Dalton Transactions

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/dalton

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Copper(I) complexes with phosphine derived from sparfloxacin. Part I – structures, spectroscopic properties and cytotoxicity.

Urszula K. Komarnicka,[a] Radosław Starosta,[a] Agnieszka Kyzioł[b] and Małgorzata Jeżowska-Bojczuk*[a]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

In this paper we present new copper(I) iodide or copper(I) thiocyanate complexes with hydroxymethyldiphenylphosphine ($PPh_2(CH_2OH)$) or phosphine derivative of sparfloxacin, a 3rd generation fluoroquinolone antibiotic agent ($PPh_2(CH_2$-Sf)) and 2,9-dimethyl-1,10-phenanthroline (**dmp**) or 2,2'-biquinoline (**bq**) auxiliary ligands. The synthesised complexes were fully characterised by NMR and UV-Vis spectroscopies as well as by mass spectrometry. Selected structures were additionally analysed using X-ray and DFT methods. All complexes proved to be stable in solution in the presence of water and atmospheric oxygen for several days. Cytotoxic activity of the complexes was tested against two cancer cell lines (CT26 - mouse colon carcinoma and A549 - human lung adenocarcinoma). Applying two different incubation times, the studies enabled a preliminary estimation of the dependence of selectivity and mechanism of action on the type of diimine and phosphine ligands. The results obtained showed that complexes with $PPh_2(CH_2$-Sf) are significantly more active than those with $PPh_2(CH_2OH)$. On the other hand, the relative impact of diimine on cytotoxicity is less pronounced. However, the **dmp** complexes are characterised by strong inhibitory properties, while the **bq** ones are rather not. This confirms the interesting and promising biological properties of the investigated group of copper(I) complexes, which undoubtedly are worthy of further biological studies.

Introduction

The design and synthesis of new drugs is a time-consuming and expensive process. Launching a new drug takes at least 10 years, while the costs can reach billions of dollars. Despite a large number of available drugs with various properties, we still struggle with many diseases, adverse drug reactions and drug resistance.[1-4] More and more often, instead of searching for new classes of compounds, an entirely different approach is used in the design of therapeutic substances.[5] It is becoming popular to modify the structures that are used in medical therapeutics by attaching other chemical moieties thereto, responsible for their more-selective transport or for changing the biological properties.[6-8]

Medicinal inorganic chemistry offers additional opportunities for the design of therapeutic agents, not available to organic chemistry.[9-11] A wide range of coordination numbers and geometries, the different redox states available, various thermodynamic and kinetic properties, as well as the intrinsic properties of metal ions may result a variety of reactions. Consequently, compounds that are very interesting from a medicinal point of view may be developed.[12-17] The widespread use of cisplatin has placed coordination chemistry of metal-based drugs in the frontline in the fight against cancer. However, the use of this drug is limited due to its high toxicity.[18] This leads, among others, to the exploration and synthesis of drugs that are based on other metal ions. Copper complexes have promising properties in this area.[19-24] They show antitumour,[23,25-26] antibacterial,[27] antiviral,[28-29] antifungal[30] and anti-inflammatory[31] activities, which make them very good candidates as drugs.

We present herein the synthesis, physicochemical properties and cytotoxicity of new copper(I) iodide and copper(I) thiocyanate complexes with phosphines and diimines. Previous studies have shown that complexes of this type have promising biological properties.[23-26,32-33] The use of a phosphine ligand prevents oxidation and hydrolysis reactions due to a strong copper-phosphine interaction.[25] What is also known is that phosphines and their complexes exhibit a high cytotoxicity and antibacterial and anti-inflammatory properties. [34-38] We used two diimine ligands (2,9-dimethyl-1,10-phenanthroline (**dmp**) and 2,2'-biquinoline (**bq**)), which are characterised by their high steric hindrance. They therefore successfully prevent the tetrahedral geometry around the copper centre from flattening in the excited state which usually leads to oxidation from Cu(I) to

Cu(II).[23,39-40] Interestingly, it has been proven that both these ligands impact, in a very different way, the biological properties of the resulting complexes.[23-24]

In this work we used two phosphine ligands: hydroxymethyldiphenylphosphine (**POH**)[41] and its derivative PPh_2CH_2Sf (**PSf**), previously synthesised and characterised by us.[34] The latter was obtained by attaching **POH** to sparfloxacin (**HSf**), a 3rd generation quinolone.[34,42] Apart from its obvious antibacterial properties, this antibiotic is a promising modulating agent in combination with anticancer drugs. It has been proven that **HSf** inhibits hERG, one of the potassium channels, which are essential proteins for the regulation of cell proliferation.[34,43] Sparfloxacin forms various metal complexes with improved antimicrobial properties: $[Fe(\mathbf{Sf})_3]$, $[Co(\mathbf{Sf})_2(H_2O)_2]$, $[Cu(\mathbf{Sf})_2]$, $[MoO_2(\mathbf{Sf})_2]$ and $[Pt(\mathbf{Sf})_2]$[44-49] (where **Sf** refers to a deprotonated form of sparfloxacin), antituberculosis activity: $[PdCl_2(\mathbf{HSf})]$,[50] and is also characterised by high cytotoxicity: $[Cu(\mathbf{HSf})(phen)(H_2O)]^{2+}$, $[AuCl_2(\mathbf{HSf})]Cl$.[51-52]

Results and discussion

Synthesis

We synthesised eight new copper(I) complexes with a general formula of [CuX(NN)P]: [CuI(dmp)POH] **1-POH**, [CuNCS(dmp)POH] **2-POH**, [CuI(bq)POH] **3-POH**, [CuNCS(bq)POH] **4-POH**, [CuI(dmp)PSf] **1-PSf**, [CuNCS(dmp)PSf] **2-PSf**, [CuI(bq)PSf] **3-PSf** and [CuNCS(bq)PSf] **4-PSf** (see Figure 1) (where NN – 2,9-dimethyl-1,10-phenanthroline (**dmp**) or 2,2'-biquinoline (**bq**); X – I or NCS; P – **POH** or **PSf**). The syntheses were carried out without light and under nitrogen, using Schlenk technique. Since phosphine oxidation is a common process during complex formation, we also synthesised phosphine oxides for comparative purposes.

Structural analysis

The X-ray molecular structures of **1-POH**, **3-POH** as well as **1-POH** and **1-PSf** in the **1-POH·1-PSf** mixed system are shown in Figure 2. Selected structural data are presented in Table 1.

The Cu atom is coordinated by the iodide anion, two nitrogen atoms of chelating diimine and the phosphorus atom of **POH** or **PSf** phosphine ligand. The coordination geometry around copper(I) central ion is a distorted tetrahedral. The distortions result from the chelating character of diimine and the difference between the steric demands of the iodine atom and phosphine ligand. The flattening deformations of the structures are small, the α values (angle between the diimine mean plane and P-Cu-I plane, see Table 1) are almost 90°. Interestingly, in the **3-POH** and in **1-POH** molecule in the **1-POH·1-PSf** system, contrary to the structure of **1-POH**, the hydroxyl group from the phosphine ligand forms a strong O1-H1...I1 intramolecular bond to iodine atom[53] (**3-POH:** DH: 0.84, HA: 2.67 DA: 3.501(4) Å DHA: 171.9°-**POH·1-PSf:** DH: 0.84, HA: 2.74 DA: 3.559(7) Å DHA: 163.8°).

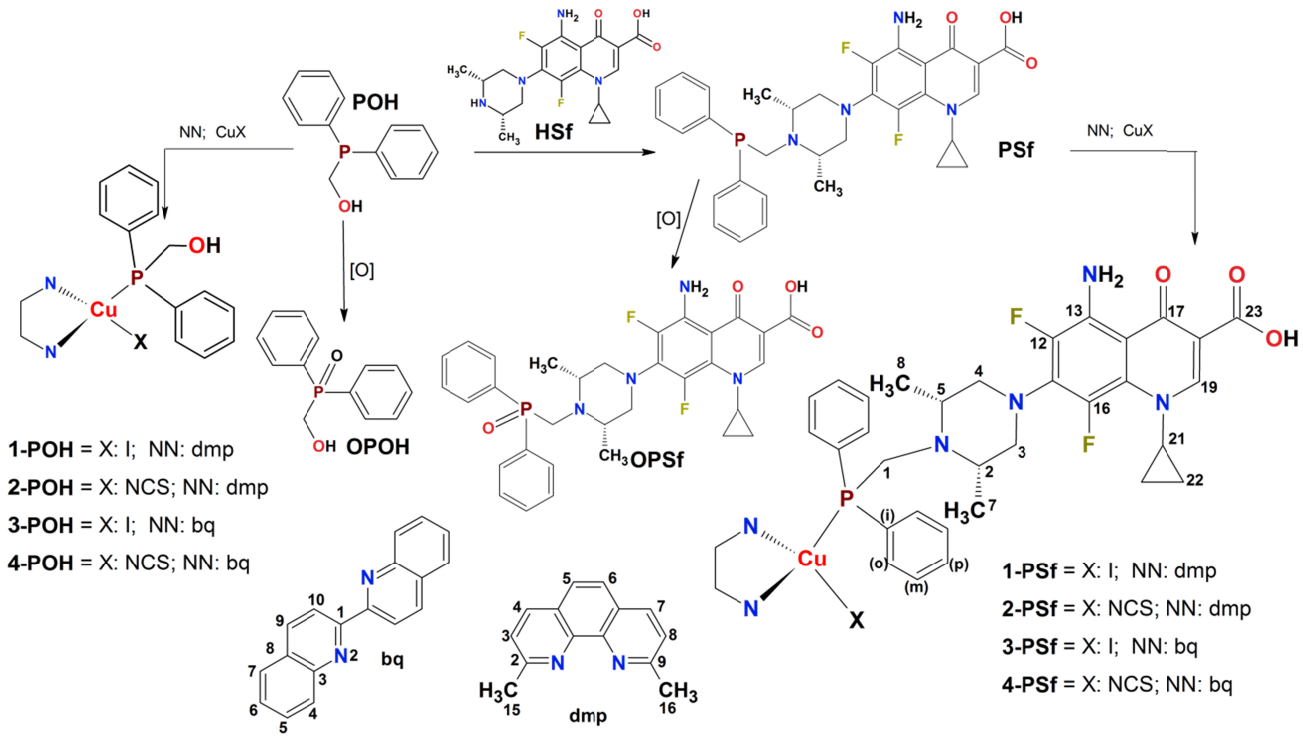

Fig. 1 Schematic view of the complexes and ligands (with atomic numeration) and synthetic routes.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

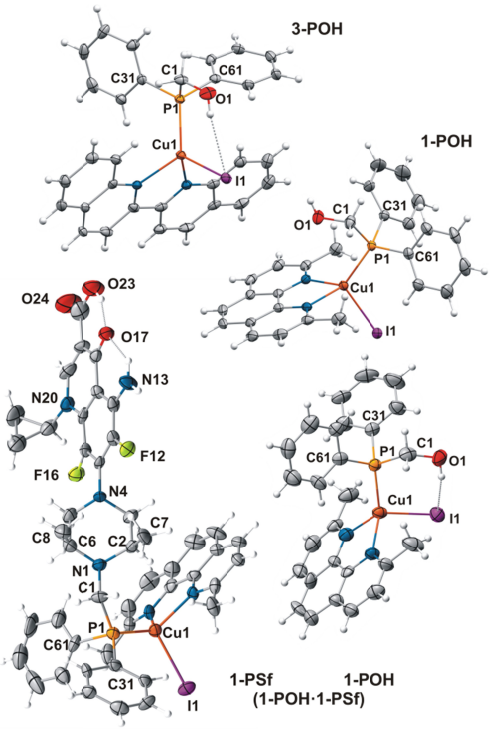

Fig.2 X-ray structures (50% thermal elipsoids) of **1-POH**, **3-POH** and the molecules of **1-PSf** and **1-POH** in **1-POH·1-PSf**.

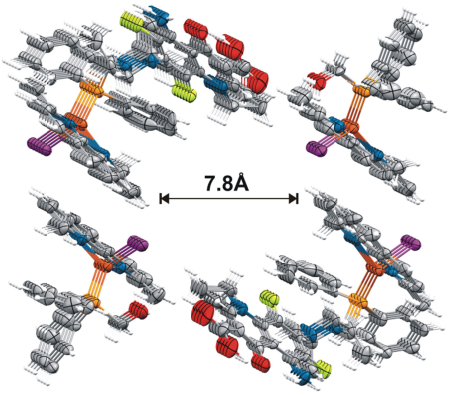

Fig.3 Molecular packing of in **1-POH·1-PSf**.

Numerous attempts to obtain a crystal structure of any of the **PSf** complexes were unsuccessful. We obtained only a mixed crystal of **1-POH·1-PSf** after two months of a slow diethyl ether diffusion into a CH_2Cl_2 solution of **1-PSf**. This process showed that **PSf** could be unstable and partially decompose under these conditions. Both molecules in **1-POH·1-PSf** form discrete dimers bound by typical π-stacking interactions, with carbon atoms both overlapping with others and placed in the centres of the stacked rings.[54] The most interesting feature of this structure is, however, the large, empty channels along the "b" axis with a diameter exceeding 7 Å (see Figure 3).

Table 1 Selected structural data for **PSf** and its complexes

X-ray:	**3-POH**	**1-POH**	**1-POH (B)***	**1-PSf (A)***
Cu1-I1	2.637(1)	2.659(1)	2.632(2)	2.630(1)
Cu1-P1	2.201(1)	2.191(1)	2.196(3)	2.203(3)
P1-C1	1.854(5)	1.830(3)	1.846(11)	1.868(10)
av. (P1-C1,31,61)	1.830(5)	1.826(3)	1.841(11)	1.845(10)
α	88.0	88.3	86.77	87.87
DFT:	**PSf-ax**	**PSf-eq**	**1-PSf-ax**	**1-PSf-eq**
E_{rel} [kcal/mol]	**1.39**	**0**	**0**	**2.27**
Cu1-I1			2.558	2.557
Cu1-P1			2.171	2.183
P1-C1	1.885	1.886	1.868	1.892
av. (P1-C1,31,61)	1.856	1.8563	1.844	1.855

* molecules A and B from the structure of **1-POH·1-PSf**) system; α - angle between the mean dmp (or bq) plane and the Cu1-P1-I1 plane

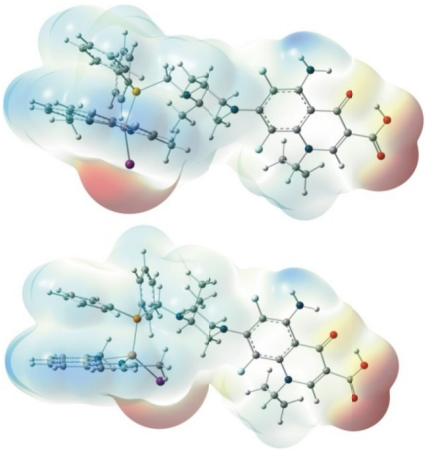

Fig.4 The DFT structures of **1-PSf**-eq (up) and **1-PSf**-ax (down) with the SCF electron density (0.0004 iso-surfase) with mapped ESP potential (in the range from -0.05 eV (red) to 0.05 eV (blue).

An analysis of the **1-PSf** structure showed that, contrary to the X-ray structures of **HSf**[55-56] and **OPSf**[34], the methyl groups on the piperazine ring occupy the axial positions. This, most probably, contributes to reducing the entire ligand size. To verify that observation, the DFT calculations (Gaussian 09 (Rev.D.01) package[57]) were performed for the **1-PSf** molecule in two variants: **1-PSf-ax** with the discussed methyl groups in the axial positions and **1-PSf-eq** in the equatorial positions (see Figure 4). Analogous calculations performed for **HSf** and its derivatives indicated that the preferred position for these groups is equatorial,[34] however the calculated energy of **1-PSf-ax** is lower

by 2.3 kcal/mol than the energy of **1-PSf-eq**. Such a difference is not large; however, a comparison of the "axial" and "equatorial" structures reveals a noticeable impact of the -CH_3 groups' orientation on the whole molecule (Table 1). Although for the uncoordinated phosphine only the longer C1-N1 bond in the **PSf-eq** is observed as the main difference, both **1-PSf** molecules differ significantly. The equatorial orientation of the methyl groups leads to elongations of the bonds around the phosphorus and copper atoms. Nevertheless, a longer Cu-P bond and higher S4 value observed in **1-PSf-eq** suggest that the phosphine - copper bond is weaker in this structure than in **1-PSf-ax**.

Spectroscopic characterisation

The synthesised compounds were thoroughly characterised with NMR and UV-Vis spectroscopies. These two techniques allowed the structures and stability of the complexes in solution to be determined. Due to the low solubility of the complexes in water, their NMR spectra were recorded in $CDCl_3$ (Figure 5 shows selected $^{31}P\{1H\}$ NMR spectra; all spectra and spectral data are presented in Tables S1-S4† and Figures S1-S10†). Formation of the Cu(I) complexes in each case resulted in significant broadening and a low-field shift of the phosphine signal observed in $^{31}P\{^1H\}$ NMR spectra. The value of the chemical shift of **POH** changed by 3-6 ppm (from -9.33 ppm for free phosphine to -5.47 (**1-POH**), -2.85 (**2-POH**), -3.01 (**3-POH**) and -3.19 ppm (**4-POH**)). In the case of **PSf** these changes were more pronounced - the signal was shifted by 24-28 ppm (from -35.88 to -11.93 (**1-PSf**), -8.76 (**2-PSf**), -10.48 (**3-PSf**) and -7.43 ppm (**4-PSf**)) upon coordination.

Unexpectedly, the phosphorus spectra of the **PSf** complexes showed additional broad signals around -30 ppm (see Figures S7-S10†). Their intensity increased in the presence of an excess of the phosphine (see Figure 7 c), which suggested a slow exchange of this ligand resulting from the high steric hindrances of **PSf**. These are caused by the presence of methyl groups on the piperazine rings. These substituents influence the orientation of the phosphine phenyl rings and lead to a weaker phosphine binding.[34] A slow ligand exchange is indirectly confirmed by analysis of the carbon and proton spectra, which show only pure complexes with both phosphines (see Tables S1-S4† and Figures S1-S10†). It should be noted that in any case we did not observe the decomposition of **PSf** to **POH** and **HSf** leading to the formation of the **x-POH** complexes (the phenomenon observed in the X-ray studies).

As expected, the formation of the complexes affected the chemical shifts of the signals originating from the ligands on the ^1H and $^{13}C\{^1H\}$ NMR spectra. In the case of the diimines, the number of carbon and proton signals did not change and the greatest changes were observed for the atoms neighbouring the nitrogens. This confirmed that these atoms coordinated to Cu(I) ions in a symmetrical, chelating mode. For the **bq** complexes (regardless of the employed phosphine) especially large signal shifts were observed for H10 (upfield) and H4 (downfield) protons (Tables S1†, S4† and Figures S5-S6†,S9-S10†). This was a result of the **bq** transition from the *s-trans* conformation preferred for the uncoordinated ligand to the *s-cis* one in complexes.[58-59, 60] Importantly, the chemical shifts of the signals for the iodide complexes with both phosphines and diimines differ significantly from the ones for $[Cu_2I_2(NN)_2]$ prepared *in situ* (see Tables S11-S12†). This suggests that the possible reaction of the phosphine dissociation according to the eq.1. does not occur or occurs to a small degree, despite a weak **PSf** binding.

$$2[CuI(NN)P] = [Cu_2I_2(NN)_2] + 2P \quad (1)$$

For phosphine ligands, analogously to the diimines, the largest changes in the ^1H and $^{13}C\{^1H\}$ NMR spectra were observed for H1 and C1 atoms. They were, however, distinct among complexes with **POH** and **PSf**. For the **POH** complexes (except **2-POH**), the signals were shifted rather strongly towards lower fields, while the **PSf** complexes, slightly towards higher fields. Interestingly, the shape of the C1 signals is a feature strongly distinguishing the **1-POH** and **2-POH** complexes, where doublets of $^1J(C\text{-}P)$ = 20 Hz coupling constant were observed. Since broad singlets were observed for the rest of complexes, this proved that both complexes with **dmp** and **POH** are characterised by an exceptionally low lability.

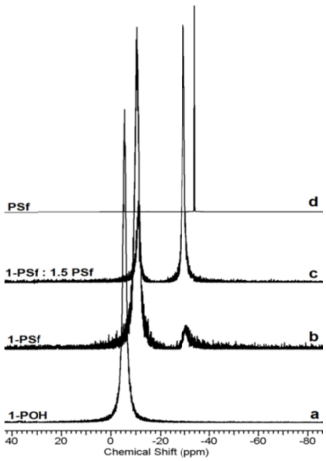

Fig.5 $^{31}P\{^1H\}$ NMR power-spectra (298 K, $CDCl_3$): a) **1-POH**, b) **1-PSf**, c) **1-PSf** + 1.5**PSf** d) **PSf**.

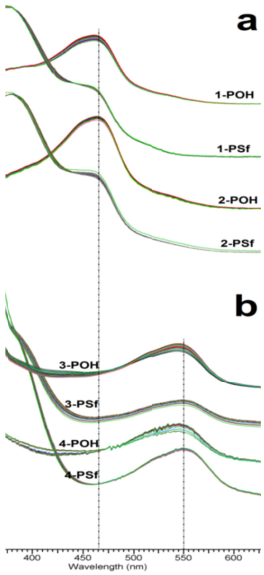

Fig.6 Electronic absorption spectra of the solutions ($5 \cdot 10^{-5}$ M) of: a) the **dmp** complexes: **1-POH**, **2-POH**, **1-PSf** and **2-PSf**; b) the **bq** ones: **3-POH**, **4-POH**, **3-PSf** and **4-PSf**. Spectra were measured every 2 h during 96 h in the DMSO:H_2O (3:1 V:V) mixture.

To conclude, the analysis of the $^{31}P\{^{1}H\}$, ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra confirmed that the Cu(I) central ion is coordinated by two nitrogen atoms of diimine (**dmp** or **bq**) and the phosphorus atom of the phosphine ligand. However, the coordination of **POH** and **PSf** ligands is different, which probably results not only from the differences in their steric hindrances, but also from the character of one of the substituents (-CH_2**O**H vs. -CH_2**N**R_2).

For biological studies (described in the next part of this article) the DMSO solutions of the compounds were used, therefore, their $^{31}P\{1H\}$, ^{1}H and $^{13}C\{1H\}$ NMR spectra were also recorded in the DMSO-d_6/D_2O (3:1 V:V) mixture under aerobic conditions (see Figure S13-S16†). They were very similar to the spectra recorded in $CDCl_3$, which showed a negligible influence of water and oxygen on all the studied complexes. Moreover, the phosphorus spectra were also recorded 3 days after dissolution of the compounds. They did not alter, indicating a high durability for the complexes in solutions. The same was observed for the UV-Vis spectra, also recorded in the DMSO/water mixture under aerobic conditions (see Figure 6). During 96 h of the experiments we observed no significant reduction in the intensity of the characteristic $(MX,MPR_3)LCT$[26, 61] absorption band. This was clear evidence of the absence of Cu(I) to Cu(II) oxidation processes under aerobic conditions in the presence of water and confirmed the validity of the choice of the diimine ligands.

Preliminary cytotoxicity studies

Cytotoxicity of the synthesised complexes, ligands (diimines, phosphines and phosphine oxides as potential decomposition products) and the starting compounds (CuNCS and Cu**I**) was tested *in vitro* towards two cancer cell lines: mouse colon carcinoma (CT26) and human lung adenocarcinoma (A549). We have undertaken to test these types of the cells because recently published statistics[62] has shown that, for the past two decades, lung cancer has ranked first and colon cancer second in terms of morbidity. However, the murine (CT26 cells) and human (A549) cancer cell lines selected for the study are examples of two of the most commonly used cell lines in preliminary studies of cytotoxicity *in vitro*. We decided to choose the murine cancer line because of its biological and pharmacological properties, which are representative of the properties of human colon carcinoma.[63]

Most of the compounds studied here are insoluble in aqueous media. Therefore, they were predissolved in DMSO for biological tests. The final concentrations of the compounds ranged between 0.1 and 0.001 mM. Cytotoxic activity was assessed on the basis of IC_{50} values (concentration of a drug required to inhibit the growth of 50% of the cells[64]). IC_{50} values were determined from the plots of cell viability in the presence of each compound (see Figure 7), with matching dose–response curves calculated using the Hill equation (Origin 9.0)[65]. The values of IC_{50} were confirmed by analysis of images of the stained tumour cells treated with the compounds at the corresponding concentrations (see Figure 8). Namely, cell viability was examined by counting the green cells (fluorescein diacetate – FDA stain) with normal nuclei, which were treated as surviving cells, and the red ones (propidium iodide – PI stain) treated as dead.

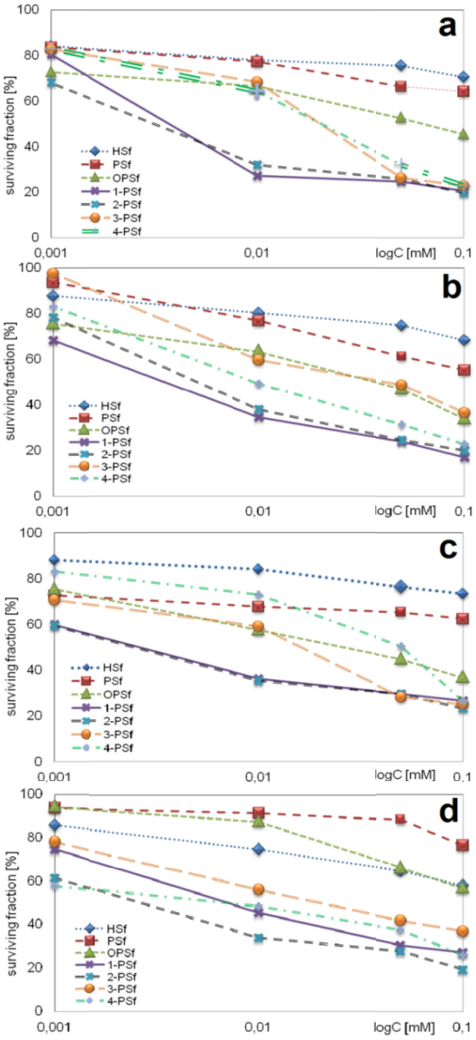

Fig.7 The percentage survival values of cancer lines in cytotoxic assay of **HSf**, **PSf**, **OPSf**, **1-PSf**, **2-PSf**, **3-PSf** and **4-PSf** for line A549: a) after 4h incubation, b) after 24h incubation, and for line CT26: c) after 4h incubation, d) after 24h incubation. X-axis: the concentration of the compound [mM] presented in the logarithmic scale; Y-axis: surviving fraction [%].

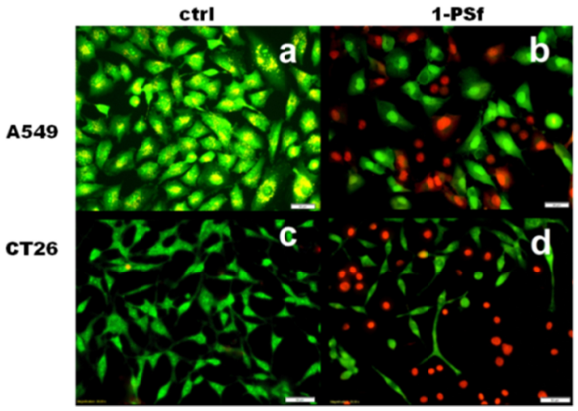

Fig.8 The photos (magnification 20.00×, bar 50 µm) of both cell lines after 24 h incubation with **1-PSf**. Line A549: a) control, b) with **1-PSf** in IC_{50} (7.84±0.16 µM) Line CT26: c) control, d) with **1-PSf** in IC_{50} (8.29±0.71 µM). The green cells with a normal morphology are viable ones (FDA), while round red cells are dead (PI) .

For a more complete description of the biological activity, two separate approaches were applied: cytotoxicity was measured after a short incubation time (4 h, then cells were washed and left in the medium for 24 h at 37°C) and a long (24 h) incubation time. This methodology can not only provide information about the toxicity of the compounds, but also about their penetration velocity to the cell, giving a closer insight into the cell death mechanism. Consequently, if after a short time a compound is characterised by high values of IC_{50}, and these values decrease with the increase of the incubation time, this means that the compound is cytotoxic and it needs a longer time to penetrate into the cell. Therefore, it is most likely that it has very weak inhibitory properties on the cells' growth. This effect can also be observed when a compound kills the cells in a particular phase of the cycle. The cells in different phases multiply, which implies a need for a longer incubation time. On the other hand, when the IC_{50} values are low and do not change with the increase in incubation time, this means that the studied compound is cytotoxic (most likely inhibiting the growth of tumour cells) and its penetration into the cell is rapid.

As shown in Figures 7-9 and Table S5†, the investigated substances exhibited a significant and diversified cytotoxic activity against both studied cell lines. The complexes had a higher cytotoxicity than the ligands, regardless of the cell type or the incubation time. Importantly, they were more cytotoxic then cisplatin. The complexes with **POH** exhibited a slightly higher cytotoxic activity than the uncomplexed ligands and starting compounds, but their cytotoxicity was relatively low in comparison with other copper(I) complexes of a general formula [CuX(NN)P][25] Moreover, almost no selective activity was observed for the **POH** complexes against the tested lines after a shorter incubation time (Fig. 9, Table S5†). The 24 h incubation gave a different picture. In the case of A549 line, **dmp** complexes (**1-POH**, **2-POH**) were twice as active as the **bq** ones (**3-POH**, **4-POH**). The activity against CT26 was slightly higher, but similar for both types of complexes (Fig. 11, Table S5†).

The cytotoxicity of the **PSf** complexes was significantly higher than the activity of **POH** ones and exhibited a different pattern. For both lines, after 4 h of incubation, the **dmp** complexes (**1-PSf**, **2-PSf**) were more cytotoxic but rather not selective. Their IC_{50} values were one order of magnitude lower compared to the values for the **bq** complexes, in which coordinating anions significantly affected the activity. The iodide complex **3-PSf** showed a similar cytotoxicity against both tested lines, while the thiocyanate complex **4-PSf** showed two times lower activity against CT26. In the case of 24 h incubation with most of **PSf** complexes, a similar cytotoxic activity, independent of the (pseudo)halide ion, was observed. Only **4-PSf** (NCS), after this time of incubation, significantly decreased its activity against A549, which attests to the high selectivity of this particular complex. Interestingly, the effect of the time prolongation on the cytotoxic activity is strongly dependent on the type of diimine. For the **dmp** complexes (**1-PSf**, **2-PSf**) only a slight increase in the IC_{50} values was observed. This suggested a significant contribution of cell growth inhibition in the general mechanism of cell death. However, in the case of the **bq** complexes (**3-PSf**, **4-PSf**), prolongation of the incubation time caused a large decrease of IC_{50}, which implied a different mechanism of cell death.

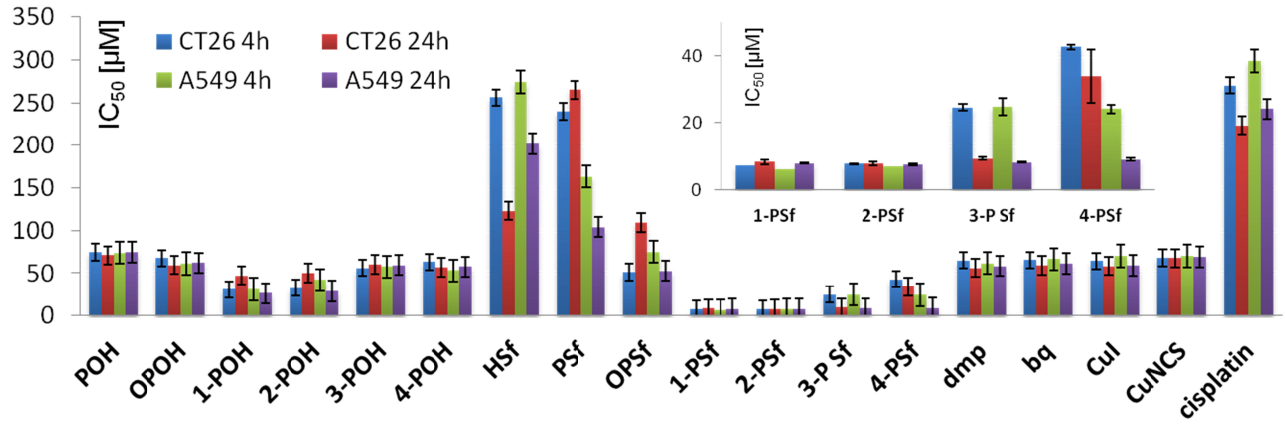

Fig.9 IC_{50} values [μM] for CT26 and A549 cell lines after 4 and 24h treatment with studied compounds, cisplatin and **HSf**, **PSf**, **OPSf**[34] (with the insert of IC_{50} for **PSf** complexes on the upper right side)

Experimental section

Materials

Reactions were carried out under a dinitrogen atmosphere using standard Schlenk techniques. $PPh_2(CH_2OH)_2Cl$[66], $PPh_2(CH_2OH)$[41], $OPPh_2(CH_2OH)$[67], $PPh_2(CH_2Sf)$ and $OPPh_2(CH_2Sf)$[34] were synthesised according to a literature procedures. Sparfloxacin, Ph_2PH and other small chemicals and solvents were purchased from Sigma-Aldrich (Germany) and used without further purifications. All cell culture fluids were purchased from IMMUNIQ (Poland).

Methods

Elemental analyses were performed on a Vario EL3 CHN analyser for C, H, and N, and they were within 0.3% of the theoretical values. Mass spectra were recorded on a Bruker

Daltonics micrOTOF–Q mass spectrometer equipped with electrospray ionization (ESI) source and operated in positive ion mode. NMR spectra were recorded on a Bruker AMX 500 spectrometer (at 298 K) with traces of solvent as an internal reference for ^{1}H ($CDCl_3$: 7.27 ppm) and ^{13}C spectra ($CDCl_3$: 77.0 ppm) and 85% H_3PO_4 in H_2O as an external standard for ^{31}P. The signals in the spectra are defined as: s = singlet (* – strongly broadened signal), d = doublet, dd – doublet of doublets, t = triplet and m = multiplet. Chemical shifts are reported in ppm and coupling constants are reported in Hz. Absorption spectra were recorded on a Cary 50 Bio spectrophotometer (Varian Inc., Palo Alto, CA) in the 800–200 nm range.

X-ray data were collected using a KM4–CCD diffractometer and graphite–monochromated MoKα radiation generated from Diffraction X–ray tube operated at 50 kV and 20 mA. The images were indexed, integrated, and scaled using the Oxford Diffraction data reduction package.[68] The structure was solved by direct methods (SHELXS Ver. 2013/1) and refined by the full–matrix least–squares method on all F2 data (SHELXL Ver. 2014/7).[69] Non H atoms were included in the refinement, with anisotropic displacement parameters and the H atoms was included from geometry of the molecule or found in a difference Fourier map. The data were corrected for absorption.[68]

Synthesis

General synthetic route of the POH complexes

Diimine (**dmp** or **bq**), copper (pseudo)halide (CuI or CuNCS) were added in equimolar ratios to the phosphine PPh_2CH_2OH (**POH** 0.200 – 0.250 g). The substrates were dissolved in 20 ml of deaerated acethonitrile. After few minutes the cloudy solutions were formed, which after half an hour became clear. After 12 h of stirring the precipitates formed. They were filtered and dried under vacuum. The complexes are well soluble in $CHCl_3$, DMSO and CH_2Cl_2, moderately in CH_3CN, poorly in ethanol and methanol and insoluble in water.

Characterisation of [CuI(dmp)POH] (1-POH):

Dark yellow solid. Yield: 65%, M = 614.92 g/mol. Anal. Calcd. for $PCuIC_{27}H_{25}N_2O$: C, 52.74; H, 4.10; N, 4.56%. Found: C, 52.71; H, 4.11; N, 4.57%.

NMR ($CDCl_3$, 298 K) : $^{31}P\{^{1}H\}$: -5.47s ^{1}H: H^{Ph}: 7.31-7.15; H^{1}: 4.76 s* (2H); $_{dmp}H^{3,8}$: 7.50 d (J = 8.20) (2H); $_{dmp}H^{4,7}$: 8.22 d (J = 8.20) (2H); $_{dmp}H^{5,6}$: 7.79 s (2H); $_{dmp}H^{15,16}$: 2.79 s (6H); $^{13}C\{^{1}H\}$: $C^{Ph(o)}$: 132.50 s*; $C^{Ph(m)}$: 128.50 d (J = 7.27); $C^{Ph(p)}$: 129.59 s; C^{1}: 63.10 d (J=23.63); $_{dmp}C^{2,9}$: 159.49 s; $_{dmp}C^{3,8}$:124.96 s; $_{dmp}C^{4,7}$: 136.64 s; $_{dmp}C^{5,6}$: 124.40 s; $_{dmp}C^{11,12}$: 143.09 s; $_{dmp}C^{13,14}$: 127.08 s; $_{dmp}C^{15,16}$: 26.77 s.

MS ($CHCl_3$): 479.1 (100% $Cu(dmp)_2^+$); 271.0 (45.5% $Cu(dmp)^+$); 199.1 (13.9%, $[PPh_2CH_2]^+$); 488.0 (5.7% $Cu(dmp)PPh_2CH_2OH^+$).

Single crystals were obtained from the CH_2Cl_2/MeOH solution.

Crystallographic data: **1-POH** ≡ $C_{27}H_{25}CuIN_2OP$, crystal size: 0.45x0.32x0.20 mm, crystal system: orthorhombic, space group Pbca, a = 9.904(3)Å, b = 14.416(4)Å, c = 34.669(8)Å, V = 4950(2) Å^3, D_{calcd}(Z = 8) = 1.650 g/cm^3, θ range for data collection: 3.055° – 28.732°, Mo Kα radiation (λ = 0.71073 Å), μ_{Mo} = 2.217 mm^{-1}, reflections collected/unique 27933 / 5928 [R(int) = 0.0267], final R indices [I>2σ(I)] R_1 = 0.0364, wR_2 = 0.0952, R indices (all data) R_1 = 0.0418, wR_2 = 0.0977, GOF = 1.056, largest diff. peak and hole: 1.997 and -0.795eÅ^{-3}, data/restraints/parameters: 5928 / 0 / 301, T = 100(2)K.

Characterisation of [CuNCS(dmp)POH] (2-POH):

Yellow solid. **Yield:** 49%, M = 546.10 g/mol. **Anal. Calcd.** for $PCuSC_{28}H_{25}N_3O$: C, 61.58; H, 4.61; N, 7.69%. Found: C, 61.56; H, 4.62; N, 7.66%. NMR ($CDCl_3$, 298 K) : $^{31}P\{^{1}H\}$: -2.85s ^{1}H: H^{Ph}: 7.32-7.14; H^{1}: 4.61 s* (2H); $_{dmp}H^{3,8}$: 7.49 d (J = 8.20) (2H); $_{dmp}H^{4,7}$: 8.21 d (J = 8.20) (2H); $_{dmp}H^{5,6}$: 7.75 s (2H); $_{dmp}H^{15,16}$: 2.71 s (6H); $^{13}C\{^{1}H\}$: $C^{Ph(o)}$: 132.32 d (J=12.72); $C^{Ph(m)}$: 128.56 d (J = 8.17); $C^{Ph(p)}$: 129.64 s; C^{1}: 62.36 d (J=20.89); $_{dmp}C^{2,9}$: 158.91 s; $_{dmp}C^{3,8}$:124.90 s; $_{dmp}C^{4,7}$: 136.76 s; $_{dmp}C^{5,6}$: 125.43 s; $_{dmp}C^{11,12}$: 142.78 s; $_{dmp}C^{13,14}$: 126.78 s; $_{dmp}C^{15,16}$: 26.27 s. MS ($CHCl_3$): 479.1 (100% $Cu(dmp)_2^+$); 271.0 (34.1% $Cu(dmp)^+$); 199.1 (17.2%, $[PPh_2CH_2]^+$); 488.0 (7.4% $Cu(dmp)PPh_2CH_2OH^+$).

Characterisation of [CuI(bq)POH] (3-POH):

Burgundy solid. Yield: 59%, M = 662.97 g/mol. Anal. Calcd. for $PCuIC_{31}H_{25}N_2O$: C, 56.16; H, 3.80; N, 4.22%. Found: C, 56.13; H, 3.82; N, 4.21%.

NMR ($CDCl_3$, 298 K) : $^{31}P\{^{1}H\}$: -3.01s* ^{1}H: H^{Ph}: 7.32-7.07; H^{1}: 4.43 s*(2H); $_{bq}H^{10}$: 8.34 d (J = 8.58) (1H); $_{bq}H^{9}$: 8.24 d (J = 8.58) (1H); $_{bq}H^{7}$: 7.90 d (J = 8.01) (1H); $_{bq}H^{6}$: 7.77 t (J=8.30) (1H); $_{bq}H^{5}$: 7.59 t (J = 7.44) (1H); $_{bq}H^{4}$: 8.86 d (J = 8.58) (1H); $^{13}C\{^{1}H\}$: $C^{Ph(i)}$: 132.84s*; $C^{Ph(o)}$: 131.32 d (J = 9.99); $C^{Ph(m)}$: 128.76 d (J = 11.81); $C^{Ph(p)}$: 129.54 s; C^{1}: 63.35 s*; $_{bq}C^{1}$: 156.23 s; $_{bq}C^{10}$: 119.42 s; $_{bq}C^{9}$: 136.74 s; $_{bq}C^{7}$: 127.64 s;$_{bq}C^{6}$: 126.94 s; $_{bq}C^{5}$: 129.50 s; $_{bq}C^{4}$: 129.92 s; $_{bq}C^{3}$: 147.92 s; $_{bq}C^{8}$: 128.44 s.

MS ($CHCl_3$): 575.1 (100% $Cu(bq)_2^+$); 319.0 (38.8% $Cu(bq)^+$); 536.06 (7.8% $Cu(bq)PPh_2CH_2OH)^+$); 199.1 (14.7%, $[PPh_2CH_2]^+$).

Crystals were obtained from the mixture of CH_2Cl_2 and MeOH (1:1 V:V).

Crystallographic data: 3-POH ≡ $C_{31}H_{25}CuIN_2OP$, crystal size: 0.18x0.15x0.10 mm, crystal system: triclinic, space group P-1, a = 10.234(1)Å, b = 10.495(1)Å, c = 14.945(2)Å, α = 103.77(1)°, β = 91.56(2)°, γ = 118.90(2)° V = 1345.7(3) Å3, D_{calcd}(Z = 2) = 1.636 g/cm^3, θ range for data collection: 2.847° – 36.841°, Mo Kα radiation (λ = 0.71073 Å), μ_{Mo} = 2.046 mm^{-1}, reflections collected/unique 22538 / 10413 [R(int) = 0.0772], final R indices [I>2σ(I)] R_1 = 0.0709, wR_2 = 0.1154, R indices (all data) R_1 = 0.1289, wR_2 = 0.1357, GOF = 1.025, largest diff. peak and hole: 1.167 and -0.917eÅ^{-3}, data/restraints/parameters: 10413 / 0 / 335, T = 100(2)K.

Characterisation of [CuNCS(bq)POH] (4-POH):

Dark Burgundy solid. Yield: 61%, M = 594.15 g/mol. Anal. Calcd. for $PCuSC_{32}H_{25}N_3O$: C, 64.68; H, 4.24; N, 7.07%. Found: C, 64.65; H, 4.25; N, 7.05%.

NMR ($CDCl_3$, 298 K) : $^{31}P\{^{1}H\}$: -3.19s* ^{1}H: H^{Ph}: 7.25-6.89 (10H); H^{1}: 4.73 s* (2H); $_{bq}H^{10}$: 8.20 s* (2H); $_{bq}H^{9}$: 8.07 s*(2H); $_{bq}H^{7}$: 7.60 s* (3H); $_{bq}H^{6}$: 7.60 s* (3H); $_{bq}H^{5}$: 7.51 s* (2H); $_{bq}H^{4}$: 8.77s* (2H); $^{13}C\{^{1}H\}$: $C^{Ph(o)}$: 132.47 s*; $C^{Ph(m)}$: 128.05 d (J = 8.17); $C^{Ph(p)}$: 128.72 s; C^{1}: 63.35 s*; $_{bq}C^{10}$: 118.96 s; $_{bq}C^{9}$: 137.93 s; $_{bq}C^{7}$: 127.30 s; $_{bq}C^{5}$: 129.43 s; $_{bq}C^{4}$: 130.64 s; $_{bq}C^{3}$: 146.16 s; $_{bq}C^{8}$: 128.36 s.

MS ($CHCl_3$): 575.1 (100% $Cu(bq)_2^+$); 319.0 (18.2% $Cu(bq)^+$); 536.06 (7.9% $Cu(bq)PPh_2CH_2OH)^+$); 199.1 (11.9%, $[PPh_2CH_2]^+$).

Preparation of the PSf complexes - general method.

Phosphine (**PSf** 0.200 – 0.250 g), diimine (**dmp** or **bq**) and copper (pseudo)halide (CuI or CuNCS) in equimolar ratios were dissolved in 25 ml of deaerated CH_3CN:$CHCl_3$ (4:1 V:V), initially forming cloudy solutions. Over some time, the solutions

became clear. They were stirred in the dark and the solid complexes precipitated out after 14 h. They are well soluble in DMSO, moderately in $CHCl_3$, CH_2Cl_2 and CH_3CN, slightly in methanol and ethanol, insoluble in water.

Characterisation of [CuI(dmp)PPh$_2$CH$_2$Sf] (1-PSf):

Dark yellow solid. Yield: 62%, M = 989.31 g/mol. Anal. Calcd. for $PCuIC_{46}H_{45}F_2N_6O_3$: C, 55.85; H, 4.58; N, 8.50%. Found: C, 55.82; H, 4.60; N, 8.48%.

NMR ($CDCl_3$, 298 K) : $^{31}P\{^1H\}$: -11.93s*, -28.42s*; ^1H: H^{Ph}: 7.87-7.15; H^1: 3.92 d (J = 5.05) (2H); $H^{2,6}$: 3.08 m (3H); $H^{3,5}$: 3.36 d (J = 13.61) (2H); $H^{7,8}$: 0.91 d (J = 6.22) (3H); H^{13}: 6.49 s* (2H); H^{19}: 8.65 s (1H); H^{21}: 3.92 m (2H); H^{22}: 1.23 m (4H); H^{22}: 1.12 d (J = 6.22) (10H); H^{23}: 14.53 s* (1H); $_{dmp}H^{3,8}$: 7.55 d (J = 8.16) (2H); $_{dmp}H^{4,7}$: 8.25 d (J = 8.16) (2H); $_{dmp}H^{5,6}$: 7.83 s (2H); $_{dmp}H^{15,16}$: 2.81 s (5H); $^{13}C\{^1H\}$: $C^{Ph(i)}$: 133.37 d (J = 15.44); $C^{Ph(o)}$: 132.71 d (J = 12.71); $C^{Ph(m)}$: 128.48 d (J = 9.08); $C^{Ph(p)}$: 129.52 s; C^1: 51.22 s*; $C^{2,6}$: 54.19 d (J = 7.27); $C^{3,5}$: 57.59 s*; $C^{7,8}$: 19.21 s; C^{12}: 136.55 dd (J = 238.5, J = 5.9); C^{13}: 134.48 dd (J = 10.9, J = 1.8); C^{14}: 105.97 d (J = 5.45); C^{15}: 128.13 dd (J = 5.9, J = 2.27); C^{16}: 139.98 dd (J = 237.0, J = 5.45); C^{17}: 180.13 s; C^{18}: 106.71 s; C^{19}: 149.32 s; C^{21}: 40.37 d (J = 14.53); C^{22}: 9.03 d (J = 8.17); C^{23}: 166.61 s; $_{dmp}C^{2,9}$: 159.46 s; $_{dmp}C^{3,8}$:124.93 s; $_{dmp}C^{4,7}$: 136.56 s; $_{dmp}C^{5,6}$: 125.37 s; $_{dmp}C^{11,12}$: 143.07 s; $_{dmp}C^{13,14}$: 127.06 s; $_{dmp}C^{15,16}$: 26.75 s. MS ($CHCl_3$): 479.1 (100% $Cu(dmp)_2^+$); 271.0 (32.5% $Cu(dmp)^+$); 861.26 (6.78% $Cu(dmp)PPh_2CH_2C_{19}H_{21}F_2N_4O_3)^+$); 199.1 (3.8%, $[PPh_2CH_2]^+$); 279.2 (56% $[C_{13}H_{10}N_2F_2O_3]^+$); 393.2 (7.6% $[C_{19}H_{23}F_2N_4O_3]^+$); 405.2 (1.1% $[C_{20}H_{25}F_2N_4O_3]^+$); 473.4 (19.2%); 529.5 (13.4% $[OPPhCH_2C_{19}H_{21}F_2N_4O_3]^+$).

Crystals of the mixed system **1-POH·1-PSf** suitable for X-ray analysis were obtained after 2 months by slow diffusion of diethyl ether into dichloromethane solution of **1-PSf**.

Crystallographic data: 1-POH·1-PSf ≡ $C_{73}H_{70}Cu_2I_2N_8O_4P_2$, crystal size: 0.12x0.10x0.05 mm, crystal system: monoclinic, space group C 2/c, a = 48.920(3)Å, b = 10.128(1)Å, c = 34.906(2)Å, β = 116.38(1)°, V = 15494(2) Å^3, D_{calcd}(Z = 8) = 1.375 g/cm^3, θ range for data collection: 2.848° – 36.986°, Mo Kα radiation (λ = 0.71073 Å), μ_{Mo} = 1.441 mm^{-1}, reflections collected/unique 64786 / 23137 [R(int) = 0.1823], final R indices [I>2σ(I)] R_1 = 0.0981, wR_2 = 0.1825, R indices (all data) R_1 = 0.2750*, wR_2 = 0.2562*, GOF = 1.008, largest diff. peak and hole: 0.777 and -0.687 eÅ^{-3}, data/restraints/parameters: 23137/0/845, T = 100(2)K. (**The crystal structure shows a three-dimensional structure with large open channels. Most probably, these channels were filled with solvent molecules (diethyl ether and dichloromethane), which during the preparation of the crystal for measurements evaporated. This led to a noticeable disorder of the structure, therefore, to refine the structure, the PLATON/SQUEEZE procedure was used.*[70,71])

Characterisation of [CuNCS(dmp)PPh$_2$CH$_2$Sf] (2-PSf):

Yellow solid. Yield: 51%, Molar mass: 920.51 g/mol. Anal. Calcd. for $PCuSC_{47}H_{45}F_2N_7O_3$: C, 61.36; H, 4.93; N, 10.66%. Found: C, 61.34; H, 4.94; N, 10.64%.

NMR ($CDCl_3$, 298 K) : $^{31}P\{^1H\}$: -8.76s*, -31.32s*; ^1H: H^{Ph}: 7.49-7.18; H^1: 3.92 d (J = 6.68) (2H); $H^{2,6}$: 3.01 m (4H); $H^{3,5}$: 3.33 d (J = 12.02) (2H); $H^{7,8}$: 0.88 d (J = 6.29) (6H); H^{13}: 6.47 s* (2H); H^{19}: 8.62 s (2H); H^{21}: 3.93 m (2H); H^{22}: 1.23 m (4H); H^{22}: 1.11 d (J = 6.29) (5H); H^{23}: 14.61 s* (1H); $_{dmp}H^{3,8}$: 7.50 d (J = 8.39) (2H); $_{dmp}H^{4,7}$: 8.19 d (J = 8.20) (2H); $_{dmp}H^{5,6}$: 7.74 s (2H); $_{dmp}H^{15,16}$: 2.76 s (5H); $^{13}C\{^1H\}$: $C^{Ph(i)}$: 133.21 d (J = 17.26); $C^{Ph(o)}$: 132.36 d (J = 12.71); $C^{Ph(m)}$: 128.59 d (J = 8.17); $C^{Ph(p)}$: 129.65 s; C^1: 51.25 s*; $C^{2,6}$: 54.32 d (J = 9.08); $C^{3,5}$: 57.47 t (J = 3.63); $C^{7,8}$: 19.26 s; C^{12}: 136.15 dd (J = 237.5, J = 5.9); C^{13}: 134.39 dd (J = 10.45, J = 4.09); C^{14}: 106.01 d (J = 5.45); C^{15}: 128.16 dd (J = 5.91, J = 2.27); C^{16}: 138.27 dd (J = 236.3, J = 5.90); C^{17}: 180.15 s; C^{18}: 106.75 s; C^{19}: 149.35 s; C^{21}: 40.41 dd (J = 14.53, 5.45); C^{22}: 9.05 dd (J = 7.72, J = 2.27); C^{23}: 166.63 s; $_{dmp}C^{2,9}$: 158.99 s; $_{dmp}C^{3,8}$:124.92 s; $_{dmp}C^{4,7}$: 136.61 s; $_{dmp}C^{5,6}$: 124.92 s; $_{dmp}C^{11,12}$: 142.90 s; $_{dmp}C^{13,14}$: 126.97 s; $_{dmp}C^{15,16}$: 26.27 s. MS ($CHCl_3$): 479.1 (100% $Cu(dmp)_2^+$); 271.0 (41.8% $Cu(dmp)^+$); 861.26 (4.78% $Cu(dmp)PPh_2CH_2C_{19}H_{21}F_2N_4O_3)^+$); 199.1 (5.1%, $[PPh_2CH_2]^+$); 279.2 (34.1% $[C_{13}H_{10}N_2F_2O_3]^+$); 393.2 (8.1.6% $[C_{19}H_{23}F_2N_4O_3]^+$); 405.2 (3.2% $[C_{20}H_{25}F_2N_4O_3]^+$); 473.4 (14.2%); 529.5 (9.1% $[OPPhCH_2C_{19}H_{21}F_2N_4O_3]^+$).

Characterisation of [CuI(bq)PPh$_2$CH$_2$Sf] (3-PSf):

Burgundy solid. Yield: 55%, Molar mass: 1036.35 g/mol. Anal. Calcd. for $PCuIC_{46}H_{45}F_2N_6O_3$: C, 57.89; H, 4.37; N, 8.10%. Found: C, 57.86; H, 4.39; N, 8.08%.

NMR ($CDCl_3$, 298 K) : $^{31}P\{^1H\}$: -10.48s*, -35.12 s*; ^1H: H^{Ph}: 7.36-6.93; H^1: 3.92 d (J = 3.43) (2H); $H^{2,6}$: 3.09 m (4H); $H^{3,5}$: 3.33 d (J = 12.02) (2H); $H^{7,8}$: 0.81 d (J = 5.72) (4H); H^{13}: 6.46 s* (3H); H^{19}: 8.63 s (2H); H^{21}: 3.92 m (2H); H^{22}: 1.21 m (4H); H^{22}: 1.11 d (J = 6.29) (4H); H^{23}: 14.60 s* (1H); $_{bq}H^{10}$: 8.15 s* (1H); $_{bq}H^9$: 7.89 s* (2H); $_{bq}H^{6,7}$: 7.49 m (6H); $_{bq}H^5$: 7.57 m (4H); $_{bq}H^4$: 8.92 (1H) s*; $^{13}C\{^1H\}$: $C^{Ph(i)}$: not observed ; $C^{Ph(o)}$: 133.51 d (J = 18.17); $C^{Ph(m)}$: 128.55 d (J = 7.27); $C^{Ph(p)}$: 128.38 s; C^1: 51.24 s*; $C^{2,6}$: 54.53 d (J = 9.99); $C^{3,5}$: 57.71 s*; $C^{7,8}$: 19.34 s; C^{11}: not observed; C^{12}: 136.59 dd (J = 237.5, J = 5.9); C^{13}: 134.48 dd (J = 13.63, J = 10.9); C^{14}: 106.01 d (J = 5.45); C^{15}: 128.16 dd (J = 5.9, J = 2.27); C^{16}: 140.01 dd (J = 236.6, J = 5.0); C^{17}: 180.16 s; C^{18}: 106.76 s; C^{19}: 149.39 s; C^{21}: 40.32 d (J = 14.53); C^{22}: 9.07 d (J = 7.27); C^{23}: 166.66 s; $_{bq}C^{10}$: 119.36 s; $_{bq}C^1$: not observed s; $_{bq}C^9$: 137.34 s; $_{bq}C^7$: 129.51 s; $_{bq}C^6$:128.70 s; $_{bq}C^5$: 130.59 s; $_{bq}C^4$: 131.02 s; $_{bq}C^3$: 146.13 s; $_{bq}C^8$: 129.66 s.

MS ($CHCl_3$): 575.1 (100% $Cu(bq)_2^+$); 319.0 (46.5% $Cu(bq)^+$); 909.26 (3.58% $Cu(bq)PPh_2CH_2C_{19}H_{21}F_2N_4O_3)^+$); 199.1 (3.8%, $[PPh_2CH_2]^+$); 279.2 (56% $[C_{13}H_{10}N_2F_2O_3]^+$); 393.2 (7.6% $[C_{19}H_{23}F_2N_4O_3]^+$); 405.2 (1.1% $[C_{20}H_{25}F_2N_4O_3]^+$); 473.4 (19.2%); 529.5 (13.4% $[OPPhCH_2C_{19}H_{21}F_2N_4O_3]^+$).

Characterisation of [CuNCS(bq)PPh$_2$CH$_2$Sf] (4-PSf):

Dark burgundy solid. Yield: 47%, Molar mass: 968.53 g/mol. Anal. Calcd. for $PCuSC_{47}H_{45}F_2N_7O_3$: C, 63.24; H, 4.68; N, 10.12%. Found: C, 63.22; H, 4.60; N, 10.11%.

NMR ($CDCl_3$, 298 K) : $^{31}P\{^1H\}$: -7.43s*, -28.69s*; ^1H: H^{Ph}: 7.33-7.01; H^1: 3.92s* (4H); $H^{2,6}$: 3.08 m (3H); $H^{3,5}$: 3.33 d (J = 11.06) (4H); $H^{7,8}$: 0.85 d (J = 6.10) (3H); H^{13}: 6.47 s* (2H); H^{19}: 8.61 s (1H); H^{21}: 3.92 m (4H); H^{22}: 1.22 m (3H); H^{22}: 1.11 d (J = 6.29) (3H); H^{23}: 14.69 s* (1H); $_{bq}H^{10}$: 8.12 s* (2H); $_{bq}H^9$: 7.62 s* (3H); $_{bq}H^{6,7}$: 7.44 m (2H); $_{bq}H^5$: 7.50 m (3H); $_{bq}H^4$: 8.65 s* (2H); $^{13}C\{^1H\}$: $C^{Ph(i)}$: 133.22 d (J = 16.35); $C^{Ph(o)}$: 132.50 d (J = 13.63); $C^{Ph(m)}$: 128.47 d (J = 9.08); $C^{Ph(p)}$: 128.60 s; C^1: 51.25 s*; $C^{2,6}$: 54.47 d (J = 4.54); $C^{3,5}$: 57.76 t (J = 3.18); $C^{7,8}$: 19.39 s; C^{11}: not observed; C^{12}: 136.02 dd (J = 237.0, J = 5.40); C^{13}: not observed; C^{14}: 106.71 s*; C^{15}: 128.16 dd (J = 6.36, J = 2.73); C^{16}: 139.35 dd (J = 236.5, J = 5.90); C^{17}: 180.23 s; C^{18}: 106.79 s; C^{19}: 149.42 s; C^{21}: 40.39 dd (J = 14.53; J = 0.91); C^{22}: 9.08 d (J = 8.17); C^{23}: 166.72 s; $_{bq}C^1$: not observed; $_{bq}C^{10}$: 118.89 s; $_{bq}C^9$: 136.98 s; $_{bq}C^7$: 129.16 s;

$_{bq}C^6$: 127.44 s; $_{bq}C^5$: 130.10 s; $_{bq}C^4$: 130.70 s; $_{bq}C^3$: 146.51 s; $_{bq}C^8$: 129.566 s.

MS ($CHCl_3$): 575.1 (100% $Cu(bq)_2^+$); 319.0 (37.2% $Cu(bq)^+$); 909.26 (4.17% $Cu(bq)PPh_2CH_2C_{19}H_{21}F_2N_4O_3)^+$); 199.1 (6.3%, $[PPh_2CH_2]^+$); 279.2 (37.9% $[C_{13}H_{10}N_2F_2O_3]^+$); 393.2 (3.1% $[C_{19}H_{23}F_2N_4O_3]^+$); 405.2 (3.1% $[C_{20}H_{25}F_2N_4O_3]^+$); 473.4 (21.5%); 529.5 (7.34% $[OPPhCH_2C_{19}H_{21}F_2N_4O_3]^+$).

DFT studies

DFT calculations were performed using the Gaussian 09 (Rev.D.01) package.[57] We employed the standalone functional of Truhlar and Zhao[72] (M06). The basis sets employed were 6-31G(d) for geometry optimization and 6-311+G(2d,p) for single-point calculations, except iodine atom, for which 6-311G(d,p) basis set[73] was used in both cases. The structures were optimized in the gas phase. Minima of energy were characterised as such by computation of the harmonic vibrational frequencies. Atomic charges were calculated using the fit to the electrostatic potential at points selected according to the Merz-Singh-Kollman scheme.[74]

Cytotoxicity studies

Cell cultures:

CT26 cell line (mouse colon carcinoma, morphology: fibroblast, ATCC: CRL-2638) and A549 cell line (human lung adenocarcinoma, morphology: epithelial, ATCC: CCL-185) were obtained from professor Luis G. Arnaut group (Chemistry Department, University of Coimbra, Portugal). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) without phenol red, supplemented with 10% fetal bovine serum (FBS) and with 1% streptomycin/penicillin. Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 (standard conditions). Cells were passaged at preconfluent density, using a solution containing 0.05% trypsin and 0.5 mM EDTA.

MTT assay:

The MTT assay (MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was carried out as described previously to measure cell viability.[65] Ten thousand cells in 200 µL of growth media were seeded in the wells of a 96-well plate. After 24 h, 200 µL of various concentrations of **POH**, **OPOH**, **1-POH**, **2-POH**, **3-POH**, **4-POH**, **1-PSf**, **2-PSf**, **3-PSf**, **4-PSf**, **dmp**, **bq**, **CuI**, **CuNCS** (0.1; 0.05; 0.01 and 0.001 mM) were added and incubated for 4 or 24 h at 37°C in a CO_2 incubator. The compounds were predissolved in DMSO and diluted in the respective medium with 1% FBS. In the first case, at the 4th hour of incubation, the contents of the plate were pipetted out carefully, cells were washed with PBS, and fresh relevant medium was added. Cells were incubated in standard conditions for 24 h and after that time MTT assay was carried out. In the second approach, after 24 h of incubation with tested compounds cells viability was assessed by MTT test. Surviving fraction was calculated as described elsewhere.[65] The viability was calculated with regard to the untreated cells control. The IC_{50} values were determined using Hill equation (Origin 9.0).[64] All experiments were replicated twice with triplicate.

Fluorescence microscopy:

Viable and dead cells were detected by staining with fluorescein diacetate (FDA, 5 mg/L) and propidium iodide (PI, 5 mg/L) for 20 min and examined using fluorescence inverted microscope (Olympus IX51, Japan) with an excitation filter of 470/20 nm. Photographs of cells after treatment with the tested compounds were taken under magnification 20×.

Conclusions

We synthesised eight new complexes of copper(I) iodide and copper(I) thiocyanate with two diimines (**dmp** or **bq**) and phosphine ligands: hydroxymethyldiphenylphosphine ($PPh_2(CH_2OH)$, **POH**) or phosphine derivative of sparfloxacin, a 3rd generation fluoroquinolone antibiotic ($PPh_2(CH_2$-Sf), **PSf**). Spectroscopic studies showed that all the complexes are stable in solutions for several days, also in the presence of water and oxygen. Analysis of NMR spectra showed that **PSf** binds to the copper(I) ion in a different pattern than other phosphine ligands we have obtained so far. For complexes described in this work two signals appeared on $^{31}P\{^1H\}$ NMR spectrum in contrast to previously synthesised compounds,[26,34,36,65] where only one phosphorus signal was observed. This can only be explained by the high lability of the **PSf** complexes caused by this ligand's great steric requirements, which are related to the presence of the $-CH_3$ groups on the piperazine ring.

An analysis of the **1-PSf** structure showed that, contrary to **HSf**[55-56] and **OPSf**[34], the methyl groups on the piperazine ring occupy the axial positions. Such conformation of the ring reduces the size of the entire ligand and probably facilitates the coordination process. DFT studies supported this explication. Unlike in the case of the free ligand, the structure of the **1-PSf** molecule with the discussed methyl groups in axial positions (**1-PSf-ax**) was characterised by a slightly lower energy than the structure with the -CH_3 groups in equatorial positions (**1-PSf-eq**).

Cytotoxic activity of the complexes and employed ligands was tested against two cancer lines (CT26 - mouse colon carcinoma and A549 - human lung adenocarcinoma). The studies, which applied two different approaches (4 and 24 h incubation times), enabled preliminary estimation of the influence of diimine and phosphine ligands on the selectivity and mechanism of action. We have found that the type of phosphine ligand had a decisive impact on the cytotoxic properties of the studied coordination compounds. The **PSf** complexes were much more active than the **POH** ones. This was undoubtedly related to the presence of the -**Sf** fragment. Although sparfloxacin, as a free molecule, was characterised by a small cytotoxic effect, its attachment to the copper(I) complex *via* -CH_2PPh_2 moiety, highly elevated the cytotoxicity. It should be emphasised that the type of diimine ligand significantly affected the overall action mechanism. However, other factors related to the cell cycles or cell aging cannot be excluded,[23,34] which needs further investigation.

Proven stability in the air and water, and the high cytotoxicity of the **PSf** complexes presented herein indicate their potential usefulness in the development of new anticancer agents. However, further, mechanistic studies are required. Work on the transport into tumour cells and the mechanism of cell death will be presented in our next paper.

Acknowledgements

The authors gratefully acknowledge financial support from the Polish National Science Centre (Grant 2011/03/B/ST5/01557). The DFT calculations have been carried out in Wroclaw Centre for Networking and Supercomputing (http://www.wcss.wroc.pl), grant No. 140. The *in vitro* research was carried out with the equipment purchased thanks to the financial support of the European Regional Development Fund in the framework of the Polish Innovation Economy Operational Program (contract no. POIG.02.01.00–12–023/08)

Notes and references

[a] *Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, E-mail: malgorzata.jezowska-bojczuk@chem.uni.wroc.pl*
[b] *Faculty of Chemistry, Jagiellonian University, R. Ingardena 3, 30-060 Kraków, Poland.*

† Electronic Supplementary Information (ESI) available: the NMR spectra and the NMR data, a table with IC_{50} values [μM] for CT26 and A549 cell lines after 4 and 24 h treatment with the complexes and ligands. See DOI: 10.1039/b000000x/. CCDC-1051189, 1051190 and 1051191 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 union Road, Cambridge CB2 1EZ, UK; e-mail: deposit@ccdc.cam.ac.uk)]

1 A. Jemal, R. Siegel, E. Ward, T. Murray, J. Xu, C. Smigal and M. J. Thun, *CA Cancer J Clin.*, 2006, **56**, 106.
2 B. K. Edwards, M. L. Brown, P. A. Wingo, H. L. Howe, E. Ward, L. A. G. Ries, D. Schrag, P. M. Jamison, A. Jemal, X. Cheng Wu, C. Friedman, L. Harlan, J. Warren, R. N. Anderson and L. W. Pickle; *J Natl Cancer Inst*, 2005, **97**, 1407.
3 M. J. Renan. *Mol. Carcinog.*, 1993, **7**, 139.
4 D. Hanahan and R. A. Weinberg, *Cell*, 2000, **100**, 57.
5 L. Juillerat-Jeanneret and F. Schmitt, *Med. Res. Rev.*, 2007, **3**, 574.
6 A. A. Rosenkranz, D.A. Jans and A.S. Sobolev, *Immunol Cell Biol.*, 2000, **78**, 452.
7 P. Dozzo, M.S. Koo, S. Berger, T.M. Forte and S.B. Kahl, *J Med. Chem.*, 2005, **48**, 357.
8 Y. Berger, L. Ingrassia, R. Neier and L. Juillerat-Jeanneret, *Bioorg. Med. Chem.*, 2003, **11**, 1343.
9 T. W. Hambley, *Dalton Trans.*, 2007, 4929.
10 C. Orvig and M. J. Abrams, *Chem. Rev.*, 1999, **99**, 2201.
11 K. H. Thompson and C. Orvig, *Dalton Trans*., 2006, 761.
12 C. Santini, M. Pellei, V. Gandin, M. Porchia, F. Tisato, and C. Marzano, *Chem. Rev.*, dx.doi.org/10.1021/cr400135x.
13 M. Navarro, J. E. Cisneros-Fajardo, T. Lehmann, R. A. Sánchez-Delgado, R. Atencio, P. Silva, R. Lira, and Julio A. Urbina, *Inorg. Chem*., 2001, 27, 6879.
14 J. B. Delehanty, J. E. Bongard, D. C. Thach, D. A. Knight, T. E. Hickey and E. L. Chang, *Bioorg. Med. Chem.*, 2008, 16, 830.
15 N.Margiotta, A.Bergamo, G. Sava, G. Padovano, E. de Clercq and G. Natile, *J Inorg. Biochem*., 2004, **98**, 1385.
16 I. Kostova, *Anti-Can.r Drug Disc*., 2006, **1**, 1.
17 A. Bakalova, *J.Uni. of Chem. Tech. and Metal.*, 2006, **41**, 119.
18 B.-E. Kim, T. Nevitt and D. J. Thiele, *Nat. Chem. Biol.*, 2008, **4**,176.
19 F. Tisato, C. Marzano, M. Porchia, M. Pellei and C. Santini, *Med.Res. Rev.*, 2010, **30**, 708.
20 C. Marzano, M. Pellei, F. Tisato and C. Santini, *Anti-Cancer AgentsMed. Chem.*, 2009, **9**, 185.
21 S. Tardito and L. Marchio, *Curr. Med. Chem.*, 2009, **16**, 1325.
22 T. Wang and Z. J. Guo, *Curr. Med. Chem.*, 2006, **13**, 525.
23 R. Starosta, A. Bykowska, A. Kyzioł, M. Płotek, M. Florek, J. Król and M. Jeżowska-Bojczuk *Chem. Biol. Drug Des.*, 2013, **82**, 579.
24 R. Starosta, K. Stokowa, M. Florek, J. Król, A. Chwiłkowska, J. Kulbacka, J. Saczko, J.Skała and M. Jeżowska-Bojczuk, *J. Inorg. Biochem.*, 2011, **105**, 1102.
25 C. Santini, M. Pellei, V. Gandin, M. Porchia, F. Tisato and C. Marzano, *Chem. Rev.*, 2014, **114**, 815.
26 R. Starosta, A. Brzuszkiewicz, A. Bykowska, U. K. Komarnicka, B. Bazanów, M. Florek, Ł. Gadzała, N. Jackulak, J. Król and K. Marycz, *Polyhedron*, 2013, 50, 481.
27 G. Borkow and J. Gabbay, *FASEB Journal*, 2004, **18**, 1728.
28 J. O. Noyce, H. Michels, and C. W. Keevil, *Appl. Environ. Microbiol.*, 2007, **73,** 2748.
29 F. Lebon, N. Boggetto, M. Ledecq, F. Durant, Z. Benatallah, S. Sicsic, R. Lapouyad, O. Kahn, A. Mouithys-Mickalad, G. Deby-Dupont and M. Reboud-Ravaux, *Biochem. Pharmacol.*, 2002, **63**, 1863.
30 B. Dudová, D. Hudecová, R. Pokorný, M. Micková, M. Palicová, P. Segĺa and M. Melník, *Folia Microbiol. (Praha)*, 2002, **47**, 225.
31 J. E. Weder, C. T. Dillon, T. W. Hambley, B. J. Kennedya, P. A. Laya, J. R. Biffin, H. L. Regtop and N. M. Davies, *Coord. Chem. Rev.*, 2002, **232,** 95.
32 V. Gandin, F. Tisato, A. Dolmella, M. Pellei, C. Santini, M. Giorgetti, C. Marzano and M. Porchia, *J. Med. Chem.*, 2014, **57**, 4745.
33 R. Bortolozzi, G. Viola, E. Porcù, F. Consolaro, C. Marzano, M. Pellei, V. Gandin and G. Basso, *Oncotarget*, 2014, **5**, 5978.
34 U. K. Komarnicka, R. Starosta, K. Guz-Regner, G. Bugla-Płoskońska, A. Kyzioł and M. Jeżowska-Bojczuk, *J. Mol. Struct.* DOI 10.1016/j.molstruc.2015.04.044
35 C. Marzano, M. Pellei, F. Tisato and C. Santini, *Med. Chem.*, 2009, **9**, 185.
36 R. Starosta, M. Florek, J. Król, M. Puchalska and A. Kochel, *New J. Chem.*, 2010, **34**, 1441.
37 F.J. Ramos-Lima, A.G. Quiroga, J.M. Perez, M. Font-Bardia, X. Solans and C. Navarro-Ranninger, Eur. *J. Inorg. Chem.*, 2003, 1591.
38 W. Henderson and S. R. Alley, *Inorg. Chim. Acta*, 2001, **322**, 106.
39 A. Lavie-Cambot, M. Cantuel, Y. Leydet, G. Jonusauskas, D. M. Bassani and N. D. McClenaghan, *Coord. Chem. Rev.*, 2008, **252**, 2572.
40 D.V. Scaltrito, D.W. Thompson, J.A. O'Callaghan and G.J. Meyer, *Coord. Chem. Rev.*, 2000, **208**, 243.
41 O. Kuhl, S. Blaurock, J. Sieler and E. Hey-Hawkins, *Polyhedron*, 2001, **20**, 2171.
42 J. J. Schentag, *Clin. Ther.*, 2000, **22**, 4.
43 J. Gong, X. Liu, B. Shang, S. Chen and Y. Zhen, *Oncol. Rep.*, 2010, **23**, 1747.
44 V. Uivarosi, *Molecules*, 2013, **18,** 11153.
45 E. K. Efthimiadou, A. Karaliota and G. Psomas, *J. Inorg. Biochem.*, 2010,**104**, 455.
46 E. K. Efthimiadou, A. Karaliota and G. Psomas, *Bioorg. Med. Chem. Lett.*, 2008, **18**,4033.
47 E. K. Efthimiadou, Y. Sanakis, C. P. Raptopoulou, A. Karaliota and N. Katsaros, G. Psomas, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 3864.
48 E. K. Efthimiadou, A. Karaliota and G. Psomas, *Polyhedron*, 2008, **27**, 349.
49 M. N. Patel, D. S. Gandhi and P. A. Parmar, *Inorg. Chem. Commun.*, 2012, **15**, 248.
50 L. M. M. Vieira, M. V. de Almeida, M. C. S. Lourenço, F. A. F. M. Bezerra and A. P. S. Fontes, *Eur. J. Med. Chem.*, 2009, **44**, 4107.
51 L. R. Gouvea, L. S. Garcia, D. R. Lachter, P. Roberta Nunes, F. de Castro Pereira, E. P. Silveira-Lacerda, S. R.W. Louro, P. J. S. Barbeira and L. R. Teixeira, *Eur. J. Med. Chem.*, 2012, **55**, 67.
52 D. Shingnapurkar, R. Butcher, Z. Afrasiabi, E. Sinn, F. Ahmed, F.Sarkar and S. Padhye, *Inorg. Chem. Commun.*, 2007, **10**, 459.
53 S. Peter, J. K. Cockcroft, T. Roisnel and H. D. Lutz, *Acta Cryst.,* 1996,. **B52**, 423.
54 C. Janiak, *J. Chem. Soc., Dalton Trans.*, **2000**, 3885.
55 A. Llinàs, J.C. Burley, T.J. Prior, R.C. Glen and J.M. Goodman, *Cryst. Growth Des*., 2008, **8**, 115.
56 A. Sivalakshmidevi, K. Vyas and G. Om Reddy; *Acta Crystallogr. Sect. C*, 2000, **56**, 115.
57 Gaussian 09, Revision D.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G.

Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, Gaussian, Inc., Wallingford CT, 2013

58 M. Yagi, M. Torii, N. Yamauchi, T. Kaneshima and J. Higuchi, *Chem. Phys. Lett.*, 1991, **187**, 604.

59 M.H. Zaghai and H.A. Qaseer, *Transition Met. Chem.*, 1991, **16**, 39.

60 K. Folting and L.L. Merritt, *Acta Cryst.*, 1977, **B33**, 3540.

61 H. Suh, D. J. Casadonte Jr., L. Hope-Weeks, H. Kim, B. Kim and T. Chang, *Inorg. Chim. Acta.*, 2013, **394**, 710.

62 R. L. Siegel, K. D. Miller and A. Jemal, *Cancer J. Clin.*, 2015, **65**, 5.

63 M. G. Brattain, J. Strobel-Stevens, D. Fine, M. Webb and A. M. Sarrif, *Cancer Res*., 1980, **40**, 2142.

64 K. Strohfeldt and M. Tacke, *Chem. Soc. Rev.*, 2008, **37**, 1174.

65 J. Weyermann, D. Lochmann and A. Zimmer, *Int. J. Pharm.*, 2005, **288**, 369.

66 J. Fawcett, P. A. T. Hoye, R. D. W. Kemmitt, D. J. Law and D. R. Russell, *Dalton Trans.*, 1993, 2563.

67 L. Feng, E. Urnezius and R. L. Luck, *J. Organomet. Chem.*, 2008, **693**, 1564.

68 Oxford Diffraction, CRYSALIS–CCD and CRYSALIS–RED, Oxford Diffraction Ltd, Abingdon, England, 2010.

69 G. M. Sheldrick, *Acta Cryst.,* 2008, **A64**, 112.

70 P. v.d. Sluis and A. L. Spek, *Acta Cryst.*, 1990, **A46**, 194

71 A. L. Spek, *Acta Cryst.*, 2009, **D65**, 148

72 Y. Zhao and D. G. Truhlar, *Theor. Chem. Acc.*, 2008, **120**, 215.

73 D. Feller, *J. Comp. Chem.*, 1996, **17**, 1571; K.L. Schuchardt, B. T. Didier, T. Elsethagen, L. Sun, V. Gurumoorthi, J. Chase, J. Li and T. L. Windus, *J. Chem. Inf. Model.*, 2007, **47**, 1045

74 B. H. Besler, K. M. Merz Jr. and P. A. Kollman, *J. Comp. Chem.*, 1990, **11**, 431.