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Cytotoxic Activities of Bis-cyclometalated *M*(III) Complexes (*M* = Rh, Ir) Containing 5-substituted 1,10-Phenanthroline or 4,4′-substituted 2,2′-Bipyridine Ligands

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Dedicated to Professor Wolfgang Schnick on the Occasion of his 65th Birthday

The synthesis and characterization of eight new bis-cyclometalated compounds $[M(ptpy)_2(N\hat{}N)]PF_6$ (ptpy = 2-(*p*-tolyl)pyridinato; N^N = 5-chloro-1,10-phenanthroline: *M* = Rh, **1**; M = Ir, **2**); N^N = 5-methyl-1,10-phenanthroline: *M* = Rh, **3**; M = Ir, **4**; N^N = 4,4′-diphenyl-2,2′-bipyridine: *M* = Rh, **5**; *M* = Ir, **6**; N^N = 4,4′-diamino-2,2′-bipyridine: *M* = Rh, **7**; *M* = Ir, **8**) are described. All compounds were characterized by spectroscopic means. Additionally, the molecular structures of compounds **4**, **5**, **6**, and **8** in the crystal were determined by single-crystal X-ray diffraction studies. To explore the cytotoxic properties of all new eight compounds, colorimetric assays (MTT assay) against prominent cancer cell lines, MCF-7 and HT-29, were performed. The determined IC_{50} values are in the low micromolar range, between 1.3-5.6 μM. The most effective compounds are **1** and **2** with 5-chloro-substituted phenanthroline ligands, whereas the diamino-substituted bipyridine ligands (**5** and **6**) are the least cytotoxic compounds. The tested complexes showed a significant increase in cytotoxicity, up to 25-fold increase in MCF-7 cancer cells, and up to 60-fold increase in the HT-29 cell line compared to the established anticancer compound cisplatin under identical conditions.

Introduction

Currently metal complexes have been widely studied in the development of new pharmaceutical metal-containing drugs.[1] In this field, cyclometalated iridium(III) complexes belong to compounds of potential candidates because they play a crucial role in studies of cancer therapy due to their high cytotoxic activities.[2] A tuning of the anticancer properties of these species can be realized by modifications of the cyclometalated as well as the ancillary ligands.[3] Moreover, these compounds are currently a well-established class of organometallic complexes with advantageous potential applications in biology and medicine.[4] In this context, they were also studied in bioimaging and biosensing applications.[5] Very recently such compounds were examined in cancer treatment procedures using the photodynamic therapy (PDT) where they act as promising candidates of photosensitizers to support the generation of reactive oxygen species (ROS).[6] In the course of related investigations we have a current interest in studies of the cytotoxic activity of bis-cyclometalated iridium(III) compounds towards some human cancer cell lines by examining the substituent influences in the sphere of the ancillary ligands.[7] Thus, we investigated the cytotoxic activities of some derivatives containing modified phenanthroline, and related bipyridine ligands respectively.[8,9] To continue our efforts in this field we describe herein the synthesis and the characterization of eight new bis-cyclometalated *M*(III) complexes (*M* = Rh, Ir) as well as the evaluation of the cytotoxic activities of these compounds. The reported species bear the ancillary ligands 5-chloro-1,10-phenanthroline (5-Cl-phen, compound **1** and **2**), 5-methyl-1,10-phenanthroline (5-Me-phen, compound **3** and **4**) on the one hand, and 4,4′-diphenyl-2,2′-bipyridine (dph-bpy, compound **5** and **6**) and 4,4′-diamino-2,2′-bipyridine (da-bpy, compound **7** and **8**) on the other hand. During these investigations the molecular structures of the compounds **4**, **5**, **6**, and **8** in the solid were determined by single-crystal X-ray diffraction studies. The main focus of the investigations therein was directed on studies of the biological activity of the new compounds **1**–**8** using MTT assays towards the prominent cancer cell lines MCF-7 (human breast adenocarcinoma) and HT-29 (colon adenocarcinoma) respectively. MTT- assay is a colorimetric assay, measuring the cell viability via metabolic

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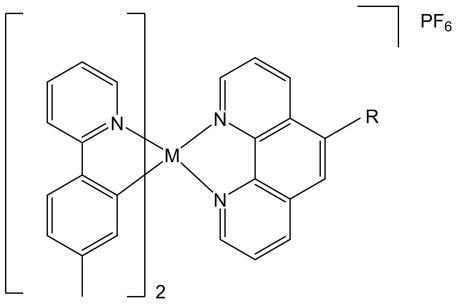
processes and hence, the cytotoxicity, due to the loss of cells. The half inhibitory concentration (IC_{50}) corresponds to the half maximum concentration of the remaining viable cells, after treatment with the tested compound for 48 h. Living cells are capable to reduce the yellow dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide) to its insoluble violet formazan, whose absorbance is quantitatively measured. The potent *in vitro* antiproliferative activities of these new compounds were compared with the reference cisplatin as the usual standard.

Results and Discussion

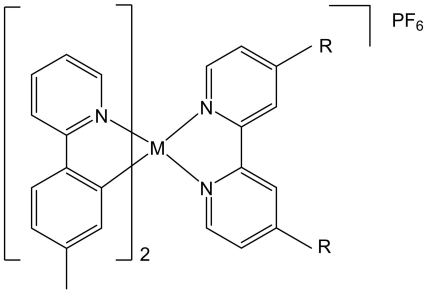
The preparation of the cationic mononuclear title complexes was realized by cleavage of the complexes $[\{M(\mu\text{-Cl})(ptpy)_2\}_2]$ (M = Rh, Ir; ptpy = 2-(*p*-tolyl)pyridinato) by the chelating phenanthroline or bipyridine ligands in a mixture of dichloromethane/methanol/water under reflux conditions. The intermediate formed chloride salts yielded after metathesis with KPF_6 their corresponding hexafluoridophosphate compounds **1**–**8** (see Eq. 1 and Scheme 1 respectively). All compounds were obtained as yellow crystalline solids in good yield and characterized by elemental analysis, ^1H and $^{13}C\{^1H\}$ NMR spectroscopy, mass spectrometry and additionally for **4**, **5**, **6**, and **8** by single-crystal X-ray diffraction studies. The ^1H and $^{13}C\{^1H\}$ NMR spectra of all new compounds confirmed their molecular constitution (see Experimental Section).

$$[\{M(\mu\text{-Cl})(ptpy)_2\}_2] + 2\ N\hat{}N + 2\ KPF_6 \rightarrow [M(ptpy)_2(N\hat{}N)]PF_6 + 2\ KCl \quad (1)$$

(M = Rh, Ir; N^N = substituted phen and bpy ligands respectively: see Scheme 1)



M = Rh, R = Cl (**1**)
M = Ir, R = Cl (**2**)
M = Rh, R = Me (**3**)
M = Ir, R = Me (**4**)



M = Rh, R = Ph (**5**)
M = Ir, R = Ph (**6**)
M = Rh, R = NH_2 (**7**)
M = Ir, R = NH_2 (**8**)

Scheme 1. Graphical overview on compounds **1**–**8**.

Molecular Structure of Compounds 4, 5, 6 and 8

Compound **4** crystallized from a mixture of dichloromethane/methanol/*iso*-hexane at room temperature by the diffusion method in the monoclinic space group $P2_1/n$ with four molecules in the unit cell. A selected ORTEP view of the molecular structure of the cation in **4** is shown in Figure 1, selected bond lengths and angles are given in the caption.

The complex cation of **4** exhibits two cyclometalated 2-(*p*-tolyl)pyridinato ligands beside the 5-methyl-1,10-phenanthroline ligand completing a distorted octahedral coordination arrangement around the iridium atom. The coordination sphere of the cation in **4** is, for example, comparable with a series of complex cations $[Ir(ppy)_2(5R\text{-phen})]^+$ (ppy = phenylpyridinato; R = Cl, Br, PhR) investigated by X-ray crystallography.[10] In the coordination sphere of the central Ir atom in **4**, the N atoms of the pyridyl rings and cyclometalated C atoms of the *p*-tolyl rings are in the trans- and cis-positions, respectively, with respect to iridium, and the two N atoms in the 5Me-phen ligand are located in the trans-position relative to the cyclometalated C atoms. With respect to the complex $[Ir(ppy)_2(5Cl\text{-phen})]^+$, the

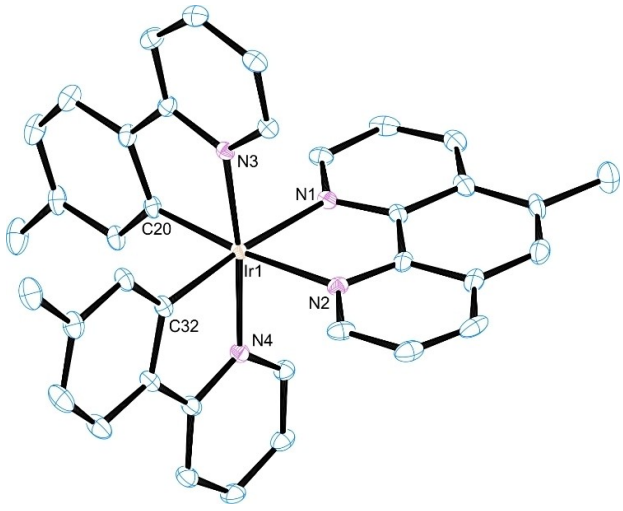


Figure 1. The molecular structure of the cation of compound **4** in the crystal (ORTEP drawing and atom labeling scheme with 25% probability level). Hexafluoridophosphate and hydrogen atoms are omitted for clarity. Selected bond lengths/Å and angles/°: Ir1–N1, 2.148(4); Ir1–N2, 2.131(4); Ir1–N3, 2.047(4); Ir1–N4, 2.047(4); Ir1–C20, 2.017(5); Ir1–C32, 2.011(5). N1–Ir1–C32, 174.35(18); N2–Ir1–C20, 173.30(18); N3–Ir1–N4, 173.32(15); C32–Ir1–N4, 80.39(18); C20–Ir1–N3, 80.41(18); N1–Ir1–N2, 77.47(16).

corresponding bond angles for the latter were reported as: C1–Ir1–N1, 80.3(2); C12–Ir1–N2, 80.1(2) and N3–Ir1–N4, 77.2(2),[10] (for **4** compare caption in Figure 1). Also the Ir–C and Ir–N bond parameters of **4** are in a good accordance with the observed ones in the before mentioned series of closely related iridium complex cations.[10]

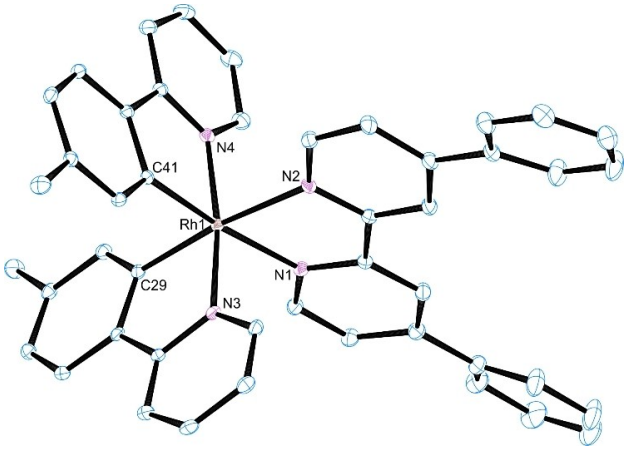


Figure 2. The molecular structure of the cation of compound **5** in the crystal (ORTEP drawing and atom labeling scheme with 25% probability level). Hexafluoridophosphate and hydrogen atoms are omitted for clarity. Selected bond lengths/Å and angles/°: Rh1–N1, 2.148(2); Rh1–N2, 2.141(2); Rh1–N3, 2.043(2); Rh1–N4, 2.047(2); Rh1–C29, 1.996(2); Rh1–C41, 1.999(2). N1–Rh1–C41, 173.81(9); N2–Rh1–C29, 173.45(9); N3–Rh1–N4, 171.86(8); N1–Rh1–N2, 75.87(8); N3–Rh1–C29, 81.19(9); N4–Rh1–C41, 81.31(9).

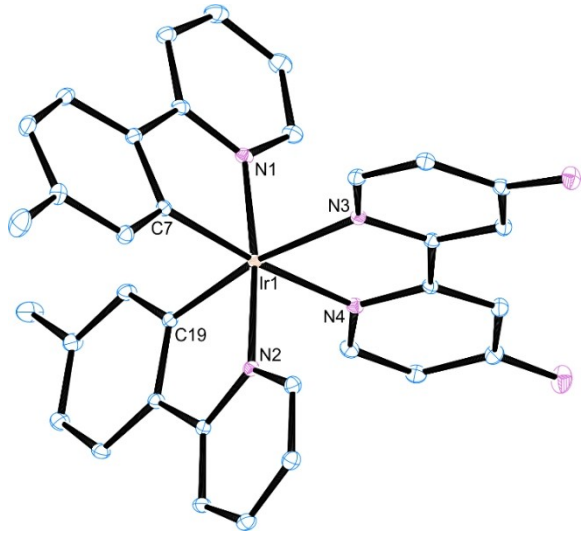


Figure 3. The molecular structure of the cation of compound **8** in the crystal (ORTEP drawing and atom labeling scheme with 25% probability level). Hexafluoridophosphate and hydrogen atoms are omitted for clarity. Selected bond lengths/Å and angles/°: Ir1–N1, 2.0411(18); Ir1–N2, 2.0503(17); Ir1–N3, 2.1429(17); Ir1–N4, 2.1318(18); Ir1–C7, 2.011(2); Ir1–C19, 2.011(2). N1–Ir1–N2, 173.35(7); N3–Ir1–C19, 169.73(7); N4–Ir1–C7, 174.54(7); N3–Ir1–N4, 76.53(7); N1–Ir1–C7, 80.53(8); N2–Ir1–C19, 80.51(8).

Even compound **5** crystallized from a mixture of dichloromethane/methanol/*iso*-hexane at room temperature by the diffusion method in the monoclinic space group $P^{-}1$ with two molecules in the unit cell. A selected ORTEP view of the molecular structure of the cation in **5** is shown in Figure 2, selected bond lengths and angles are given in the caption.

Compound **6** crystallized from a mixture of dichloromethane/methanol/*iso*-hexane at room temperature in the triclinic space group $P^{-}1$ with two molecules in the unit cell. In the literature, the closely related compound $[Ir(ppy)_2(dph\text{-}bpy)]PF_6$ (ppy = phenylpyridinato) was described including the determination of its molecular structure in the crystal.[11] For this reason, we refrain of a deeper discussion of the molecular structure of **6** at this point (more details are deposited with the Supporting Information of this paper).

Compound **8** crystallized from a mixture of dichloromethane/methanol/*iso*-hexane at room temperature in the monoclinic space group $P2_1/n$ with four molecules in the unit cell. A selected ORTEP view of the molecular structure of the cation in **8** is shown in Figure 3, selected bond lengths and angles are given in the caption. In all the therein described substituted bipyridine complexes, the bite angle of the ancillary bpy ligand (~76°) is somewhat smaller than that in the cyclometalated one (~80°) which is consistent with findings reported in the literature.[12] In the last years, we confirmed these observations for some closely related rhodium(III) and iridium(III) compounds bearing 4,4′-dichloro-2,2′-bipyridine as the ancillary ligand.[13] All in all, the inspection of the Ir–N and Ir–C bond parameters including the corresponding bond angles of compounds **5**, **6**, and **8** afforded a very good accordance with the usually observed ones in closely related compounds reported by us.[7]

Biological Activity of Compounds 1–8

The antiproliferative activity of the new synthesized rhodium(III) and iridium(III) compounds (**1**–**8**) was investigated by determining the IC_{50} value of each species against the cancer cell lines MCF-7 (human breast adenocarcinoma) and HT-29 (colon adenocarcinoma) via MTT assays. The experiments were all performed as triplicates with cisplatin as a positive control. An overview of the resulting IC_{50} values is shown in Table 1 and

Table 1. IC_{50} values in µM of **1**–**8** for the antiproliferative activity towards the cancer cell lines MCF-7 and HT-29 with cisplatin as control reference. MTT assay, 48 h incubation.

Compound	MCF-7	HT-29
1 (Rh-5-Cl-phen)	2.57 ± 0.12	1.28 ± 0.36
2 (Ir-5-Cl-Phen)	1.64 ± 0.58	1.27 ± 0.32
3 (Rh-5-Me-Phen)	3.78 ± 0.75	2.84 ± 0.68
4 (Ir-5-Me-Phen)	3.97 ± 0.92	1.79 ± 0.08
5 (Rh-Dph-Bpy)	3.74 ± 0.99	1.82 ± 0.06
6 (Ir-Dph-Bpy)	4.38 ± 0.60	1.78 ± 0.08
7 (Rh-Da-Bpy)	5.55 ± 0.21	2.37 ± 1.20
8 (Ir-Da-Bpy)	5.63 ± 0.17	3.98 ± 0.21
Cisplatin ($Mean_{total}$)	39.72 ± 2.55	77.86 ± 10.14

corresponding diagrams in Figure 4 (MCF-7) and Figure 5 (HT-29).

Overall, all compounds **1**–**8** show relatively similar cytotoxicity values between 1.2–5.6 μM against both cell lines, significantly better than the established anticancer compound cisplatin (40 and 78 μM against MCF-7 and HT-29, respectively). Due to limited solubility, the metal complexes in this study were dissolved in DMSO stock solutions. Although it is known that cisplatin is somewhat deactivated by DMSO,[14] the Pt control compound was treated in exactly the same way as our sample compounds to ensure identical biological conditions across all samples. Consequently however, it should be noted that IC_{50} values for cisplatin are higher than they would be in a DMSO-free experiment.

A comparison between the metal centers of the compounds bearing the same ligand system (**1** vs. **2**, **3** vs. **4**, **5** vs. **6**, **7** vs. **8**), shows similar values and cytotoxicity, with minor differences in both cell lines. The overall most effective compounds against these two cancer cell lines were **1** and **2** with the 5-chloro-1,10-phenanthroline ligand system, while the least effective compounds were **5** and **6** with 4,4′-diamino-2,2′-bipyridine ligands (~5.6 μM for MCF-7 and 3.2 μM for HT-29). In comparing the phenanthroline and bipyridine ligands, a slight difference is noticeable. The compounds with phenanthroline-based ligands (**1**–**4**) seem to have an overall higher cytotoxicity than those with substituted bipyridine ligands (**5**–**8**). Considering the different substituents of phenanthroline, either 5-chloro or 5-methyl, the 5-chloro-substituted phenanthroline systems (**1**, **2**) seem to be marginal more active, particularly in MCF-7 cells. For the diphenyl-substituted bipyridine compounds **5** and **6**, a modest increase in cytotoxicity with lower IC_{50} values was observed. In total, these data clearly demonstrate that each tested compound is more active against the colon carcinoma HT-29 cell line than against the breast cancer cell line MCF-7, although all values were found in a small micromolar range, between 1.3–5.6 μM. For cisplatin it is already well-known that it does not exhibit high antiproliferative activity towards slow-growing colon cancer cells, such as HT-29.[15,16] However, this effect could be beneficial, since slightly lower cytotoxicity can be combined with cell targeting moieties, such as peptides (*e.g.* cell-penetrating peptides), to increase cellular uptake and cell selectivity.[17] To this end, diamino-substituted bipyridine ligands, such as in **5** and **6**, can be additionally modified by formation of organometallic metal-peptide bioconjugates.

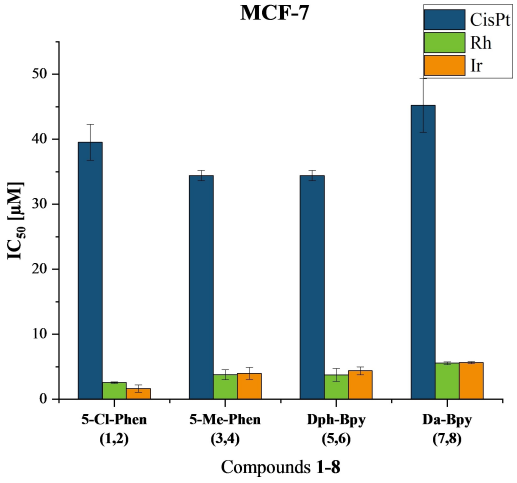


Figure 4. IC_{50} values [in μM] of compounds **1**–**8** (*M* = Rh, green; *M* = Ir, orange), grouped according to their ancillary ligand system, against the cancer cell line MCF-7 in comparison to cisplatin (blue).

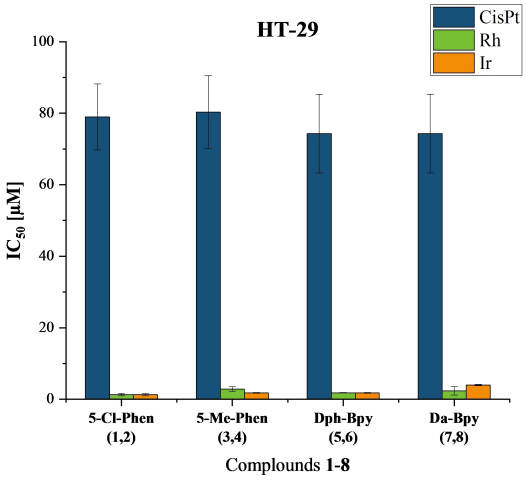


Figure 5. IC_{50} values [in μM] of compounds **1**–**8** (*M* = Rh, green; *M* = Ir, orange), grouped according to their ancillary ligand system, against the cancer cell line HT-29 in comparison to cisplatin (blue).

Conclusions

The synthesis and characterization of eight new bis-cyclometalated compounds of the formula $[M(ptpy)_2(N^N)]PF_6$ (ptpy = 2-(*p*-tolyl)pyridinato; N^N = 5-chloro-1,10-phenanthroline: *M* = Rh, **1**; M = Ir, **2**); N^N = 5-methyl-1,10-phenanthroline: *M* = Rh, **3**; M = Ir, **4**; N^N = 4,4′-diphenyl-2,2′-bipyridine: *M* = Rh, **5**; *M* = Ir, **6**; N^N = 4,4′-diamino-2,2′-bipyridine: *M* = Rh, **7**; *M* = Ir, **8** was reported. During their characterization the molecular structure in the solid of compounds **4**, **5**, **6**, and **8** was confirmed by single-crystal X-ray diffraction studies. Furthermore, the biological activity of compounds **1**–**8** was investigated by MTT assays. All compounds exhibit significant cytotoxicity towards the two tested cell lines MCF-7 and HT-29, with IC_{50} values ranging between 1.3-5.6 μM. The values found for cisplatin are significantly higher, with values at ~40 mM and ~78 μM against MCF-7 and HT-29 cancer cell lines, respectively. In comparison to cisplatin, the new rhodium(III) and iridium(III) compounds are significantly more cytotoxic, and all compounds are more active in MCF-7 than in HT-29 cancer cells. The highest cytotoxic effect was observed for the compounds bearing substituted phenanthroline ligand, as represented by **1** and **2**. In those, the 5-chloro substitution seems to be marginal more active in both cancer cell lines than the compounds bearing the 5-methyl-substituted phenanthroline ligand. The compounds with the highest IC_{50} values, thus lowest cytotoxicity have been **5** and **6** with 4,4′-diamino-2,2′-bipyridine ligand, nevertheless

with excellent values in the low micromolar range. Moreover, compounds **5** and **6** can be further developed by modifying the amino groups to obtain organometallic bioconjugates via amide bond formation. Hereby, cell selectivity and cellular uptake could be increased. This series of complexes demonstrates the impact of even minor structural modifications on their biological properties. The major aim of this project was to obtain preliminary cytotoxic values of the synthesized complexes. Therefore, we selected the cell lines according to our cell culture standards and especially for comparative purposes. For the past studies, we tested each compound against a cisplatin active cancer cell line and selected the well-established cell line MCF-7, and a more cisplatin-resistant cancer cell line, such as HT-29. The choice of these standard cell-lines was kept constantly during collaboration. Overall, more specific biological studies are crucial to reveal which exact influence each ligand system could have on the cells. Localization studies under high biological conditions, for instance, is one of the relevant experiments, important to investigate on the mode of actions of the novel iridium(III) and rhodium(III) complexes. Moreover, the selectivity of the complexes towards cancer cells could be further investigated by testing the compounds against MCF-10 (non-tumorigenic) cells. Unfortunately, herein our laboratory feasibility reaches its limitations, and further collaboration with groups in the biological field is necessary.

Experimental Section

General: All manipulations were performed under an atmosphere of dry nitrogen using conventional Schlenk techniques. Solvents were dried with standard procedures and stored under nitrogen. The substituted 1.10-phenanthroline and substituted 2.2′-bipyridine ligands were purchased from Aldrich and used as received. The starting complexes [{*M*(μ-Cl)(ptpy)$_2$}$_2$] (*M* = Rh, Ir) were prepared following literature methods.[18] NMR spectra were recorded in CD_2Cl_2 using a Jeol Eclipse 400 instrument operating at 400 MHz (^{1}H) and 100 MHz (^{13}C) respectively. Chemical shifts are given in ppm, referenced to the solvent signals of dichloromethane at δ = 5.36 (^{1}H) and 53.5 ppm (^{13}C). Mass spectra were measured using a JeolMstation JMS 700 instrument. Elemental analyses (C, H, N) were performed by the Microanalytical Laboratory of the Department of Chemistry, LMU Munich, using a Heraeus Elementar Vario EL instrument.

Biological activities

The cells of MCF-7 and HT-29 were treated as following. For cell maintenance Dulbecco's Modified Eagle's Medium (DMEM), containing 10% fetal calf serum and 1% of penicillin/streptomycin was used. The cells were, after detaching from wells via trypsin and EDTA, washed and plated out on 96 well plates in a concentration of 6,000 cells/well. After an incubation time of 24 h, at 37 °C, 10% CO_2, the cells were attached to the wells. Under sterile conditions the cells were treated with compound **1**–**8** in a certain concentration (2–250 μM) to a total volume of 200 μL/well. The DMSO concentration was maintained at 0.5% in each experiment (except for highest concentrations). As a control for 100% cell viability, a set of wells were solely treated with 0.5% DMSO. As a positive control of the assay, cisplatin was treated in each experiment to confirm drug sensitivity of the cancer cell lines. After 48 h of incubation, MTT (2.5 mg/mL) was added to each well and incubated for another 2 h. After removing media, 200 μL of DMSO was added to each well, dissolving the formazan crystals. The resulting adsorption was measured at 550 nm and 620 nm as the reference for media. Each experiment was measured in at least triplicates and three independent experiments.

Synthesis of compounds 1–8: To a solution of [{*M*(μ-Cl)(ptpy)$_2$}$_2$] (*M* = Rh, Ir) (0.15 mmol) in 25 mL of a mixture of CH_2Cl_2/MeOH/H_2O (1 : 1 : 0.5) the phenanthroline or the bpy ligands (0.3 mmol) was added and the mixture was refluxed with stirring for 2 h. After cooling to room temperature KPF_6 (0.4 mmol) was added and the mixture stirred for 30 minutes. The solvent was removed to dryness in vacuo, the residue dissolved in dichloromethane, and finally chromatographed on alumina with CH_2Cl_2/acetone (9 : 1) as the eluent. The resulting solution was evaporated to dryness and the residue was re-dissolved in 5 ml of dichloromethane/methanol and the product precipitated by slow diffusion of *iso*-hexane at room temperature.

[Rh(ptpy)$_2$(5-Cl-phen)]PF$_6$ (1): Yield: 130 mg (54.4%). *Anal.* $C_{36}H_{27}ClF_6N_4PRh$ (798.96): C, 54.12; H, 3.41; N, 7.01. Found: C, 53.94; H, 3.57; N, 6.94%. **MS** (FAB$^+$): *m/z* = 654.1 [M$^+$] complex cation. **^{1}H NMR** (400 MHz, CD_2Cl_2): δ = 8.95 (dd, *J* = 1.2 Hz, *J* = 8.6 Hz, 1H), 8.55 (dd, *J* = 1.6 Hz, *J* = 8.2 Hz, 1H), 8.41 (dd, *J* = 1.2 Hz, *J* = 5.0 Hz, 1H), 8.35 (dd, *J* = 1.2 Hz, *J* = 5.0 Hz, 1H), 8.27 (s, 1H), 7.80 (m, 8H), 7.23 (m, 2H), 6.98 (d, *J* = 8.0 Hz, 2H), 6.83 (m, 2H), 6.18 (d, *J* = 2.8 Hz, 2H), 2.14 (s, 6H). **^{13}C{^{1}H} NMR** (100 MHz, CD_2Cl_2): δ = 166.5 (d, J_{RhC} = 32.4 Hz), 166.3 (d, Rh–C, 32.5 Hz), 165.0, 164.9, 151.2, 150.8, 148.5, 145.9, 144.2, 141.1, 141.0, 140.9, 138.2 (2), 137.9, 135.9, 133.5, 133.4, 132.0, 130.3, 129.3, 127.2, 126.9, 126.8, 124.9, 124.8, 124.6, 122.9, 122.8, 122.7, 119.7, 119.6, 21.7.

[Ir(ptpy)$_2$(5-Cl-phen)]PF$_6$ (2): Yield: 140 mg (52.6%). *Anal.* $C_{36}H_{27}ClF_6IrN_4P$ (888.27): calcd. C, 48.68; H, 3.06; N, 6.31. Found: C, 48.27; H, 3.08; N, 6.09%. **MS** (FAB$^+$): *m/z* = 743.1 [M$^+$] complex cation. **^{1}H NMR** (400 MHz, CD_2Cl_2): δ = 8.92 (dd, *J* = 1.6 Hz, *J* = 8.4 Hz, 1H), 8.53 (dd, *J* = 1.6 Hz, *J* = 8.6 Hz, 1H), 8.38 (dd, *J* = 1.2 Hz, *J* = 5.0 Hz, 1H), 8.32 (m, 1H), 8.27 (s, 1H), 7.78 (m, 8H), 7.23 (m, 2H), 6.93 (d, *J* = 8.4 Hz, 2H), 6.78 (m, 2H), 6.16 (d, *J* = 3.2 Hz, 2H), 2.12 (s, 6H). **^{13}C{^{1}H} NMR** (100 MHz, CD_2Cl_2): δ = 167.8, 167.7, 151.9, 151.4, 149.2, 148.9, 148.4, 148.3, 147.5, 145.9, 141.1, 141.0, 138.1 (2), 137.7, 135.6, 132.6, 132.5, 132.4, 130.7, 129.9, 127.4, 127.3, 127.2, 124.9, 124.8, 124.0, 123.9, 122.7, 122.6, 119.6, 119.5, 21.5.

[Rh(ptpy)$_2$(5-Me-phen)]PF$_6$ (3): Yield: 110 mg (44.8%). *Anal.* $C_{37}H_{30}F_6N_4PRh$ x 0.5 CH_2Cl_2 (778.55 + 0.5 CH_2Cl_2): C, 54.86; H, 3.81; N, 6.82. Found: C, 54.91; H, 3.81; N, 6.82%. **MS** (FAB$^+$): *m/z* = 633.1 [M$^+$] complex cation. **^{1}H NMR** (400 MHz, CD_2Cl_2): δ = 8.70 (dd, *J* = 1.6 Hz, *J* = 8.6 Hz, 1H), 8.50 (dd, *J* = 1.6 Hz, *J* = 8.2 Hz, 1H), 8.35 (dd, *J* = 1.2 Hz, *J* = 4.8 Hz, 1H), 8.27 (dd, *J* = 1.6 Hz, *J* = 5.0 Hz, 1H), 7.81 (m, 9H), 7.23 (m, 2H), 6.97 (d, *J* = 7.6 Hz, 2H), 6.80 (m, 2H), 6.19 (s, br, 2H), 2.87 (s, 3H), 2.14 (s, 6H). **^{13}C{^{1}H} NMR** (100 MHz, CD_2Cl_2): δ = 167.1 (d, J_{RhC} = 32.3 Hz), 166.9 (d, J_{RhC} = 32.3 Hz), 165.1, 165.0, 150.1, 149.6, 148.4, 145.6, 144.7, 140.9, 140.8, 140.7, 138.0 (2), 137.7, 136.1, 135.4, 133.5, 133.4, 131.2, 130.7, 126.9, 126.2, 125.9, 124.7, 124.6, 124.5, 124.4, 122.5, 122.6 119.6, 119.5, 21.7, 18.7.

[Ir(ptpy)$_2$(5-Me-phen)]PF$_6$ (4): Yield: 120 mg (46.3%). *Anal.* $C_{37}H_{30}F_6IrN_4P$ (867.86): C, 51.21; H, 3.48; N, 6.46. Found: C, 51.06; H, 3.72; N, 6.33%. **MS** (FAB$^+$): *m/z* = 723.2 [M$^+$] complex cation. **^{1}H NMR** (400 MHz, CD_2Cl_2): δ = 8.67 (dd, *J* = 1.6 Hz, *J* = 8.6 Hz, 1H), 8.48 (dd, *J* = 1.2 Hz, *J* = 8.2 Hz, 1H), 8.32 (dd, *J* = 1.2 Hz, *J* = 5.0 Hz, 1H), 8.24 (dd, *J* = 1.2 Hz, *J* = 5.2 Hz, 1H), 7.80 (m, 9H), 7.24 (m, 2H), 6.92 (d, *J* = 8.0 Hz, 2H), 6.78 (m, 2H), 6.20 (s, br, 2H), 2.89 (s, 3H), 2.16 (s, 6H). **^{13}C{^{1}H} NMR** (100 MHz, CD_2Cl_2): δ = 167.9, 167.8, 150.8, 150.2, 150.0, 149.7, 148.4, 148.3, 147.0, 146.1, 141.2, 141.1, 138.0 (2), 137.5,

136.5, 135.3, 132.5, 132.4, 131.8, 131.2, 127.1, 126.6, 126.4, 124.8, 124.7, 123.8, 123.7, 122.6, 122.5 119.5, 119.4, 21.5, 18.6.

[Rh(ptpy)$_2$(dph-bpy)]PF$_6$ (5): Yield: 140 mg (52.4%). *Anal.* $C_{46}H_{36}F_6N_4PRh$ (892.70): C, 61.89; H, 4.06; N, 6.28. Found: C, 61.79; H, 4.15; N, 6.02%. **MS** (FAB$^+$): m/z = 747.20 [M$^+$] complex cation. **^{1}H NMR** (400 MHz, CD_3OD): δ = 8.61 (d, J = 0.4 Hz, 2H), 8.08 (d, J = 1.4 Hz, 2H), 7.92 (d, J = 1.9 Hz, 2H), 7.83 (m, 2H), 7.77 (m, 4H), 7.69 (d, J = 1.9 Hz, 2H), 7.65 (dd, J = 5.8 Hz, J = 1.8 Hz, 2H), 7.57 (m, 8H), 7.01 (m, 2H), 6.93 (m, 2H), 6.15 (s, 2H), 2.14 (s, 6H); **^{13}C{^{1}H} NMR** (100 MHz, CD_2Cl_2): δ = 167.3 (d, J_{RhC} = 32.4 Hz), 165.1, 154.7, 152.0, 150.5, 148.6, 141.0, 140.9, 138.2, 136.0, 133.4, 130.7, 129.7 (2x), 127.4 (2x), 125.5, 124.7, 124.6, 122.8, 121.4, 119.7, 21.7.

[Ir(ptpy)$_2$(dph-bpy)]PF$_6$ (6): Yield: 155 mg (52.7%). *Anal.* $C_{46}H_{36}F_6IrN_4P$ (982.00): C, 56.26; H, 3.70; N, 5.71. Found: C, 56.88; H, 3.96; N, 5.63%. **MS** (FAB$^+$): m/z = 837.25 [M$^+$] complex cation. **^{1}H NMR** (400 MHz, CD_3OD): δ = 8.61 (d, J = 0.4 Hz, 2H); 8.05 (d, J = 1.4 Hz, 2H), 7.91 (d, J = 2.0 Hz, 2H), 7.76 (m, 6H), 7.65 (m, 4H), 7.57 (m, 8H), 6.96 (m, 2H), 6.91 (dd, J = 1.2 Hz, J = 8.0 Hz, 2H), 6.31 (s, 2H), 2.15 (s, 6H). **^{13}C{^{1}H} NMR** (100 MHz, CD_2Cl_2): δ = 167.9, 156.1, 151.6, 151.0, 150.4, 148.4, 141.3, 141.1, 138.1, 135.7, 132.4, 130.9, 129.7 (2x), 127.4 (2x), 126.1, 125.0, 123.8, 122.7, 122.0, 119.6, 21.6.

[Rh(ptpy)$_2$(da-bpy)]PF$_6$ (7): Yield: 120 mg (46.9%). *Anal.* $C_{34}H_{30}F_6N_6PRh$ x CH_2Cl_2 (770.53 + CH_2Cl_2): C, 49.14; H, 3.77; N, 9.82. Found: C, 49.56; H, 3.77; N, 9.82%. **MS** (FAB$^+$): m/z = 625.15 [M$^+$] complex cation. **^{1}H NMR** (400 MHz, CD_3OD): δ = 7.84 (d, J = 2.0 Hz, 2H), 7.77 (m, 2 H), 7.61 (s, 1H), 7.59 (m, 5H), 7.40 (d, J = 1.6 Hz, 2H), 6.97 (m, 2H), 6.86 (d, J = 1.9 Hz, 2H), 6.45 (dd, J = 6.4 Hz, J = 2.4 Hz, 2H), 6.08 (s, 2H), 4.90 (s, 4H, 2 x NH_2), 2.10 (s, 6H); **^{13}C{^{1}H} NMR** (100 MHz, CD_2Cl_2): δ = 169.0 (d, J_{RhC} = 32.4 Hz), 165.2, 155.4, 155.2, 149.4, 148.7, 141.1, 140.2, 137.5, 133.5, 124.1, 124.0, 122.3, 119.1, 111.5, 108.4, 21.6.

[Ir(ptpy)$_2$(da-bpy)]PF$_6$ (8): Yield: 130 mg (48.1%). *Anal.* $C_{34}H_{30}F_6IrN_6P$ x 0.5 CH_2Cl_2. (859.8 + 0.5 CH_2Cl_2): C, 45.92; H, 3.46; N, 9.31. Found: C, 45.47; H, 3.32; N, 9.01%. **MS** (FAB$^+$): m/z = 715.21 [M$^+$] complex cation. **^{1}H NMR** (400 MHz, CD_3OD): δ = 7.83 (d, J = 2.0 Hz, 2H), 7.69 (m, 2H), 7.58 (m, 6H), 7.36 (d, J = 1.6 Hz, 2H), 7.43 (m, 2H), 6.81 (d, J = 2.0 Hz, 2H), 6.44 (dd, J = 6.4, J = 2.4 Hz, 2H), 6.10 (s, 2H), 4.94 (s, 4H, 2 x NH_2), 2.10 (s, 6H); **^{13}C{^{1}H} NMR** (100 MHz, CD_2Cl_2): δ = 168.1, 161.9, 156.4, 155.0, 149.46 148.5, 141.4, 140.6, 137.3, 132.5, 124.5, 122.9, 122.3, 119.0, 111.7, 109.0, 21.5.

X-ray Structural Determination: Crystals of **4**, **5**, **6**, and **8** suitable for X-ray diffraction were obtained by crystallization from mixtures of dichloromethane/methanol/*iso*-hexane at ambient temperature. Crystals were selected by means of a polarization microscope, mounted on a MiTeGen MicroLoop, and investigated with a Bruker D8 Venture TXS diffractometer using Mo-Kα radiation (λ = 0.71073 Å). The structures were solved by direct methods (SHELXT)[19] and refined by full-matrix least-squares calculations on F^2 (SHELXL-2014/7).[20] Crystals of **4** contained solvate molecules of dichloromethane which could not be assigned properly and has been squeezed-out.[21] The voids are filled with approximately 1.5 CH_2Cl_2. Thus, there is approximately $^3/_4$ CH_2Cl_2 per formula unit. In crystals of **8** there are two disordered CH_2Cl_2 in the asymmetric unit. Split models have been applied. The moiety containing C35, Cl1 and Cl2 had the best geometry which has been slightly improved by restraints causing similar C–Cl distances (SADI). Then this moiety has been used as geometric model for all remaining CH_2Cl_2 moieties (SAME). SIMU restraints have been applied to improve anisotropic displacement parameters of some of the disordered atoms. The site occupation factors were refined to 0.56/0.64 and 0.74/0.26. The structure of **5** is almost isostructural with the analogous iridium compound **6** (for details see SI of this paper). The latter has been used as model for solution and refinement of the structure of **5**. An initial refinement led to a site occupation factor of approximately 0.5 for dichloromethane. Comparable to the Ir-structure, the remaining solvent electron densities could not be modelled properly. Applying SQUEEZE to this structure involving dichloromethane showed, that considerable electron densities (2.5 e·Å^{-3}) could not be squeezed-out due to too close distance from dichloromethane. As a consequence, the final R values were quite high. Hence, dichloromethane was removed from the structure model before SQUEEZE was applied. Refinement of that squeezed-out model finally revealed a slight disorder in one of the phenyl rings. This has been described by a split model. The ratio of site

Table 2. Crystal data and structure refinement details for compounds **4**, **5** and **8**.

Compound	**4**	**5**	**8**
Empirical formula	$C_{37}H_{30}F_6IrN_4P$	$C_{46}H_{36}F_6N_4PRh$	$C_{36}H_{34}Cl_4F_6IrN_6P$
M/g·mol^{-1}	867.82	892.67	1029.66
Temperature/K	102(2)	102(2)	102(2)
Crystal system	monoclinic	triclinic	monoclinic
Space group	$P2_1/n$	P^-1	$P2_1/n$
a/Å	8.8809(4)	9.5014(5)	10.7965(4)
b/Å	31.0918(11)	11.8958(6)	9.1667(4)
c/Å	13.1514(5)	21.3471(12)	38.4532(16)
α/°	90	102.550(2)	90
ß/°	93.277(2)	94.910(2)	96.4820(10)
γ /°	90	111.011(2)	90
V/Å^3	3625.5(2)	2162.9(2)	3781.3(3)
Z	4	2	4
$\rho_{calcd.}$/g·cm^3	1.590	1.371	1.809
μ/mm^{-1}	3.789	0.494	3.923
θ range for data collection/°	2.645 to 28.282	2.981 to 28.281	2.464 to 33.114
Reflections observed	7891	9478	11924
Reflections in refinement	8997	10732	14405
S	1.227	1.131	1.094
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0391, wR_2 = 0.0832	R_1 = 0.0412, wR_2 = 0.0977	R_1 = 0.0257, wR_2 = 0.0565
$\Delta\rho_{fin}$ (max/min)/e·Å^{-3}	1.506 /−2.340	0.578/−0.683	1.517/−1.039

occupation factors of the two parts has been refined to 0.90/0.10. In order to get a stable refinement for the less-occupied phenyl ring, some restraints (SAME instruction with the main part as model for good geometry) and constraints (same coordinates and anisotropic displacement parameters (EXYZ and EADP instructions) for C11/C11B and C14/C14B, respectively, had to be applied. The remaining four C-atoms of the less-occupied phenyl ring have been refined isotropically. According to PLATONs SQUEEZE routine, there is one big void with a volume of 367 A^3 with 131 squeezed-out electrons. Because dichloromethane, *iso*-hexane and methanol have been used as solvents, the voids could contain one dichloromethane (42 electrons), one *iso*-hexane (40 electrons) and two methanol (twice 18 electrons). The less-occupied part of the disorder has been neglected for the preparation of figure 2. Details of the crystal data, data collection, structure solution, and refinement parameters of compound **4**, **5**, and **8** are summarized in Table 2 (data for **6** see Table S1 in the SI). Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB21EZ, UK. Copies of the data can be obtained free of charge upon quoting the depository number CCDC-2169485 (**4**), CCDC-2169486 (**5**), CCDC-2169487 (**6**), and CCDC-2169488 (**8**) (Fax: +44-1223-336-033; E-Mail: deposit@ccdc.cam.ac.uk, http://www.ccdc.cam.ac.uk).

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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