock solution in DMSO at 4 °C for 2–8 days in order to evaluate the stability of the biological activity of the compounds tested.

4.3. Antifungal tests

The *C. albicans* American Type Culture Collection 90028 (ATCC 90028) and *Candida glabrata* (#0500024220) were used to evaluate the antifungal activity of the 15 first molecules. A micro-dilution method adapted from the Clinical and Laboratory Standards Institute (NCCLS-M27A) [38] was used. The culture medium used was RPMI 1640 (Sigma, France) with 2 mM L-Glutamine and 0.165 M morpholinopropanesulfonic acid (MOPS) buffer (Sigma, France). Prior to testing, the isolate was sub-cultured on Sabouraud dextrose agar (Bio-Mérieux, France) and was incubated at 35 °C during 48 h. Inoculates were prepared by suspending the yeast in 1.0 mL of a sterile saline solution and adjusting to a final concentration of 2.5×10^6 yeast cells/mL. Each well of 96-well plates received 100 μL of this suspension and 100 μL of various concentrations of drugs (with the maximum concentration at 50 μg/mL), and the plates incubated for 48 h at 35 °C. All antifungal assays were only performed from drug solutions diluted extemporaneously. The results were determined using a spectrophotometer (Elx 808, Vetra Microplate Reader, Avantec) at a wavelength of 550 nm. The MIC (Minimal Inhibitory Concentration) values were determined graphically by plotting concentrations of tested drugs versus percentage of yeast inhibition. For comparison, the control 5-fluorocytosine was routinely tested as a reference in this test and gave MIC similar to the ATCC reference values.

4.4. Hemolysis assays

The evaluation of hemolysis induced by compounds was performed on parasite cultures after 48 h-incubation time at 37 °C. This test permitted to detect the hemolytic compounds (with concentration tested from 0.01 μg/mL to 50 μg/mL) that weaken red blood cells and induce erythrocyte membrane bursting with subsequent release of hemoglobin. Water known to involve the lysis of red blood cells was used as positive control and RPMI alone was used as negative control. Hemolysis was considered as positive when the supernatant of the parasite culture was brown and the erythrocyte pellet totally liquid.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.11.038.

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