Novel Phosphine Sulphide Gold(I) Complexes: Topoisomerase I Inhibitors and Antiproliferative Agents.

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This work describes the synthesis of gold(I) complexes of phosphine sulphides. The formation of these new derivatives has been confirmed by X-ray crystallography. The coordination of gold(I) with the sulphur atom of phosphine sulphides favors the inhibition of topoisomerase I, as well as a high cytotoxicity of the gold(I) complexed compounds against cancer line A549 with IC_{50} values in the nanomolar range and with IC_{50} values below 5 μM against the SKOV3 cell line. It should be noted that the cytotoxicities observed for the new gold(I) complexes are higher than those observed for phosphine sulphide ligands before binding to gold. Furthermore, the results presented also indicate that the presence of a nitrogenated heterocycle, such as tetrahydroquinoline or quinoline, is also necessary for the TopI inhibition to be maintained. In addition, no toxicity was observed when the non-cancerous lung fibroblast cell line (MRC5) was treated with the new phosphine sulphide gold(I) complexes prepared.

Introduction

Cancer is a complex disease which is still considered the second leading cause of death worldwide.[1] Despite much has been investigated in the development of organic compounds that could stop the progression of the disease, the searching for alternatives to current drugs it is still necessary. In this sense, it could be indicated that many of actual drugs could increase their activity with the complementarity of metals in their structure.

Many transition metal complexes have been designed for DNA studies and in several biomedical research areas.[2] Likewise, metal complexes received interest in the recent decades for the development of new chemotherapeutical drugs after the success of cisplatin (I Figure 1),[3] carboplatin, paraplatin and oxaliplatin, as established drugs in therapy of various solid cancers.[4] However, these compounds showed several side effects such as nephrotoxicity or neurotoxicity and they were restricted.[5] Due to these limitations, different researches are focused on the use of other metal for the preparation of therapeutics agents. In this sense, gold complexes have received increasing attention in the last decade as antiproliferatives.

Gold compounds[6] are important traditional medicines and their use, known as chrysotherapy or aurotherapy, has been widely applied for the treatment of diseases such as cancer,[7] arthritis[8] and microbial infection.[9] After the FDA approval of auranofin (II, Figure 1), these compounds have been used for the therapy of rheumatoid arthritis, for the study of their anticancer activity towards cancer cell lines[10] and for clinical and preclinical investigations of parasitic[11] and microbial infections.[12]

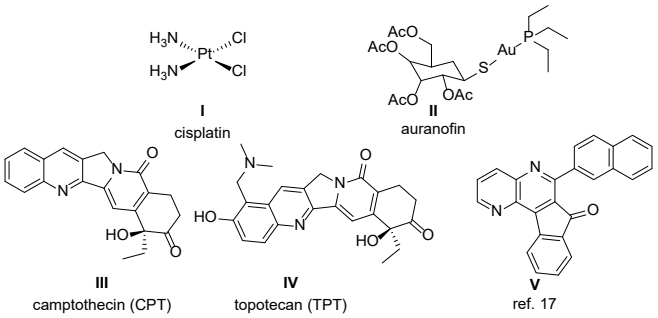

Figure 1. Structures of cisplatin (**I**), auranofin (**II**); CPT (**III**); TPT (**IV**) and optical DNA biosensor for human TopI inhibitors (**V**).[17]

TopI, an overexpressed enzyme in cancer cells, reduces superhelical stress as well as other topological consequences generated in the separation of DNA strands in metabolic processes such as replication, transcription and recombination. As a result, TopI could represent an effective target for cancer therapy,[13] and many TopI inhibitors are the basis of some chemotherapeutical combinations widely used in a broad spectrum of tumors.[14] Among inhibitors, the camptothecin (CPT, **III**, Figure 1) and its derivatives (CPTs), as topotecan (TPT, **IV**, Figure 1), are some of the most studied drugs as inhibitors

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of TopI and are commonly used in the treatment of colon, ovarian and small-cell lung cancers.[15] However, these heterocycles have different disadvantages, such as, for example, serious side effects and structural instability due to the opening of the lactone ring at physiological pH.[16]

From a chemical structural point of view, both camptothecin and its derivatives have an almost planar structure because they are formed by fused heterocycles. Moreover, with regard to the proposed inhibition mechanism of TopI, the presence of polycyclic heterocycles seems to be relevant in the effectiveness of the antiproliferative activity, probably due to an improvement in π-π* stacking interactions with the DNA base pairs.[14] Regarding polycyclic heterocycles, recently, our group has reported the synthesis of an optical DNA biosensor (**V**, Figure 1) for human TopI inhibitors,[17] as well as fused heterocycles with good inhibitory activity against human TopI (hTopI) enzyme and antiproliferative [18] or antileishmanial response.[19]

Several gold(III) macrocycles[20] and gold(III) complexes[21] have been reported as human TopI inhibitors. However, very few examples of gold(I) complexes of this type of inhibitors with anticancer activity have been reported, such as acridine thiourea gold(I) complexes[22] (**VI**, Figure 2), a new posphole gold(I) complex derived from auronafin[23] (**VII**, Figure 2) with antiglioma activity, as well as phosphane gold(I) dithiocarbamates and carbonimidothioates[24] (**VIII** and **IX**, Figure 2).

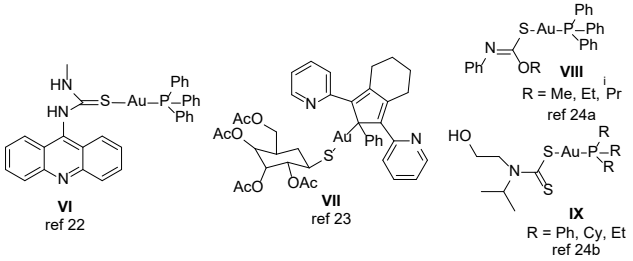


Figure 2. Structure of gold(I) complexes **VI**-**IX** TopI inhibitors.[22-24]

For the development of new molecules with enhanced anticancer properties,[25] one efficient strategy may be combining the properties of gold with other biologically active molecules (molecular hybridization).[26] Based on this approach, recently we designed and prepared new hybrid molecules as anticancer agents[27] and for the treatment of infectious diseases.[19]

With these considerations in mind, we believed that the development of new hybrid molecules[28] based on gold(I) complexes derived from quinolinyl- and tetrahydroquinolinyl-phosphine sulphides[18a,19a] may be privileged scaffolds for pharmaceuticals[29] and may improve the antiproliferative cytotoxic properties with respect to other biologically active structures.

Gold(I) is a soft Lewis Acid with great affinity for soft ligands (i.e. thiolates, phosphines, phosphine sulphides...), and the formation of lineal two-coordination complexes may be important for medicinal purposes.[30]

The first thiophosphinoyl-gold(I) complex (**X**, Figure 3) was prepared in 1965,[31a] and in the last years new phosphine sulphide-Au(I) complexes (i.e. **XI**, Figure 3)[31b-i] have been described. However, the biological activity of these complexes has not been reported. Only recently, the anti-inflammatory and antitumor activity of the first cycloaurated phosphine sulphide-gold(III) complexes (**XII**, Figure 3) have been reported.[32]

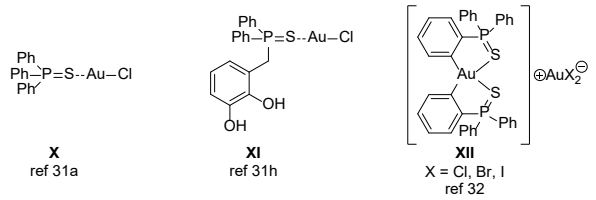


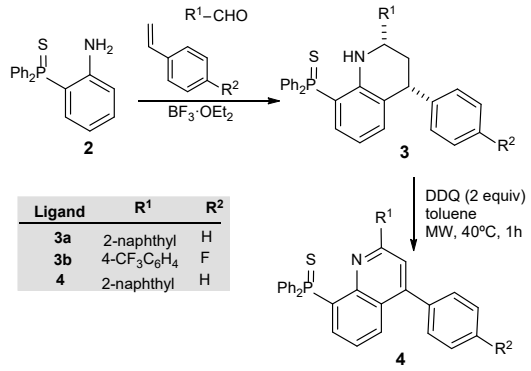
Figure 3. Structures of thiophosphinoyl-gold(I) complexes (**X**, **XI**) and cycloauraeted gold(III) complexes (**XII**).[31-32]

With this background, we designed new hybrid biologically active molecules based on gold(I) complexes containing triphenylphosphine sulphide, (2-aminophenyl)diphenylphosphine sulphide, quinolinyl and tetrahydroquinolinylphosphine sulphides and we report, for the first time, the preparation of new simple and functionalized phosphine sulphide gold(I) complexes, as well as, the TopI inhibition and the antiproliferative behaviour of this new family of compounds, that may be privileged scaffolds for pharmaceuticals.

Results and Discussion

Gold(I) complexes of phosphine sulphide tetrahydroquinolines and quinolines.

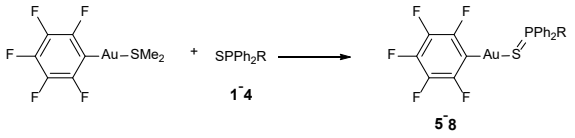
The synthesis and characterization data of functionalized tetrahydroquinoline (THQ) and quinoline (QUIN) containing phosphine sulphide group by multicomponent Povarov type [4+2]-cycloaddition reaction were communicated by us earlier (Scheme 1).[18a,19a,33]



Ligand	R^1	R^2
3a	2-naphthyl	H
3b	4-$CF_3C_6H_4$	F
4	2-naphthyl	H

Scheme 1. Syntheses of ligands **3** and **4**.[18a,19a,33]

The reaction of $[Au(C_6F_5)(SMe_2)]$ with an equimolar amount of the corresponding sulphide ligand $SPPh_2R$ (Scheme 2), such as triphenyl- **1** (R = Ph), 2-anilinodiphenyl- **2** (R = 2-$NH_2C_6H_4$), tetrahydroquinolinyldiphenyl- **3** and quinolinyldiphenylphosphine sulphide **4** in CH_2Cl_2 afforded the desired gold(I) complexes $[Au(C_6F_5)(SPPh_2R)]$ **5**-**8** (see Chart 1).



Scheme 2. Syntheses of gold(I) complexes **5**-**8**.

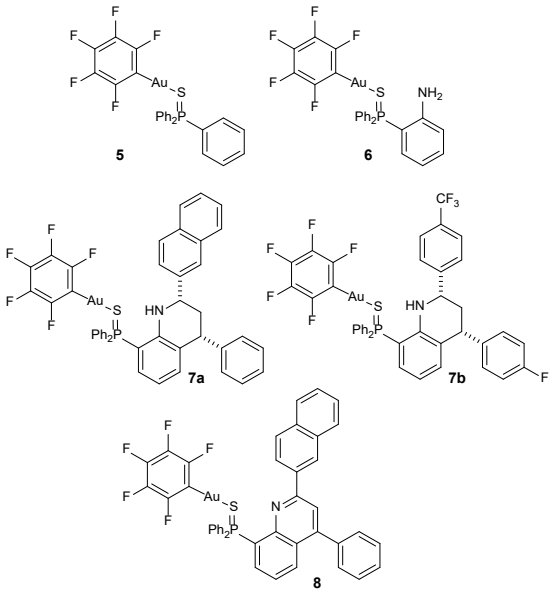


Chart 1. Gold(I) complexes **5**-**8**.

The new synthesized compounds were characterized by multinuclear NMR spectroscopy and some of them by X-ray crystallography. Their ^1H and $^{31}P\{^1H\}$ spectra were similar to those of the free ligands and their ^{19}F NMR spectra show the characteristic resonances expected for a free rotating C_6F_5 group linked to Au^I (three signals in a 2:1:2 ratio corresponding to an AA'MXX'spin system). In addition, for complex **7b** signals due to CF_3 and C_6H_4F were identified. The molecular structures of **5**, **6**, and **8** are shown in Figure 3 confirming the proposed assignments by spectroscopic means. Selected bond lengths and angles are given in Table 1. All the complexes show a nearly linear geometry for gold, with C–Au–S angles close to the ideal value of 180°. Other X-ray structures with pentafluorophenyl-gold(I) and phosphine sulphide are very scarce and only the binuclear complex $[Au(C_6F_5)\{SPPh_2CH[Au(C_6F_5)]PPh_2Me\}]$ has been reported.[34] The Au–C distances are within the range found for related pentafluorophenyl-gold(I) complexes.[34,35] The Au–S bond distances in the range 2.3039(16)–2.3148(13) Å are greater than those found for $[AuX(SPPh_3)]$ (X = Cl, 2.256 Å; Br, 2.286 Å)[36] showing the higher *trans*-influence of C_6F_5 group compared to Cl or Br. The shortest Au–Au intermolecular distance between two metallic centers (> 5.3 Å) clearly excludes any Au···Au interaction. However, the crystal packing of the complexes shows intermolecular π-stacking of the C_6F_5 rings (see Supporting Information) and these interactions define dimers in the solid-state structure.

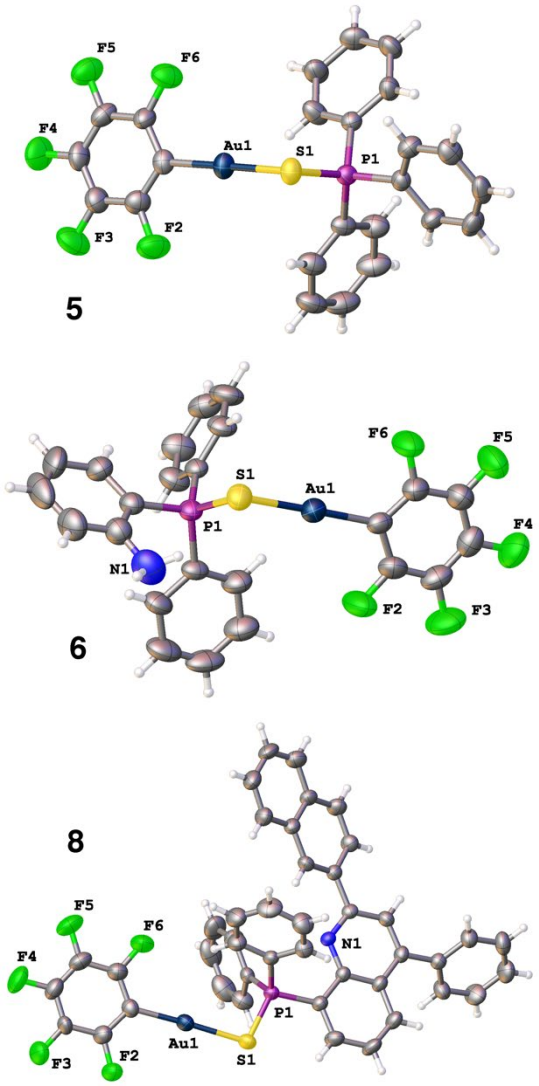


Figure 3. ORTEP view of molecular structure of complexes **5, 6** and **8** with thermal ellipsoids at 50% probability.

Table 1. Selected interatomic distances (Å) and angles (°) for complexes **5**, **6** and **8**.

Compound	Au–C	Au–S	P–S	C–Au–S	Au–S–P
5	2.025(6)	2.3105(16)	2.011(2)	177.88(15)	102.77(9)
6	2.018(6)	2.3039(16)	2.0282(19)	177.71(14)	104.26(7)
8	2.030(5)	2.3148(13)	2.0175(16)	177.56(14)	101.21(7)

Inhibition of Topoisomerase I.

Once the gold complexes were prepared from the phosphine sulphides, the inhibitory effect of the complexes on topoisomerase type I (TopI) was investigated by using a conventional supercoiled plasmid relaxation assay (Figure 4). In this assay, the inhibitory activity of human TopI enzymatic activity by the ligands **1**-**4** and the corresponding gold(I) complexes **5**-**8** is determined by observing the conversion of the supercoiled plasmid DNA to relaxed DNA (see Table S1 in the ESI). In these experiments, compound samples were mixed with enzyme followed by addition of supercoiled plasmid DNA substrate and continued incubation for increasing time periods (15 s, 1 min and 3 min). The reaction was finished by the addition of SDS. DNA relaxation products were then resolved by gel electrophoresis in 1% agarose gel and visualized by gel red staining. Camptothecin (CPT) was used as a positive control (Figure 4).

TopI inhibitory activity of new gold(I) complexes was tested by detecting the conversion of supercoiled DNA (Sc, Figure 4) to its relaxed form in the presence of the purified enzyme and expressed in quantitative fashion relative to the TopI inhibitory activity of camptothecin (Table S1). As shown in Figure 4, TopI relaxes supercoiled DNA even in the presence of DMSO (Figure 4, lanes 1-3), and camptothecin (CPT) inhibits the relaxation, as indicated by the increased intensity of the band corresponding to the supercoiled DNA (Figure 4, lanes 4-5). However, it can be seen for CPT that the inhibitory activity over TopI released at longer enzymatic reaction time (3 min), as electrophoresis gel indicated only some topoisomers are seen at the top while no supercoiled DNA (Sc, Figure 4, lane 6) was detected.

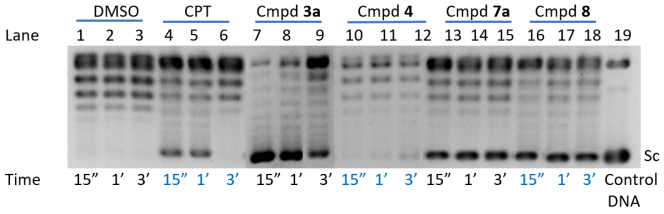


Figure 4. Inhibition of TopI activity along the time (15", 1' and 3') by compounds **3a**, **4**, **7a** and **8** and camptothecin at 100 μM: lanes 1-3, DNA+TopI+DMSO; lanes 4-6, DNA+TopI+camptothecin 100μM; lanes 7-9, DNA+TopI+**3a** 100μM; lanes 10-12, DNA+TopI+**4** 100μM; lanes 13-15, DNA+TopI+**7a** 100μM; lanes 16-18, DNA+TopI+**8** 100μM; lane 19, control DNA. Reaction samples were mixed with enzyme at 37ºC before adding the supercoiled DNA substrate and separated by electrophoresis on a 1% agarose gel, and then stained with gel red, and photographed under UV light as described in the TopI mediated DNA relaxation assay.

In previous works, we published the inhibitory TopI activity of the phosphine sulphide THQs **3** and QUIN **4**, on both human and leishmania TopI.[18a,19a] It was observed that some derivatives presented good inhibition of TopI, as THQ **3a** or THQ **3b** (Table S1, entries 4 and 5, respectively). However, more aromatized quinolinylphosphine sulphide **4** presented no inhibitory activity at any tested enzymatic reaction time (Table S1, entry 6).

So, we started studying the effect of the precursor compounds such as, triphenylphosphine sulphide **1** and (2-aminophenyl)diphenylphosphine sulphide **2** and we could observe no inhibition of TopI enzymatic activity (Table S1, entries 2 and 3, respectively).

Afterwards, we studied the effect as TopI inhibitors of the new gold(I) complexes, such as triphenylphosphine sulphide gold(I) complex **5**, (2-aminophenyl)diphenylphosphine sulphide gold(I) complex **6**, tetrahydroquinolinylphosphine sulphide gold complexes **7** and quinolinyldiphenylphosphine sulphide gold(I) complex **8** (Table S1).

Based on the results of the relaxation assays, the gold(I) complexes of tetrahydroquinolinylphosphine sulphides **7** (Table S1, entries 9 and 10) maintain the excellent inhibitory activity showed by the naked ligands **3** (without gold). Noteworthy, gold(I) complex of quinolinylphosphine sulphide **8** (Table S1, entry 11) showed excellent inhibitory activity of TopI, enhancing the lack of activity showed by the ligand **4**. Moreover, the inhibitory activity can be compared with that presented by the natural TopI inhibitor, CPT, being observed slightly better activity at short enzymatic reaction times (15 s and 1 min) and even higher inhibition response than CPT at longer reaction time (3 min).

In this regard, we decide to study the effect of gold metal on the inhibition of the enzymatic activity of TopI. That is, we wondered if the positive inhibitory effect of gold(I) complexes of THQs **7** and QUIN **8** was just due to the presence of gold(I), or if it was also due to the presence of a nitrogen heterocyclic moiety, such as the THQ or QUIN ring. To confirm this, then, we studied the biological activity of the starting triphenylphosphine sulphide **1** (Ph_3P=S, Table S1, entry 2) and the (2-aminophenyl)diphenylphosphine sulphide **2** (Table S1, entry 3) and we compared the obtained results with the activity obtained for the corresponding gold(I) complexed derivatives, **5** and **6**, respectively (Table S1, entries 7 and 8). Surprisingly, it can be seen in Table S1 that none of the compounds without heterocyclic ring show TopI inhibitory activity, while as it was mentioned before compounds with THQ or QUIN ring presented good inhibition (entries 4 to 6, and 8 to 11). Therefore, with these findings we could say that the inhibitory activity is substantially improved by adding a metal such as gold in the structure of the new compounds, and that the presence of nitrogen ring is essential to maintain the inhibition. As far as we know, these results represent the first example of gold(I) complexes coordinate to simple and functionalized phosphine sulphides with TopI inhibitory activity.

In Vitro Cytotoxicity.

The cytotoxicity of the new gold(I) complexes **7**, as well as more unsaturated compound **8** was investigated *in vitro* by testing the antiproliferative activities against two human cancer cell lines: A549 (carcinomic human alveolar basal epithelial cell) and SKOV3 (human ovarian carcinoma). The cell counting kit (CCK8) assay was employed to assess growth inhibition and, the cell proliferation inhibitory activities of the compounds are listed in Table 2 as IC_{50} values.

Table 2. Antiproliferative activity of compounds **1**-**8** and CPT.

Entry	Cmpd	R^1	R^2	Cytotoxicity IC_{50} (μM)[a]		
				A549	SKOV3	MRC5
1		cisplatin[b]		5.32+-0.39	13.29+-1.24	
2		auranofin[b]		6.49+-0.71	5.28+-0.51	
3	**1**	-	-	>50	>50	>50
4	**2**	-	-	>50	>50	>50
5	**3a**	2-naphthyl	H	0.27± 0.05	>50	>50
6	**3b**	4-$CF_3C_6H_4$	F	20.22± 0.01	>50	>50
7	**4**	2-naphthyl	H	>50	>50	>50
8	**5**	-	-	0.03± 0.01	1.22 ± 0.22	>50
9	**6**	-	-	0.04± 0.01	4.48 ± 1.27	>50
10	**7a**	2-naphthyl	H	0.07± 0.01	2.82 ± 0.30	>50
11	**7b**	4-$CF_3C_6H_4$	F	0.18 ± 0.01	2.42 ± 0.08	>50
12	**8**	2-naphthyl	H	0.11 ± 0.09	3.35 ± 0.66	>50

[a] IC_{50} values obtained for human cancer cell lines A549 (lung carcinoma) and SKOV3 (ovarian adenocarcinoma), and for non-malignant human lung fibroblasts MRC5. Samples were incubated in the presence of compounds for 48 hours and the cell cytotoxicity was measured by CCK-8 assay. The cytotoxicity IC_{50} values listed are the concentrations corresponding to 50% growth inhibition.
[b] Previously reported data.[39]

First we studied the cytotoxicity of ligands **1**-**4** before being complexed to gold(I). Results obtained for triphenylphosphine sulphide **1** (Ph_3P=S, Table 2, entry 3), the (2-aminophenyl)diphenylphosphine sulphide **2** (Table 2, entry 4) and QUIN **4** (Table 2, entry 7) showed a very low cytotoxicity of these compounds with IC_{50} over 50 μM.

The only presence of pentafluorophenyl ligand do not usually affects the cytotoxicity of gold complexes, for this reason the antiproliferative activity of the gold pentafluorophenyl species, [Au(C6F5)(SMe2)] has not been tested.[37]

Afterwards, we studied the cytotoxicity of gold(I) complexes of previously tested ligands **5**-**8** and high cytotoxicity has been detected in all derivatives against A549 cell line, with IC_{50} values in the nanomolar range, and under 5 μM against the SKOV3 cell line (Table 2, entries 8 to 12). For example, excellent cytotoxicity of new gold(I) complex derivatives **5**, **6** and **7a** against human lung adenocarcinoma cell line (A549) in the range of 30-70nM and in the human ovarian carcinoma cell line (SKOV3) in the range of 1.22-2.82 μM for compounds **5** and **7** has been observed. An enhancing effect of CF_3 group[38] in the cytotoxicity has been observed in the SKOV3 cell line with a IC_{50} value of 2.42±0.08. Noteworthy, the cytotoxicity observed for new complexes is higher in new gold(I) complexes compared with that observed previously for other metal complexed derivatives with antitumor activity such as cisplatin (Table 2, entry 1) or auranofin (Table 2, entry 2).[37]

According to the data presented in Table 2, in general cytotoxicity is higher against the human lung adenocarcinoma cell line (A549) than in the human ovarian carcinoma cell line (SKOV3). Moreover, MRC-5 non-malignant lung fibroblasts were tested for studying selective toxicity[40] and no toxicity against this cell line was observed. As far as we know, these results represent the first example of gold(I) complexes derived from phosphine sulphides with antitumor activity reported up to date.

Computational analysis.

Taking into account that theoretical calculations allowed the estimation of Molecular Electrostatic Potential Surface (MEPS), HOMO-LUMO energy gap and related parameters, which depicted the potential kinetic stability and reactivity of the target compounds,[41] theoretical studies using Density Functional Theory (DFT)[42] involving the well-known Becke three-parameter Lee-Yang-Parr function (B3LYP)[43] and 6-311G (d, p) level of theory for the synthesized compounds were carried out.

***Stereoelectronic properties*.** The molecular DFT-based parameters such as electronic chemical potential (μ), chemical hardness (η), global electrophilicity (ω), maximum number of accepted electrons (ΔNmax), dipole moment, polarizability and Free energy in gas and in aqueous medium for compounds **3**, **4**, **7** and **8** are reported in Table S2 in ESI.

Upon a general analysis of obtained results, tetrahydroquinolin-8-yldiphenylphosphine sulphides **3** and **7** present higher chemical potentials (μ) and less electrophilicity (ω) than quinolines **4** and **8**. Moreover, results obtained for more aromatic quinolin-8-yldiphenylphosphine sulphide compounds **4** and **8** are similar to those obtained for CPT (Table S2 in ESI).

On the other hand, when the computational results of the neat ligand compounds **3** and **4** are compared with those of the gold(I) complexes **7** and **8**, it is observed that the gold complexes present lower chemical potential (μ), higher electrophilicity (ω), higher number of accepted electrons (ΔNmax), much higher dipole moment and higher polarizability.

We must bear in mind that experimentally, these last compounds, gold(I) complexes **7** and **8**, have an excellent inhibition of Top I (see Table S1). These factors could indicate a different behaviour of the two types of compounds with respect to their interaction with the target, the compounds presenting the ligand being more active.

***Molecular Electrostatic Potential Surface (MEPS) analysis*.** The corresponding Molecular Electrostatic Potentials for 1,2,3,4-tetrahydroquinolin-8-yl-diphenylphosphine sulphide derivatives **3** and quinolin-8-yl-diphenylphosphine sulphide derivative **4** have been calculated (see Supporting Information, Figure S1), as well as, the corresponding MEPS of auranofin and gold complexes **7** and **8** (see Supporting Information, Figure S2), using DFT[42] with the standard basis set B3LYP / 6-311G (d, p) level of theory.

The calculations show that neat ligands, compounds **3** and **4**, have the most negative local electrostatic potential close to the sulphur atom of the phosphine sulphide group. The positive local electrostatic potentials are located, in general, over the hydrogens of the aromatic substituents of the

tetrahydroquinolinic core. These compounds **3** and **4** are less cytotoxic than the corresponding compounds **7** and **8** having the Au-perfluorophenyl fragment (Table 2) and their TopI inhibition values are also lower than for compounds **7** and **8**, with the Au-perfluorophenyl fragment (Table S1).

On the other hand, these MEPS change significantly in the complexes having the Au-perfluorophenyl fragment **7** and **8** (Figure S2 in ESI). In these cases, the positioning of local negative electrostatic potential has varied from place, being situated on the fluorine atoms of perfluorophenyl group. In addition, an extremely positive zone is observed over the aromatic substituents in the tetrahydroquinoline or quinoline core.

This suggests that the interactions with the target are different in each case and probably the presence of Au-perfluorophenyl fragment may favour the formation of the ligand –Top1–DNA ternary complex or a change in the conformation of the protein, which prevents cleavage or religation. These computational results are in agreement with the experimental inhibition results, since, as previously indicated, π-π stacking interactions between heterocyclic compounds and TopI favour the inhibition of the enzyme.[13a]

Conclusions

In summary, new biologically active hybrid molecules as gold(I) complexes of triphenyphosphine sulphide **5**, (2-aminophenyl)diphenylphosphine sulphide **6**, and tetrahydroquinolinylphosphine sulphides **7** and quinolinylphosphine sulphide **8** have been designed and synthesized.

It has been shown that the coordination of gold(I) with the sulphur atom of phosphine sulphides together with the presence of a nitrogen heterocycle, such as tetrahydroquinoline or quinoline, favor the inhibition of TopI. Based on the results of the relaxation assays, the tetrahydroquinolinylphosphine sulphide gold complexes **7** maintain the excellent inhibitory activity shown by the bare ligands **3** (without gold). Likewise, it should be noted that the quinolinylphosphine sulphide gold complex **8** showed excellent TopI inhibitory activity, improving the lack of activity shown by ligand **4**. Furthermore, the inhibitory activity can be compared with that presented by the natural TopI inhibitor, CPT, being observed slightly better activity at short enzymatic reaction times and even higher inhibition response than CPT at longer reaction time.

Regarding the cytotoxicity of these new derivatives, all the gold(I) complexes **5**, **6**, **7** and **8** showed IC_{50} values in the nanomolar range against the A549 cancer line and IC_{50} values below 5 μM against the SKOV3 cell line. It should be noted that the cytotoxicities observed for gold complexes are higher than those observed for phosphine sulphide ligands before binding to gold. Furthermore, no toxicity was observed when the non-cancerous lung fibroblast cell line (MRC5) was treated with the newly prepared phosphine sulphide gold(I) complexes.

Considering the previous literature, gold plays a fundamental role and from the cytotoxicity point of view, the results obtained with gold complexes are generally superior to the standards. However, it is possible that not only the TopI but also other biological targets may be involved in the activity of these gold complexes.

Experimental

All reactions were performed under air using dry glassware. If necessary, solvents were dried using a solvent purification system SPS PS-MD-5 or distilled from appropriate drying agents under nitrogen, prior to use. Reagents and solvents used were of commercially available reagent quality. The complex $[Au(C_6F_5)(SMe_2)]$[44] and the phosphine sulphide ligands[18a,19a,33] were prepared as reported in the literature. ^1H, ^{19}F and $^{31}P\{^1H\}$ NMR spectra were recorded at 298 K with a Varian Inova 500-MR instrument (LTI, University of Valladolid). Chemical shifts (in δ units, parts per million) were referenced to the residual solvent signal (^1H), to $CFCl_3$ (^{19}F), and to 85% H_3PO_4 ($^{31}P\{^1H\}$). MALDI-TOF mass spectrometry was carried out using a Bruker Autoflex instrument (LTI, University of Valladolid). The elemental analyses were performed with a Carlo Erba 1108 microanalyzer (Vigo University).

Experimental procedure for X-ray Crystallography. A crystal was attached to a glass fibre and transferred to an Agilent Supernova diffractometer with an Atlas CCD area detector (LTI, University of Valladolid). Data collection was performed with Mo-Kα radiation (λ = 0.71073 Å). Data integration, scaling and empirical absorption correction was carried out using the CrysAlisPro program package.[45] The crystals were kept at 293 K during data collection. Using Olex2,[46] the structure was solved with the olex2.solve[47] and refined with Shelx program.[48] The non-hydrogen atoms were refined anisotropically and hydrogen atoms were placed at idealized positions and refined using the riding model. Refinement proceeded smoothly to give the residuals shown in Table S3 (ESI, Supporting Information). CCDC 1995587, 1995588, 1995589 contains the supporting crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (internat.) +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

General procedure for the preparation of the gold(I) complexes using phosphine sulphides as ligands. A mixture of the corresponding phosphine sulphide ligand $SPPh_2R$ (**1**-**4**) and one equivalent of $[Au(C_6F_5)(SMe_2)]$ (42.6 mg, 0.1 mmol) in CH_2Cl_2 (15 mL) was stirred in a bottom flask at room temperature for 3 min. Then the mixture was concentrated under vacuum to dryness. The residue obtained was dissolved in the minimum amount of CH_2Cl_2 and the solution was again evaporated to dryness in order to remove all the SMe_2. The solid obtained was recrystallized in CH_2Cl_2/*n*-hexane giving $[Au(C_6F_5)(SPPh_2R)]$ (**5**-**8**) as a microcrystalline white solid. Suitable single crystals of **5**, **6** and **8** for X-ray Crystallography were obtained by slow diffusion of *n*-hexane into concentrated solutions of the compounds in dichloromethane at room temperature.

Complex 5. Yield: 56.0 mg (85%). Analysis calculated for $C_{24}H_{15}AuF_5PS$: C, 43.78; H, 2.30. Found: C, 44.21; H, 2.39. MS (MALDI-TOF): *m/z* calcd for $C_{24}H_{15}AuF_5NaPS$ $[M+Na]^+$: 681.0110. Found: 681.0116. ^1H NMR (499.70 MHz, CD_2Cl_2, 298 K) δ = 7.87 (m, 6 H, Ph), 7.71 (m, 3 H, Ph), 7.58 (m, 6 H, Ph). ^{19}F NMR (470.17 MHz, CD_2Cl_2, 298 K) δ = –116.70 (m, 2 F, F^{ortho}), –161.18 (t, $^3J_{FF}$ = 20.0 Hz, 1 F, F^{para}), –163.76 (m, 2 F, F^{meta}). $^{31}P\{^1H\}$ NMR (202.31 MHz, CD_2Cl_2, 298 K) δ = 44.86 (s, 1 P).

Complex 6. Yield: 57.9 mg (86%). Analysis calculated for $C_{24}H_{16}AuF_5NPS$: C, 42.81; H, 2.39; N, 2.08. Found: C, 43.00; H, 2.36; N, 1.90. MS (MALDI-TOF): *m/z* calcd for $C_{24}H_{16}AuF_5NNaPS$ $[M+Na]^+$: 696.0219. Found: 696.0221. ^1H NMR (499.70 MHz, CD_2Cl_2, 298 K) δ = 7.94 (m, 4 H, Ph), 7.72 (m, 2 H, Ph), 7.60 (m, 4 H, Ph), 7.44 (m, 1 H, C_6H_4), 6.87–6.78 (m, 2 H, C_6H_4), 6.72 (m, 1 H, C_6H_4), 4.91 (s, 2 H, NH_2). ^{19}F NMR (470.17 MHz, CD_2Cl_2, 298 K) δ = –116.64 (m, 2 F, F^{ortho}), –161.09 (t, $^3J_{FF}$ = 20.0 Hz, 1 F, F^{para}), –163.72 (m, 2 F, F^{meta}). $^{31}P\{^1H\}$ NMR (202.31 MHz, CD_2Cl_2, 298 K) δ = 40.63 (s, 1 P).

Complex 7a. Yield: 77.0 mg (89%). Analysis calculated for $C_{43}H_{30}AuF_5NPS$: C, 56.40; H, 3.30; N, 1.53. Found: C, 56.76; H, 3.56; N, 1.45. MS (MALDI-TOF): *m/z* calcd for $C_{43}H_{30}AuF_5NNaPS$ $[M+Na]^+$: 938.1315. Found: 938.1313. ^1H NMR (499.70 MHz, CD_2Cl_2, 298 K) δ = 2.14 (ddd, $^2J_{HH}$ = 13.1 Hz, $^3J_{HH}$ = 12.6 Hz, $^3J_{HH}$ = 11.4 Hz, 1 H, CH_2), 2.34 (dm, $^2J_{HH}$ = 13.1 Hz, 1 H, CH_2), 4.40 (dd, $^3J_{HH}$ = 11.4 Hz, $^3J_{HH}$ = 3.0 Hz, 1 H, CH), 4.91 (dd, $^3J_{HH}$ = 12.6 Hz, $^3J_{HH}$ = 4.7 Hz, 1 H, CH), 6.16 (s, 1 H, NH), 6.50 (td, $^3J_{HH}$ = 7.8 Hz, $^4J_{HP}$ = 3.2 Hz, 1 H, C_6H_3), 6.68 (ddm, $^3J_{HP}$ = 16.1 Hz, $^3J_{HH}$ = 7.8 Hz, 1 H, C_6H_3), 6.88 (d, $^3J_{HH}$ = 7.8 Hz, 1 H, C_6H_3), 7.08 (dd, $^3J_{HH}$ = 8.5 Hz, $^4J_{HH}$ = 1.8 Hz, 1 H, Naph), 7.23-8.10 (21 H, 3 Ph + Naph). ^{19}F NMR (470.17 MHz, CD_2Cl_2, 298 K) δ = –116.43 (m, 2 F, F^{ortho}), –161.11 (t, $^3J_{FF}$ = 20.0 Hz, 1 F, F^{para}), –163.72 (m, 2 F, F^{meta}). $^{31}P\{^1H\}$ NMR (202.31 MHz, CD_2Cl_2, 298 K) δ = 40.14 (s, 1 P).

Complex 7b. Yield: 81.4 mg (86%). Analysis calculated for $C_{40}H_{26}AuF_9NPS$: C, 50.48; H, 2.75; N, 1.47. Found: C, 50.88; H, 2.85; N, 1.41. MS (MALDI-TOF): *m/z* calcd for $C_{40}H_{26}AuF_9NNaPS$ $[M+Na]^+$: 974.0938. Found: 974.0836. ^1H NMR (499.70 MHz, CD_2Cl_2, 298 K) δ = 1.97 (ddd, $^2J_{HH}$ = 12.9 Hz, $^3J_{HH}$ = 12.5 Hz, $^3J_{HH}$ = 11.4 Hz, 1 H, CH_2), 2.25 (dm, $^2J_{HH}$ = 12.9 Hz, 1 H, CH_2), 4.36 (dd, $^3J_{HH}$ = 12.5 Hz, $^3J_{HH}$ = 4.6 Hz, 1 H, CH), 4.82 (dd, $^3J_{HH}$ = 11.4 Hz, $^3J_{HH}$ = 3.2 Hz, 1 H, CH), 6.06 (s, 1 H, NH), 6.53 (td, $^3J_{HH}$ = 7.8 Hz, $^4J_{HP}$ = 3.2 Hz, 1 H, C_6H_3), 6.71 (dd, $^3J_{HP}$ = 16.2 Hz, $^3J_{HH}$ = 7.8 Hz, 1 H, C_6H_3), 6.85 (d, $^3J_{HH}$ = 7.8 Hz, **1** H, C_6H_3), 6.95-8.10 (18 H, 2 Ph + C_6H_4F + $C_6H_4CF_3$). ^{19}F NMR (470.17 MHz, CD_2Cl_2, 298 K) δ = –62.85 (s, 3 F, CF_3) –116.35- –116.59 (m, 3 F, 2 F^{ortho} + C_6H_4F), –160.95 (t, $^3J_{FF}$ = 20.0 Hz, 1 F, F^{para}), –163.64 (m, 2 F, F^{meta}). $^{31}P\{^1H\}$ NMR (202.31 MHz, CD_2Cl_2, 298 K) δ = 39.89 (s, 1 P).

Complex 8. Yield: 80.2 mg (88%). Analysis calculated for $C_{43}H_{26}AuF_5NPS$: C, 56.65; H, 2.87; N, 1.54. Found: C, 57.19; H, 3.03; N, 1.52. MS (MALDI-TOF): *m/z* calcd for $C_{43}H_{26}AuF_5NPS$ $[M]^+$: 911.1104. Found: 911.1100. ^1H NMR (499.70 MHz, CD_2Cl_2, 298 K) δ = 7.45 (dd, $^3J_{HH}$ = 8.6 Hz, $^4J_{HH}$ = 1.8 Hz, 1 H, Naph), 7.47-7.79 (16 H, Ph + Naph + C_6H_3), 7.85 (m, 1 H, Naph), 8.00 (s br, 1 H, Naph), 8.04 (s, 1 H, CH), 8.13 (dd, $^3J_{HP}$ = 14.3 Hz, $^3J_{HH}$ = 8.3 Hz, 4 H, H^{ortho}, PPh_2), 8.31 (dm, $^3J_{HH}$ = 8.4 Hz, 1 H, C_6H_3), 8.64 (ddd, $^3J_{HP}$ = 17.0 Hz, $^3J_{HH}$ = 7.2 Hz, $^4J_{HH}$ = 1.4 Hz, 1 H, C_6H_3). ^{19}F NMR (470.17 MHz, CD_2Cl_2, 298 K) δ = –116.45 (m, 2 F, F^{ortho}), –161.62 (t, $^3J_{FF}$ = 20.0 Hz, 1 F, F^{para}), –163.96 (m, 2 F, F^{meta}). $^{31}P\{^1H\}$ NMR (202.31 MHz, CD_2Cl_2, 298 K) δ = 44.93 (s, 1 P).

Experimental procedure for biological experiments.

Materials. Reagents and solvents were used as purchased without further purification. Camptothecin was purchased from Sigma-Aldrich. All stock solutions of the investigated compounds were prepared by dissolving the powered materials in appropriate amounts of DMSO. The final concentration of DMSO never exceeded 5% (v/v) in reactions. Under these conditions, DMSO was also used in the controls and was not seen to affect TopI activity. The stock solution was stored at 5°C until it was used.

Expression and purification of Human Topoisomerase IB. The yeast Saccaromyces cerevisiae TopI null strain RS190, which was used for expression of recombinant human TopI was a kind gift from R. Sternglanz (State University of New York, Stony Brook, NY). Plasmid pHT143, for expression of recombinant TopI under the control of an inducible GAL promoter was described.[49] The plasmids pHT143 were transformed into the yeast S.cerevisiae strain RS190. The proteins were expressed and purified by affinity chromatography essentially as described.[50] The protein concentrations were estimated from Coomassie blue-stained SDS/polyacrylamide gels by comparison to serial dilutions of bovine serum albumin (BSA).

DNA relaxation assays. TopI activity was assayed using a DNA relaxation assay by incubating 110 ng/μL of TopI with 0.5 μg of negatively supercoiled pUC18 in 20 μl of reaction buffer (20 mM Tris–HCl, 0.1 mM Na_2EDTA, 10 mM MgCl2, 50 μg/ml acetylated BSA and 150 mM KCl, pH 7.5). The effect of the synthesized compounds and gold complexes **5**, **6**, **7** and **8** derivatives on topoisomerase activity was measured by adding the compounds, at different time points as indicated in the text. The reactions were performed at 37°C, stopped by the addition of 0.5% SDS after indicated time intervals. The samples were protease digested, electrophoresed in a horizontal 1% agarose gel in 1xTBE (50 mM Tris, 45 mM boric acid, 1 mM EDTA) at 26V during 20 hours. The gel was stained with gel red (BIOTIUM, 5 μg/ml), destained with water and photographed under UV illumination.

Since all drugs were dissolved in dimethyl sulphoxide (DMSO), a positive control sample containing the same DMSO concentration as the samples incubated with the drugs was included in all experiments. As a control for drug inhibition the well know TopI specific drug camptothecin was included.

Cytotoxicity assays. Cells were cultured according to the supplier´s instructions. Cells were seeded in 96-well plates at a density of 2-2.5 x 103 cells per well and incubated overnight in 0.1 mL of media supplied with 10% Fetal Bovine Serum (Lonza) in 5% CO_2 incubator at 37°C. On day 2, drugs were added and samples were incubated for 48 hours. After treatment, 10 μL of cell counting kit-8 was added into each well for additional 2 hours incubation at 37°C. The absorbance of each well was determined by an Automatic Elisa Reader System at 450 nm wavelength. Camptothecin, purchased from Sigma-Aldrich, was used as positive control.

Experimental procedure for computational methodology. Molecular modeling. All calculations included in this paper were carried out with Gaussian 16 program[51] within the density functional theory (DFT) framework[42] using the B3LYP,[43] along with the standard 6-31G** basis set. All minima were fully characterized by harmonic frequency analysis.[52] The solvent effect in DFT calculations was evaluated by means of the Polarizable Continuum Model (PCM)[53] using water as solvent. The pKa values were studied to determine the dominant species (ionization states) at physiological pH (pH = 7.4) using Epik[54] and these were the species used in each case. After a conformational search with MacroModel[55] the most stable conformations were chosen and optimized at the B3LYP/6-31G** + ΔZPVE level of theory and also were computed at the B3LYP(PCM)/6-31G** + ZPVE level using water as solvent. Among them, the most stable of each compound was chosen to calculate the molecular DFT-based parameters, molecular electrostatic potential energetics and docking studies. The obtained results for the molecular electrostatic potential surfaces were generated using GaussView Rev 5.0.9.[56]

Conflicts of interest

There are no conflicts to declare.

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