Department of Chemistry, University of Wroclaw

The Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara

Sandra Kozieł

Iridium(III) complexes with phosphine-fluoroquinolone conjugates - design, synthesis, bioactivity and nanoformulation as a potential platform for drug delivery.

Supervisor: Dr. Alina Bieńko, prof. UWr (University of Wrocław)
Supervisor: prof. Stefano Caramori (University of Ferrara)
Auxiliary supervisor: Dr. UrszulaKomarnicka (University of Wrocław)

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Streszczenie

Na całym świecie, choroby nowotworowe powodują kilka milionów zgonów rocznie. Przewiduje się, że przed końcem 2023 roku pojawi się więcej niż dwadzieścia dwa miliony nowych przypadków chorób nowotworowych. Wiele leków przeciwnowotworowych jest obecnie stosowanych w leczeniu klinicznym, ale ponad 50% z nich to leki oparte na bazie jonów platyny. Ich skuteczność nadal utrudniają problemy kliniczne, w tym nabyta lub wrodzona oporność, ograniczone spektrum działania oraz wysoka toksyczność prowadząca do działań niepożądanych. Jedną ze strategii przyjętych w celu przezwyciężenia tych ograniczeń jest opracowanie nowych alternatywnych środków przeciwnowotworowych na bazie jonów metali przejściowych (np. Cu, Ru, Pd i Au).

Obecnie spośród badanych kompleksów metali przejściowych to związki irydu są prawdopodobnie najbardziej obiecującą grupą potencjalnych leków przeciwnowotworowych. W porównaniu do związków platyny charakteryzują się one zredukowanym stopniem toksyczności w stosunku do komórek zdrowych, a zatem wyższą selektywnością działania na komórki zmienione nowotworowo. Ponadto wykazują właściwości przeciwnowotworowe poprzez indukcję apoptozy oraz interakcje z DNA lub kinazami białkowymi.

Fluorochinolony to antybiotyki o szerokim spektrum działania stosowane w leczeniu infekcji bakteryjnych nie tylko u ludzi, ale także u zwierząt. Oprócz silnego działania przeciwdrobnoustrojowego antybiotyki te wykazują również działanie immunomodulujące i przeciwnowotworowe. Dlatego też, połączenie fluorochinolonów poprzez ugrupowanie fosfinowe z kompleksami irydu(III) może dodatkowo zmniejszyć ogólną toksyczność i umożliwić selektywne dostarczanie do komórek nowotworowych.

Celem nadrzędnym moich badań było opracowanie metod syntezy kompleksów irydu(III) z fosfinowymi pochodnymi fluorochinolonów, a następnie ich charakterystyka fizykochemiczna oraz biologiczna. W kolejnym kroku dla związków o najwyższej aktywności przeciwnowotworowej zaprojektowano metody ich selektywnego dostarczania poprzez enkapsulację w nanoformulacjach.

W ramach pracy doktorskiej zsyntetyzowałem trzy związki koordynacyjne irydu(III) zawierające fosfinowe pochodne fluorochinolonów. W celu porównania wpływu antybiotyków, zsyntetyzowano również jeden kompleks irydu(III) bez antybiotyku. Otrzymane związki koordynacyjne scharakteryzowano z wykorzystaniem analizy elementarnej, spektrometrii mas (ESI-MS) oraz wybranych metod spektroskopowych (np. IR, NMR, UV-Vis

oraz fluorescencyjnej). Co warte podkreślenia, w przypadku wszystkich kompleksów, udało się otrzymać monokryształy odpowiednie do badań krystalograficznych. Kompleksy zawierają jon Ir(III), do którego koordynuje ligand aromatyczny (cyklopentadienyl), oraz dwa ligandy chlorkowe i jedna cząsteczka fosfiny – wykazując geometrię pseudooktaedryczną typu „half-sandwich". Kompleksy homojądrowe Ir(III) zawierające motyw fluorochinolonowy są stabilne w roztworze wodnym, w porównaniu do kompleksu bez tego motywu. Kompleks ten hydrolizował w roztworze wodnym, co zostało udowodnione za pomocą ^{1}H NMR przy różnych stężeniach roztworu NaCl.

Aktywność przeciwnowotworową *in vitro* zsyntezowanych kompleksów i cisplatyny przebadano wobec pięciu linii komórek nowotworowych: MCF7 (ludzki gruczolakorak piersi), A549 (ludzki gruczolakorak płucny), PANC-1 (ludzki rak przewodowy trzustki) oraz DU-145 (ludzki rak prostaty), WM2664 (ludzki rak skóry) a także wobec pierwotnej linii embrionalnych ludzkich komórek nerek (HEK293T). Badane kompleksy wykazywały obiecującą cytotoksyczność *in vitro* z wartościami IC_{50} znacznie niższymi niż dla cisplatyny. Co warte podkreślenia, wprowadzenie motywu fluorochinolonowego do kompleksów znacząco zwiększyło cytotoksyczność przeciwnowotworową finalnych związków wobec linii komórkowej płuc, piersi i czerniaka. Badanie to umożliwiło wyselekcjonowanie z puli wszystkich zsyntetyzowanych związków, kompleksu o najlepszym działaniu przeciwnowotworowym (**IrPCp**), a następnie podjęcie próby określenia mechanizmu działania cytotoksycznego. Wstępne badania skoncentrowane na wyjaśnieniu sposobu działania pozwoliły na sformułowanie następujących wniosków: ***(i)*** kompleksy irydu(III) akumulują się w komórce zarówno w jądrze, jak i w cytoplazmie, ***(ii)*** na podstawie analizy cytometrycznej wykazano, że w komórce zachodzi głównie apoptoza (programowana śmierć komórki), ***(iii)*** aktywacja kaspazy-3/7 wraz ze spadkiem potencjału błony mitochondrialnej również potwierdziła apoptotyczną śmierć komórki, ***(iv)*** kompleksy irydu(III) mogą indukować zmiany w cyklu komórkowym prowadzące do fazy G2/M ***(v)*** potwierdzono generację reaktywnych form tlenu (z udziałem rodnika hydroksylowego, tlenu singletowego i anionorodnika ponadtlenkowego) jako prawdopodobnego szlaku odpowiedzialnego za cytotoksyczność, ***(vi)*** wykazano skuteczne działanie przeciwnowotworowe na trójwymiarowych wielokomórkowych zespołach sferoidalnych guza (3D), ***(vii)*** związki irydu(III) wykazują multimodalną interakcję DNA z przewagą wiązania do małego rowka, ***(viii)*** ponadto wiążą się z resztami tryptofanu HSA w miejscu I (subdomena II A) oraz wiążą się do apo-Tf.

W celu przezwyciężenia słabej rozpuszczalności, poważnych skutków ubocznych związanych z ogólnoustrojową cytotoksycznością kompleksów i nabyciem oporności komórek rakowych, powstałe kompleksy irydu(III) zamknięto w nanoemulsjach i micelach. Zamknięcie związków w micelach (**IrPCp_M**) poprawiło skuteczną akumulację leków w ludzkim gruczolakoraku płuc i ludzkim raku prostaty oraz zwiększyło ich cytotoksyczność.

Summary

Cancer is a group of diseases classified as one of the most life-threatening worldwide. Many anti-cancer medicines are currently used in clinical treatment, but more than 50% of them are platinum-based drugs. Their effectiveness is still hampered by clinical problems, including acquired or congenital resistance, a limited spectrum of action, and high toxicity leading to adverse effects. One strategy adopted to overcome these limitations is the development of new alternative transition metal (*e.g*. Cu, Ru, Pd, and Au) anti-cancer agents.

Nowadays, transition metal complexes and iridium compounds are probably the most promising group of potential medicine. They appear to be an attractive alternative to their platinum counterparts, mainly because they are less toxic and exhibit anticancer properties through the induction of apoptosis and interactions with DNA or protein kinases.

Fluoroquinolones are broad-spectrum antibiotics used to treat bacterial infections not only in humans but also in animals. In addition, to their strong antimicrobial activity, these antibiotics have also shown immunomodulatory and antitumor effects. Consequently, the linking of fluoroquinolones *via* phosphine moiety to iridium(III) complexes may decrease the overall toxicity and may enable selective delivery to neoplastic cells.

The main aim of my work was to design and synthesis organometallic iridium(III) complexes with phosphines derived from fluoroquinolone antibiotics possessing anticancer potential. In the next step, for the compounds with the highest antitumor activity, methods for their selective delivery using encapsulation in nanoformulations were designed.

During my work, I prepared four iridium(III) complexes containing phosphine ligands with/without a fluoroquinolone motif. The physicochemical properties in both solution and solid-state of each obtained complex were investigated using elemental analysis, mass spectrometry, cyclic voltamperometry, and spectroscopic methods (NMR, IR, UV-Vis, fluorescence). The crystal structures of every synthesized complex were obtained using the X-ray single-crystal diffraction method. The mononuclear iridium(III) complexes adopt half-sandwich pseudo-octahedral “three-leg piano-stool” geometry with an η^5-coordinated cyclopentadienyl and three additional sites of ligation occupied by one phosphine ligand and two chloride ligands. Homonuclear Ir(III) complexes containing the fluoroquinolone motif are stable in an aqueous solution. In the case of the complex without the fluoroquinolone motif, hydrolysis was observed in an aqueous solution, which was monitored in the presence of various concentrations of NaCl (mimicking the most important environment in the body).

The cytotoxicity of all compounds was tested *in vitro* against the five most common cancer cell lines: MCF7 (human breast adenocarcinoma), A549 (human lung adenocarcinoma), PANC-1 (human pancreatic/duct carcinoma), WM2664 (metastatic human melanoma) and DU-145 (human prostate carcinoma) as well as one normal, human embryonic kidney (HEK293T). Based on these results, examined complexes exhibited promising cytotoxicity *in vitro* with IC_{50} values significantly lower than that of the cisplatin. It is worth emphasizing that the introduction of the fluoroquinolone motif in complexes significantly increased the antitumor cytotoxicity of the final compounds against the lung, breast, and melanoma cell line. This study made it possible to select from a pool of all compounds with the best effect (**IrPCp**) and to make an attempt to determine their mechanism of cytotoxic action. Furthermore, preliminary investigation focused on elucidation of the mode of action allowed to formulate the following conclusions: ***(i)*** iridium(III) complexes are accumulated in both nucleus and cytoplasm, ***(ii)*** cytometric analysis showed clear evidence for predominance of apoptosis in the induced cell death, ***(iii)*** the activation of caspase-3/7 along with the decrease of mitochondrial membrane potential also confirmed the apoptotic cell death, ***(iv)*** iridium(III) complexes may induce the changes in cell cycle leading to G2/M phase arrest, ***(v)*** ROS generation (involving hydroxyl radical, singlet oxygen and superoxide anion radical) as plausible pathway responsible for the cytotoxicity ***(vi)*** efficient anticancer action on 3D multicellular tumor spheroids assemblies was demonstrated, ***(vii)*** inorganic compounds exhibited multimodal DNA interaction with predominance of minor groove binding, ***(viii)***and they bind to HSA tryptophan residues at site I (subdomain II A) and bind to all four possible apo-Tf binding sites containing tyrosine or tryptophan residues.

To overcome poor solubility, serious side effects related to the systemic cytotoxicity of the complexes, and the acquisition of cancer cell resistance, the resulting homonuclear complexes were encapsulated in nanoemulsions and Pluronic-123 micelles. The enclosure of compounds in micelles (**IrPCp_M**) improved the effective accumulation of drugs in human lung adenocarcinoma and human prostate cancer and increased their cytotoxicity by an order of magnitude.

Table of Contents

List of abbreviations

χ_M	magnetic susceptibility
·OH	hydroxyl radical
$^1O_2$	singlet oxygen
A2780	ovarian cancer cell line
A549	human lung adenocarcinoma cell line
acac	acetylacetonato
apo-tf	apo-transferrin
Arg	arginine residue
Asp	aspartate residue
B_0	magnetic induction
CDDP	cisplatin
cod	1,5-cyclooctadiene
Cp*	pentamethylcyclopentadienyl
CT DNA	circulating tumor DNA
CT26	mouse colon carcinoma cell line
CV	cyclic voltammetry
DACHPt	Dichloro(1,2-Diaminocyclohexane)Platinum(II)
DAPI	4',6-diamidino-2-phenylindole
$DCFH_2$-DA	2'-7'dichlorofluorescin diacetate
DMEM	Dulbecco's Modified Eagle Medium
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DOX	doxorubicin
DU145	human prostate carcinoma
EB	ethidium bromide
EMU	the units of magnetization
EPR	electron paramagnetic resonance
ESI-MS	electrospray ionization mass spectrometry
Fe_3O_4	Iron(II, III) oxide
H	magnetic field strength
HCp	ciprofloxacin
HDVV	Heisenberg-Dirac-Van Vleck
HEK293T	human embryonic kidney
HeLa	immortal cell line
HepG2	hepatocellular carcinoma cell line
HFQ	fluoroquinolones
His	histidine residue
HLm	lomefloxacin
HNr	norfloxacin
HSA	human serum albumin

I	intensity
I_0	intensity in the absence of dye
IC_{50}	a quantitative measure that indicates how much of a particular inhibitory substance (*e.g.* drug) is needed to inhibit, in vitro, a given biological process or biological component by 50%
ICP-MS	inductively coupled plasma mass spectrometry
ImM	imidazole
IR	infrared spectroscopy
IrPCp	$Ir(\eta^5\text{-}Cp^*)Cl_2PCp$, figure 14
IrPCp_M	IrPCp encapsulated in Pluronic P-123
IrPCpCu	$Ir(\eta^5\text{-}Cp^*)Cl_2PCp$-Cu(phen), figure 30
IrPCpCu_L	IrPCpCu encapsulated in liposome
IrPLm	$Ir(\eta^5\text{-}Cp^*)Cl_2PLm$, figure 14
IrPLmCu	$Ir(\eta^5\text{-}Cp^*)Cl_2PLm$-Cu(phen), figure 30
IrPNr	$Ir(\eta^5\text{-}Cp^*)Cl_2PNr$, figure 14
IrPNrCu	$Ir(\eta^5\text{-}Cp^*)Cl_2PNr$-Cu(phen), figure 30
IrPOH	$Ir(\eta^5\text{-}Cp^*)Cl_2PPh_2CH_2OH$, figure 14
K	kelvin
LC	ligand-centered transitions
Lys	lysine residue
M	magnetization
MCF7	human breast adenocarcinomacell line
Me	metal
MG	green methyl
MLCT	metal-ligand charge transfer
MMP	mitochondrial membrane potential
MNPs	magnetic nanoparticles
MRI	magnetic resonance imaging
MTT	3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide
NAD^+	the oxidised form of nicotinamide adenine dinucleotide
NADH	1,4-Dihydronicotinamide adenine dinucleotide
NAMI-A	imidazolium-trans-tetrachloro(dimethylsulfoxide)imidazoleruthenium(III)
NaN_3	sodium azide
NMR	nuclear magnetic resonance spectroscopy
$O_2^{\cdot -}$	superoxide radical anion
Oe	oersted
PANC-1	human pancreatic/duct carcinoma cell line
PCp	PPh_2CH_2Cp, figure 11
Ph_2PCH_2OH	diphenyl(hydroxymethyl)phosphine
phen	phenantroline
PHI	computer program designed for the calculation of the magnetic properties of paramagnetic coordination complexes
PLm	PPh_2CH_2Lm, figure 11
PNr	PPh_2CH_2Nr, figure 11

POH	Ph_2PCH_2OH
PPOA	phosphine phosphonic amide ligand
ROS	reactive oxygen species
S	spin
SIM	Single Ion Magnet
SMM	Single Molecule Magnet
SOD	superoxide dismutase
SQUID	superconducting quantum interference device
T	tesla
TBAP	tetrabutyl ammonium perchlorate
TEM	transmission electron microscopes
Tyr	tyrosine residue
UV-Vis	ultraviolet–visible spectroscopy
μ	magnetic moment
WHO	World Health Organization
WM2664	human melanoma cell line

1. Introduction

Cancer has been one of the biggest global issues facing humankind in recent decades. It is estimated that in 2020 they caused approximately 10.0 million deaths worldwide [1]. Currently, only cardiovascular disease is responsible for the greater number of deaths. However, it is predicted that in the coming years the mortality caused by neoplastic diseases will increase significantly, thus distancing the death caused by cardiovascular diseases in this respect [2]. Despite many years of intensive research on the introduction of new active inorganic anticancer drugs, there is still only one group of transition metal complexes currently used in cancer chemotherapy – cisplatin and its derivatives (carboplatin, oxaliplatin, nedaplatin and lobaplatin) (**Fig. 1**). Cisplatin was introduced into clinical treatment in the late 1970s, mainly thanks to Rosenberg's discovery [3].

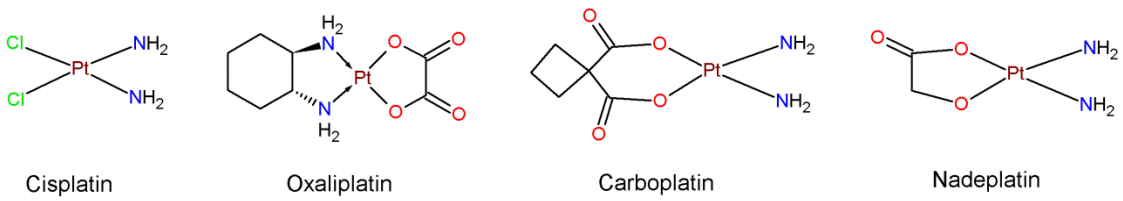


Figure 1 Overview of approved or clinically investigated drugs based on platinum ions.

Today, this inorganic compound is still used as one of the best chemotherapeutics agents for many types of cancers. Unfortunately, the use of platinum-based drugs has many limitations. The disadvantage of chemotherapy is the ability of neoplastic cells to become resistant to the action of platinum therapeutics, and also the significant toxicity of these complexes toward healthy cells [4-7]. Therefore, it is necessary to search for new chemical compounds that could replace the platinum ones in the future. Such compounds are sought from among metal complexes due to their broad and diverse structural types and distinct ligand binding modes, which may lead to the improvement of their biological properties (*e.g.* antitumor activity) [8-11]. Currently, the scientific attention focuses on iridium compounds, which seem to be the most promising future antitumor compounds [10, 12-14]. The effectiveness of the therapeutics is also determined by the way it is delivered to the body [15].

The conventional approach is mostly often based on the oral ingestion or direct injection into the blood of the appropriate dose of the drug to obtain the desired effect in the affected area [16,17]. Unfortunately, as it is difficult to control the distribution of the drug in the body, the drug substance "incidentally" also reaches other places. It can lead to the side effects of such kind of treatment and also the patients being forced to take higher doses of medication to be

sure that the desired therapeutic effect will be achieved. There are also problems with the kinetics of drug release. The standard approach initially releases the drug in high concentration very rapidly, after which the dosage of the drug becomes approximately sinusoidal [5-7]. These data show the necessity to search for new, effective solutions in the fight against cancer, in particular drugs with a high selectivity profile.

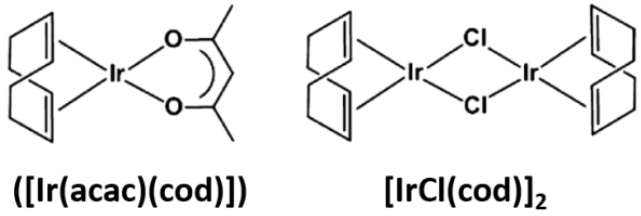
One effective means of delivering drugs to a target in the body is through the use of nanostructures. From a pharmacological point of view, there is a noticeable better potential for improving drug bioavailability, spreading drug circulation time, regulatory drug release, and targeting neoplastic cells [18-22]. Smart systems (*e.g.* micelles, liposomes, *etc.)* characteristic feature is the ability to change their properties in a controlled manner in response to environmental stimuli, such as oxidative stress [23], pH [24], ultrasound [25], temperature [26], magnetism [27] or glucose level [28]. However, among the aforementioned nanoparticles (*e.g.* polymers, liposomes, micelles, *etc.*), magnetic nanoparticles (MNPs) are among the most used in biomedical applications [29-31]. Compared to others, magnetic nanoparticles can be easily transported through the magnetic field even after drug administration [27].

1.1. Iridium compounds as anticancer agents

Over the past decades, many iridium complexes have been synthesized and have attracted much attention in many fields, especially catalysis, *e.g.* Crabtree's hydrogenation catalyst [32,33]. However, the relatively unexplored chemistry of organo-iridium compounds differs from others due to their highly tunable properties making them suitable for use as potential anticancer drugs. First off, iridium exhibits potential redox properties by adopting various degrees (most common oxidation states are +3 and +4) of oxidation and kinetic stability in a biological environment. Thus, complexes with iridium ions can generate reactive oxygen species (ROS), leading to cell apoptosis induction by reducing mitochondrial membrane potential [13,34]. They are well soluble in water - therefore, they have higher cellular absorption efficiency [12]. Furthermore, they are characterized by long emission lifetimes, large stokes shifts, and non-linear absorption, which makes them used as bio-imaging and bio-detection agents [35]. Octahedral cyclometalated Ir^{III} complexes not only serve as cell imaging agents but can also inhibit tumor necrosis factor α, promote DNA oxidation, and exhibit good anticancer activity *in vivo*/*vitro*. The above-described scientific findings indicate that iridium compounds

have a different mechanism of action than cisplatin and could break cancer cell resistance [34,36-38].

Initial studies of anticancer activity of iridium complexes focused on square-planar Ir^{I} complexes because of their structural and electronic similarity to Pt^{II} anticancer complexes such as cisplatin, *e.g.* [Ir(acac)(cod)] [39] and binuclear $[IrCl(cod)]_2$ [40] (**Fig. 2**). Both compounds had antitumor activity comparable to cisplatin and inhibited the growth of lung cancer [39,40].



([Ir(acac)(cod)]) **$[IrCl(cod)]_2$**

Figure 2 Example of structure of iridium(I) complexes with square-planar geometry [39,40].

Recently, the half-sandwich Ir^{III} complexes have attracted continuous research interest [12], because of their higher anticancer activity than Ir^{I} compounds. The name reflects the pseudo-octahedral structure they resemble a stool, where the "top of the stool" is a six-electron aromatic ligand (pentamethylcyclopentadienyl (Cp*) or its derivatives) while the last three coordination sites belong to halides (or other labile groups), nitrogen-, oxygen-, sulfur- or phosphorous-donor ligands (monodentate or chelating) forming "legs" (**Fig. 3**). However, the most frequent complexes are those possessing all three monodentate ligands or one monodentate and one chelating ligand [12,41].

Every component of a half-sandwich iridium complex allows adjusting its biological properties. The π-bound negatively charged pentamethylcyclopentadienyl or its derivatives stabilize the entire structure by modifying the electronic behavior of the remaining ligands. Additionally, the presence of Cp* can cause fine-tuning of the electronic properties of the iridium center, which may increase the complex binding to biomolecules [14]. Usually, only inside the cell, one of the ligands is labile (Cl–, Br–, I–, SCN–) allowing interaction of iridium compound with biomolecules, as cisplatin does. Moreover, this aromatic ring facilitates iridium complex penetration through the membranes, which enhances cellular uptake, and may play a role in interactions with the target, incl. through DNA and affects the rate of hydrolysis [12, 14].

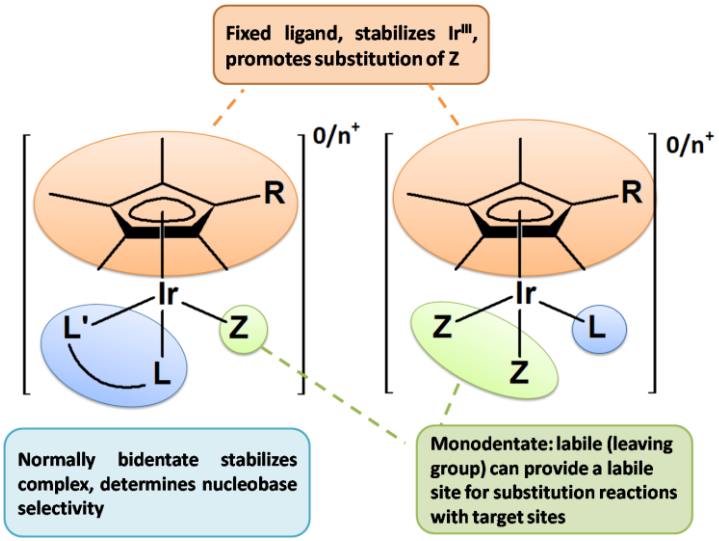


Figure 3 Iridium half-sandwich structure-activity relationships.

The above-described scientific findings clearly indicate possible various mechanisms of cellular response (biological activity) dependent on the type of the ligand, or spatial structure of complexes (geometry) [12,42].

P. Sadler group reported a series of half-sandwich iridium(III) complexes [12], with the formula $[(\eta^5\text{-Cp*})\text{Ir(L^L')Cl}]^{0/+}$ containing N^N- (*e.g.*1,10-phenanthroline (phen)) and modified the Cp* ring by introducing of a phenyl (Cp^{xph}; orange circle) or biphenyl (Cp^{biph}; purple circle) substituent (**Fig. 4**). These studies have shown that organometallic compounds undergo rapid hydrolysis, which activates the Me-Cl (Me: metal) bond. This process distinguishes these compounds from cisplatin, which contains two labile Pt-Cl bonds. Cytotoxic activity toward human ovarian cancer cells (A2780) increases with phenyl substitution on pentamethylcyclopentadienyl moiety: **3**> **2**> **1 (Fig. 4)**. This increase in cytotoxic activity corresponds to an increase in DNA binding and hydrophobicity, which increases the accumulation in cells [12].

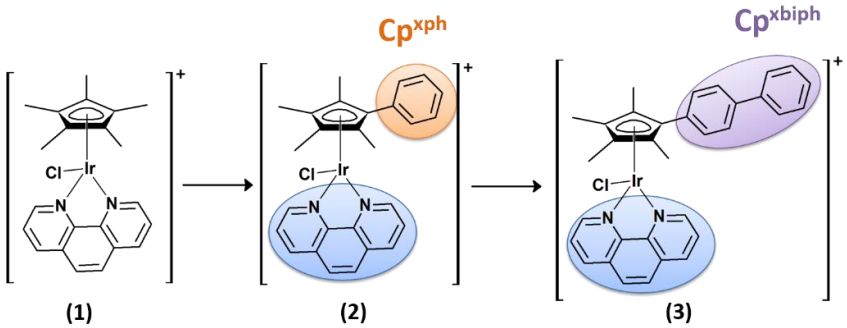
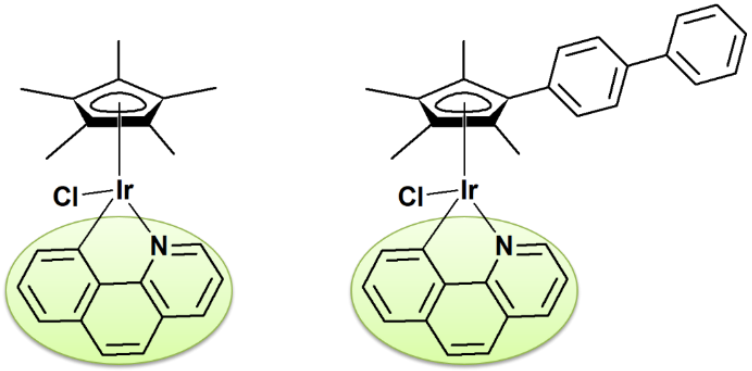


Figure 4 Structures of Iridium (III) complexes with general formula $[(\eta^5\text{-Cp}^x)\text{Ir(N^N)Cl}]^+$.

The same group also showed that the anticancer activity of Ir(III) complexes can also be achieved by changing the chelating ligand. They modified complexes **1** and **3** by replacing the neutral N, N-chelating ligand (**Fig. 4,** blue circle) with the negatively charged C, N-chelating ligand, resulting in the formation of complexes **4** and **5** (**Fig. 5**, green circle) [43,44]. This resulted in a change in binding to nucleobases and increased hydrophobicity leading to higher cellular uptake and consequently increased cytotoxicity to A2780 cancer cells. For example, complex **2** showed good cytotoxicity with an IC_{50} of 10.5 mM toward human ovarian cancer cells (A2780), whereas complex **1** was inactive. Cytotoxicity activity trend was found to be in the order: **5**> **3**> **4**> **1** [12,43,44]. Results show that these complexes induce apoptosis through ROS-dependent mitochondrial pathway and protein synthesis disruption [43,44].



(4) (5)

Figure 5 Structures of iridium(III) complexes with general formula [(η^5-Cp^x)Ir(C^N)Cl].

Yanjing Yang and co-workers synthesized and characterized half sandwich zwitter ionic iridium(III) complexes containing different substituent's in the Cp* rings (**Fig. 6**) [45]. These researchers similarly to the group of prof. Sadler [12] investigated the effect of Cp* ring elongation on the biological activity of cationic iridium(III) half-sandwich complexes [45]. It was found that the presence of fluorine substituents significantly increased the hydrophobicity of the obtained complexes. As it has been mentioned earlier, this causes a significant increase in the accumulation of complexes in cells and an increased antitumor activity [45].

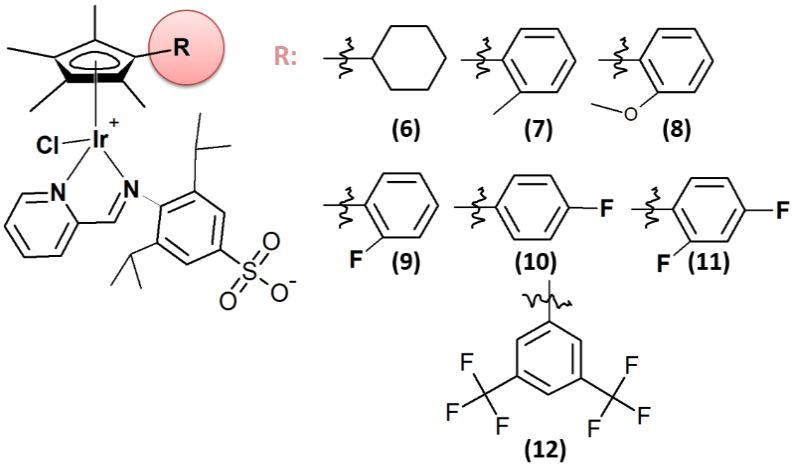


Figure 6 Structures of iridium(III) complexes with general formula $[(\eta^5\text{-}C_5Me_5)Ir(XY)Cl]^{0/+}$.

The cytotoxicity of these complexes (**6-12, Fig. 6**) was tested against hepatoma (HepG2), lung (A549), and immortal (HeLa) cell lines. Complexes **9-12** (**Fig. 6**) showed significant antitumor activity, in contrast to complexes **6-8** (**Fig. 6**) which were inactive. Inorganic compounds **9-12** can convert NADH to NAD+, exhibit fluorescence emission and induce intercellular ROS. It had been proven that complex **9** successfully entered lung cells *via* an energy-dependent pathway. In addition, it may induce cell death *via* the disruption of lysosomes [45].

Zhe Liu and co-workers synthesized a series of half sandwiched iridium(III) complexes containing P-O- chelating ligand (phosphine phosphonic amide ligand (PPOA)) (**Fig.7**) [46]. Compared to the previously discussed complexes (**1-12**) [12,43-45], the presence of elongated phenyl rings in this resulting Ir system only slightly changed the antitumor activity of the obtained complexes.

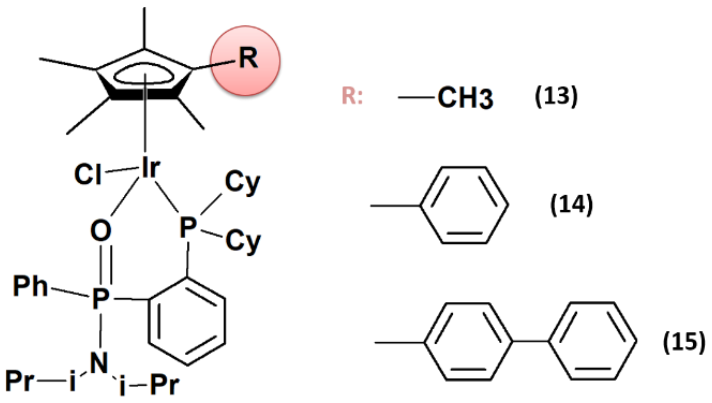


Figure 7 Structure of iridium complexes $[(Cp^x)Ir(P\text{^}O)Cl]PF_6$ with phosphine phosphonic amide ligand.

The cytotoxicity of **13-15** complexes (**Fig. 7**) was tested on immortal (HeLa) and lung (A549) cells with average IC_{50} values 1 μM and 4.4 μM, respectively. These results were comparatively better than the IC_{50} values calculated for cisplatin against a lung cancer cell line. The **13-15** complexes were able to generate ROS by converting NADH to NAD^+ by catalytic hydride transfer from NADH coenzyme to oxygen. Studies on the mechanism of action of these complexes showed that they induce oxidative stress through cell cycle perturbation at S and G2/M phase, and apoptosis in HeLa cancer cells [46].

1.2. Aminomethylphosphines derived from fluoroquinolone

The goal of science is to design a therapeutic agent whose mechanism of action will be different from that of all drugs obtained so far. However, the design and synthesis of new therapeutic substances are very time-consuming and expensive. It takes at least 10 years to bring a new drug to market and costs up to millions of dollars. To paraphrase Confucius saying "When it is obvious that the goals cannot be reached, don't adjust the goals, adjust the action steps", to achieve goals, instead of looking for new classes of therapeutic compounds, it is better to modify the structure of a drug currently used in medicine [47,48]. This can be done by attaching to it another structural motif responsible for selective transport or changing biological properties. It is noted that the most common modification is the secondary nitrogen atom that is part of the piperazine ring of the fluoroquinolone antibiotic [49-58].

1.2.1. Fluoroquinolones

In 1962, George Lesher accidentally discovered the first quinolone antibiotic, nalidixic acid, which was a by-product of the reaction of the antimalarial chloroquine. This discovery led to extensive research on many thousands of compounds based on the quinolone skeleton (**Fig. 8A**), of which over two dozen have found clinical application. Since the 1960s, quinolone antibiotics have been one of the most frequently used antibiotics, especially in urinary tract infections, but also in the treatment of malaria or with immunomodulatory and anticancer effects [59,60].

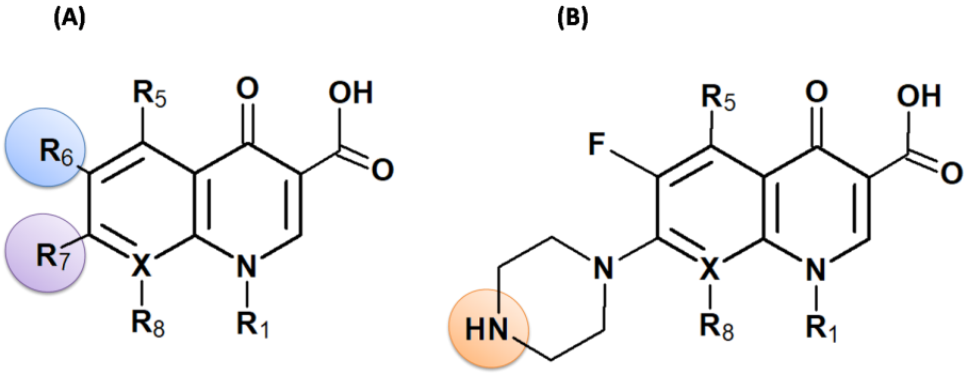


Figure 8 (A) The core structure of quinolone antibiotics with important positions for modifications to improve the activity of the drug. **(B)** The core structure of fluoroquinolones with positions for modifications. R_1, R_5, R_6, R_7, R_8, and X are drug modification positions that improve their biological properties.

One of the first modifications was the introduction of a fluorine atom in the 6-position (**R6**, **fig. 8A**, blue circle, **8B**) of the quinolone ring as well as the piperazine ring in the 7-position ((**R7**, **fig. 8A**, purple circle, **8B**). These changes resulted in a significant increase in the activity of the antibiotics against microorganisms. Quinolones containing a fluorine atom or atoms are called fluoroquinolones (HFQ) (*e.g.* ciprofloxacin, norfloxacin, lomefloxacin **Fig. 9**). The action mode of quinolone antibiotics inhibits the bacterial activity of enzymes (topoisomerase II (DNA gyrase) and topoisomerase IV) by causing the breakage of bacterial chromosomes. As a result of these processes, the activity of enzymes is blocked leading to inhibition of replication, transcription, repair, and recombination of DNA, which results in the death of the bacterial cell [59,61,62].

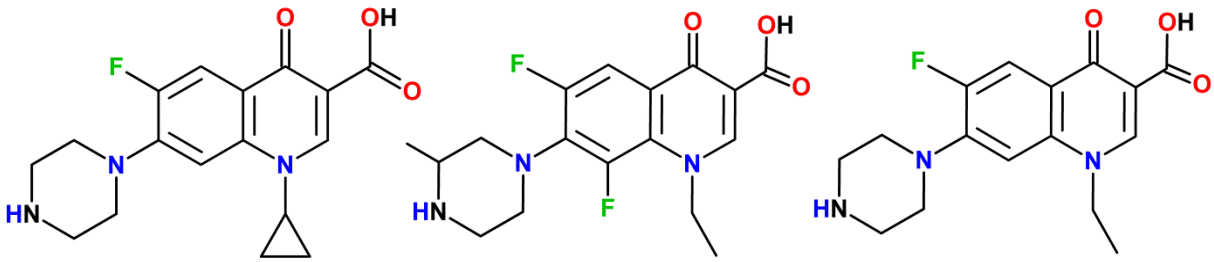


Figure 9 Structure of the molecule of ciprofloxacin, lomefloxacin, and norfloxacin (on the left).

Due to the quinolones' ability to inhibit topoisomerase II - and thus the DNA repair activity - their antitumor activity in recent years has been thoroughly investigated [59,61]. Many complexes obtained by coordinating quinolones with metal ions have been characterized by

their ability to interact with DNA. This fact makes them promising in terms of anticancer properties [58, 62]. Unfortunately, the widespread and frequent use of antibiotics has led to a significant increase in bacterial resistance to antimicrobial agents [63]. Bacteria have developed a variety of resistance systems which are making antibiotic therapy slowly ineffective [64,65]. That is why nowadays scientists put great attention to modifying structures of these antibiotics that can lead to the breakdown bacterial resistance but also strengthen and increase their bacterial but also anticancer action [63,66].

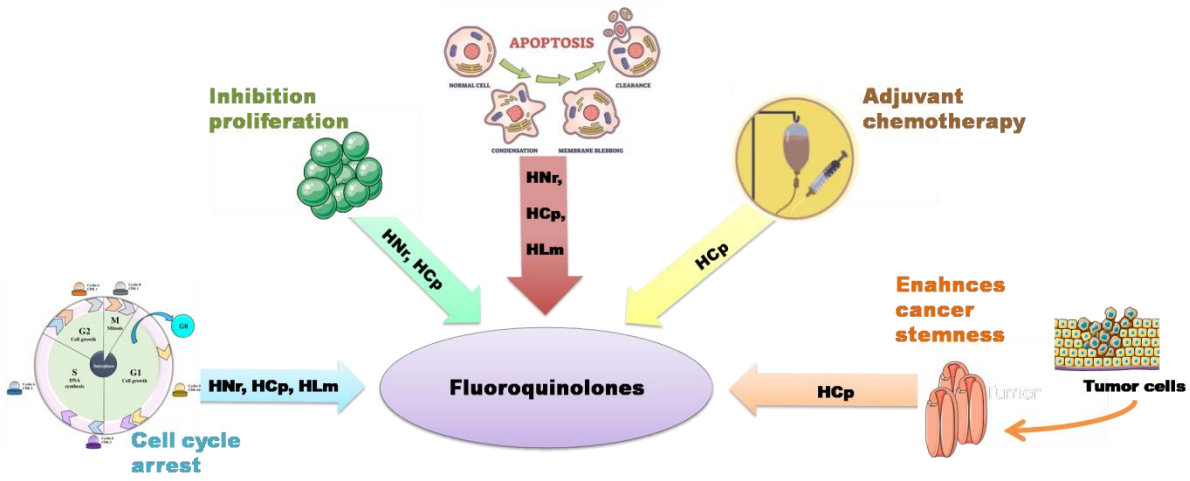


Figure 10 Anticancer activities of fluoroquinolones antibiotics: HNr - norfloxacin, HCp - ciprofloxacin, HLm – lomefloxacin.

The subject of the research undertaken in this work is second-generation antibiotics: ciprofloxacin, lomefloxacin, and norfloxacin (**Fig. 9**). For example, ciprofloxacin is the antibiotic most commonly used in respiratory and urinary tract infections but has also been shown to have anticancer properties against melanoma [67], breast [68], colorectal [69], pancreatic [70], and human prostate [71] cancer cell lines. Studies have shown that ciprofloxacin induced apoptosis by mitochondrial-dependent pathways or S/G2 [72] and G2/M phase arrest [67,68,73]. Other studies have shown a similar S/G2 phase for the antibiotic lomefloxacin in human epithelial tumor cells [74]. The third antibiotic I chose is norfloxacin, which can inhibit cytokine synthesis even at lower concentrations. Additionally, like lomefloxacin, it can also induce apoptosis by decreasing the level of cellular reduced glutathione, increasing mitochondrial dysfunction and oxidative stress in cancer cells [72] (**Fig. 10**).

1.2.2. Phosphinesdervied from fluoroquinolones

The purpose of introducing various structural modifications to quinolone molecules is not only to avoid the phenomenon of drug resistance but also to increase their biological activity. An example of such modification can be an attachment of a phosphine motif - one of the strongest electron-pair donors – to a fluoroquinolone drug. Phosphine ligands, in general, can be easily functionalized, in particular, aminomethylphosphanes derived from aminoacids [75-78] or prepared from the highly water-soluble aliphatic secondary amines [79, 80]. It has been already proven that aminomethylphosphanes derived from fluoroquinolones (**Fig. 11**) can be characterized by better antibacterial properties than parent drugs but also possess new anticancer activity [54, 55, 56, 57, 73, 81]. Those phosphines derived from ciprofloxacin, norfloxacin, and lomefloxacin (**Fig. 11**) were found nontoxic toward healthy cells but at the same time have weak biological activity, especially anticancer [81].

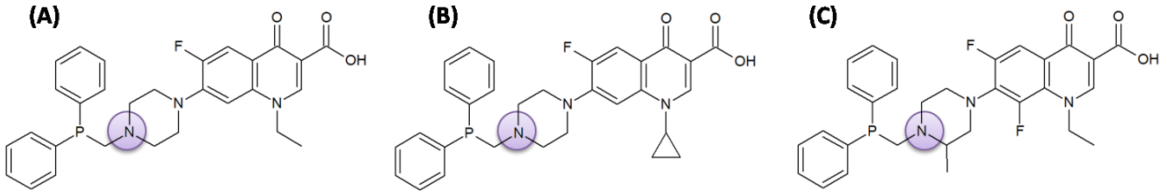


Figure 11 Sructures of aminomethylphosphanes derived from fluoroquinolones: **(A)** norfloxacin, **(B)** ciprofloxacin, **(C)** lomefloxacin.

However, coordination compounds of such aminomethylphosphaneswith ions: Cu(I), Cu(II), Se(II), and Ru(II) significantly increased their antimicrobial and anticancer *in vitro* activity. Such complexes are in most cases, air-stable, and importantly, more cytotoxic *in vitro* against cancer cells than cisplatin. Studies have shown that the cytotoxicity of complexes with phosphines derived from antibiotics against tumor cell lines is much higher than all starting ligands, antibiotics, and cisplatin [55, 57, 58, 81-83]. For example, IC_{50} values for structures depicted below on (investigated by U.Komarnicka *et al.* [58]) are 30 times higher than for cisplatin against human lung adenocarcinoma and mouse colon carcinoma cell lines. It was proved that they penetrate the tumor cells which generate ROS and intercalate with DNA, consequently, causing its degradation. Moreover, treatment of tumor cells with compounds has shown that these compounds cause apoptosis in the induced cell death along with a reduction in the potential of the mitochondrial membrane [58]. The above facts were the reason for my decision to continue work on increasing the biological activity of those phosphines by coordinating them to Ir(III).

1.3. Towards increased anticancer selectivity

One way to enhance the therapeutic effect of drugs is to develop carriers that enable their delivery in the appropriate concentration to pathologically changed tissues, but without affecting normal cells [84]. For this purpose, nanomaterials can be used, which can be categorized into four types(*i*) inorganic, (*ii*) carbon, (*iii*) organic, and (*iv*) composite [85]. Among nanomaterials we can include polymer nanoparticles, liposomes, polymeric micelles, dendrimers, and nanotubes. In my dissertation, it has been used polymeric micelles and liposomes, therefore I will focus only on discussing these two structures (**Fig. 12**).

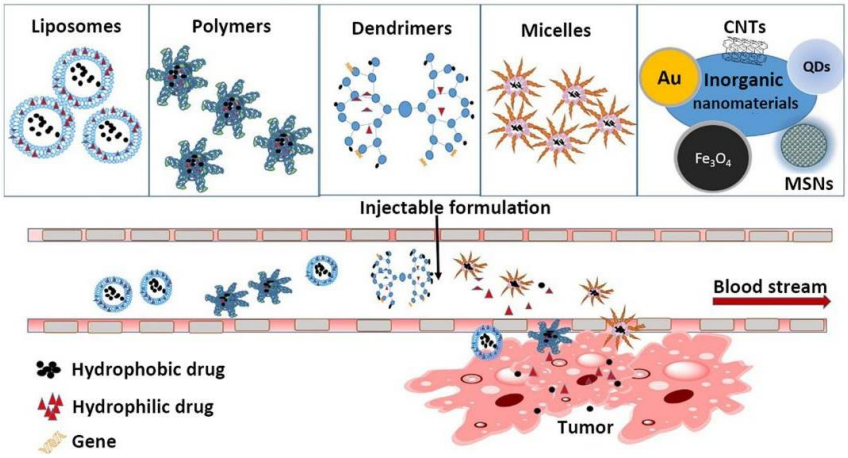


Figure 12 Nanomaterials used as drug carriers for cancer therapy [86].

Polymer micelles consist of a hydrophobic inner part - a core - and a hydrophilic outer part - a shell. Therefore, compounds with poor water solubility can accumulate in the hydrophobic part. In addition, polymer micelles can be functionalized with targeted ligands to increase accumulation within the tumor [87]. Depending on the composition, the polymer micelles are internalized by the neoplastic cells and then induce the action of the drug in an organelle-specific manner. Thus, polymer micelles potentially avoid drug efflux or intracellular uptake mechanisms, overcoming drug resistance in neoplastic cells. Consequently, these micelles have found wide application as drug delivery systems in anti-cancer therapy due to their excellent physicochemical and biological properties [84, 87-89]. Examples of drugs encapsulated inside micelles that have entered clinical trials are CDDP (cisplatin) and DACHPt loading micelles (active oxaliplatin complex), which are in phase III and I clinical trials, respectively. Following systemic administration, micelles have been shown to achieve remarkable *in vivo* antitumor efficacy with reduced side effects on healthy cells due to prolonged circulation and efficient tumor accumulation [90, 91].

Another modified version of the lipid bilayer nanocarriers, liposomes, as a drug delivery platform is also gaining increasing importance in cancer therapy. Briefly, this is because of its excellent biocompatibility and the ability to select particle size and surface modification to overcome biological barriers and reach destinations [92]. Currently, there are many products available on the market and in the clinical development phase for use as drug carriers with anti-cancer properties. Pegylated liposomal doxorubicin is one such example used in epithelial ovarian cancer. The encapsulated doxorubicin significantly improved drug accumulation in tumor tissues, minimizing many side effects, *e.g.* it reduced the cardiological toxicity caused by non-liposomal doxorubicin [93]. Interestingly, the combination of magnetic inorganic nanoparticles (MNPs) with liposomes offers many advantages. As a result of this connection, the magnetic surfaces of the nanoparticles are modified, which increases their solubility in the aqueous environment and increases the intracellular uptake [94]. On the other hand, because the phospholipids in liposomes are biocompatible, the loading of MNPs in the liposomes will not cause any adverse toxicity, and will additionally assist them in targeted anticancer therapy using a magnetic field [95]. For example, H. Nobuto and co-workers have developed novel multifunctional magnetic liposomes containing doxorubicin (magnetic DOX liposomes) that treat osteosarcoma-bearing hamsters. To maximize the DOX concentration in the neoplastic tissue, the authors used a DC dipole magnet applying a magnetic field strength of 0.4 T for 1 h. It should be emphasized that this therapy inhibited tumor growth for two weeks and significantly suppressed metastasis for three weeks after treatment [96].

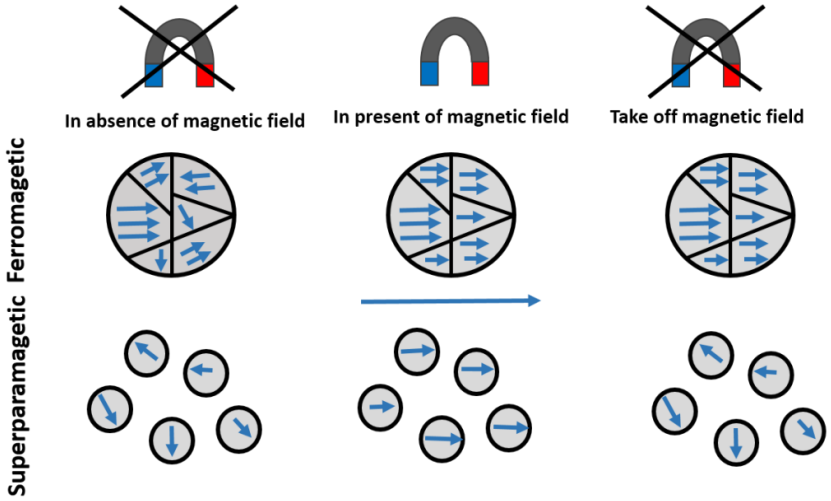


Figure 13 Behavior of magnetic domains in ferromagnets and magnetic moments in superparamagnetic nanoparticles in relation to an external magnetic field.

In the last 20 years, MNPs (Magnetic Nanomaterials) have already been used in modern medicine as (*i*) biosensors - to detect a specific type of biomolecules [97, 98], (*ii*) drug carriers

[99, 100], (*iii*) tumor biomarkers in photodynamic therapy [101, 102] (*iv*) an important element of hyperthermia [103,104] or as (*v*) contrast agents in computed tomography and MRI imaging [105].

Typically, MNPs are considered to exhibit ferrimagnetic, ferromagnetic, or superparamagnetic properties, however, from a medical point of view, the use of superparamagnetic particles is the preferred approach (**Fig. 13**) [106]. The majority of nanoparticles used in these applications are superparamagnetic iron oxides magnetite Fe_3O_4 or maghemite Fe_2O_3, because due to their known metabolic pathways and low overall toxicity [99, 106].

2. Aims of this Work

The principal aim of this desideration was to design and synthesize organometallic iridium(III) complexes with phosphines derived from fluoroquinolone antibiotics possessing anticancer potential.

In the next step, for the compounds with the highest antitumor activity, methods for their selective delivery using encapsulation in nanoformulations were designed.

Successfully obtained materials will be good candidates for the next stage of *in vivo* research and in the future may be considered as medicines.

To achieve the main goal of the work, it was necessary to develop many intermediate steps:

- Synthesis diphenyl(hydroxymethyl)phosphine (Ph_2PCH_2OH) and three phosphine ligands derived from fluoroquinolones (e.g. lomefloxacin, ciprofloxacin and lomefloxacin);
- Synthesis new mononuclear Ir(III) with phosphine-fluoroquinolone conjugate. A hydrophobic phosphine unit (Ph_2PCH_2OH) will be used as the linker between the cytotoxic metal complex and the fluoroquinolone carrier;
- Physicochemical characterization of the synthesized complexes in the solid-state as well as in solution (X-ray, elemental analysis, MS spectrometry, NMR, UV-Vis, luminescence, IR spectroscopies);
- Determination of the stability of the complexes in water solution and the air;
- Description of the properties of luminescent complexes;
- Description of electrochemical properties;
- Encapsulation of the resulting Ir(III) complexes in micelle and liposome structure;
- Examine biological activity *in vitro* against several tumor and normal cell lines for all obtained compounds;
- Determination of cell viability in a 3D model of cell culturing (spheroids)
- Establish the mechanism of cancer cell death induced by the tested compounds:
    - Type of cell death characterization of initiator and effector caspase expressions in cancer cell;
    - Analysis of the cell cycle;
    - ROS production inside the cancer cell and mitochondrial damages;
    - Analysis of DNA interaction with complexes;
    - Investigation of interaction of complexes with human protein such as album and transferrin.

Publications constituting the basis of the doctoral dissertation

The research results obtained for the above-mentioned goals have been described in the form of three published scientific articles.

[S1] Synthesis, physicochemical characterization and preliminary *in vitro* antitumor activity of phosphine Ru(II) and Ir(III) complexes, ***Dalton Transactions***, advanced article, 2022, DOI: 10.1039/D2DT01055K; **back cover**

[S2] Anticancer potency of novel organometallic Ir(III) complexes with phosphine derivatives of fluoroquinolones encapsulated in polymeric micelles; ***Inorganic Chemistry Frontiers***, 2020, 7, 3386-3401; DOI:10.1039/d0qi00538j

[S3] Interaction between DNA, albumin and apo-transferrin and iridium(III) complexes with phosphines derived from fluoroquinolones as a potent anticancer drug; ***Pharmaceuticals***, 2021, 14, 685/1-685/25; DOI:10.3390/ph14070685

In addition, some unpublished results will be presented also in this report to show the applicative nature of the Ir(III)-phosphine complexes.

It is worth mentioning, that presented in this paper data have been obtained thanks to NCN grant preludium "*Homo- and heterometallic phosphine ruthenium and iridium complexes - design, synthesis, bioactivity, and magnetic-nanoformulation as a potential platform for dual-targeted drug delivery*", which I am the leader.

[S4] Liposomal formulation of magnetic iridium-copper complexes with phosphine derived from fluoroquinolones for human prostate carcinoma treatment; ***Inorganic Chemistry Frontiers***, submitted

3. Experimental section

Several organic and inorganic compounds have been synthesized using Schlenk techniques. In the next step, the obtained compounds have been characterized in detail using many physicochemical methods.

The conditions of the conducted experiments were described in detail in the articles that will be the basis of the scientific achievement. Only the most important data are presented below.

- All synthetic works with the use of aminomethylphosphines and mononuclear iridium(III) compounds were carried out using the Schlenk technique in an inert gas (nitrogen) atmosphere to avoid oxidation of any of the reactants in the presence of atmospheric oxygen. The solvents used for the synthesis were each time deaerated.
- All synthesized mononuclear complexes were subjected to detailed NMR analysis taking into account the phosphorus, proton, and carbon spectra. In problematic situations, two-dimensional measurements were also used.
- The structure of all compounds was confirmed by electrospray ionization mass spectrometry (ESI-MS), and the purity of the compounds was each time checked by elemental analysis.
- In justified cases, additional measurements were used with the methods of IR or UV-Vis spectroscopy. In addition, using spectroscopic methods, I also determined the stability of compounds in an aqueous solution (UV-Vis, NMR), and characterized their fluorescent properties as well as their electrochemical potential (CV).
- The fluorescent properties of the complexes can provide useful information on the distribution, accumulation, and uptake of anti-cancer drugs in living cells or organisms.
- All synthesized compounds were obtained in the form of single crystals, which made it possible to perform X-ray diffraction measurements and solve the crystal structure. These measurements were carried out in cooperation with Dr. Agnieszka Skórska-Stania from the Faculty of Chemistry of the Jagiellonian University.
- To overcome poor solubility, serious side effects related to the systemic cytotoxicity of the complexes, and the acquisition of cancer cell resistance, the resulting homonuclear complexes were encapsulated in nanoemulsions and polymer micelles.
- Determination of the cytotoxicity of compounds as well as an attempt to explain the mechanism of action was carried out at the Faculty of Chemistry of the Jagiellonian University (in the team of Prof. GrażynaStochel) and in cooperation with the Małopolska Biotechnology Center with Dr. Barbara Pucelik: ***(i)*** determination of cytotoxicity against healthy and cancer cells (determination of IC_{50} using the MTT test): human breast adenocarcinoma (MCF7), human lung adenocarcinoma (A549), mouse colon carcinoma (CT26), human pancreatic/duct carcinoma (PANC-1), (human prostate carcinoma (DU145), metastatic human melanoma (WM2664), human embryonic kidney (HEK293T); ***(ii)*** use of a fluorescence microscope to visualize and confirm the results on tumor cells stained with propidium iodide and fluorescein diacetate; ***(iii)*** metal uptake and intracellular localization (ICP-MS, confocal microscopy, commercially used

tests); ***(iv)*** determination of the type of cell death (flow cytometer, commercially used kits); ***(v)*** characterization of initiator and effector caspase expressions in cancer cell; ***(vi)*** analysis of the cell cycle ***(vii)*** and apoptosis-related proteins e.g. caspase -3/7; ***(viii)*** study of level of mitochondrial membrane; ***(ix)*** drug cytotoxicity analysis on 3D tumor spheroids.

- The ability to interact with selected iridium complexes with blood plasma proteins: albumin and transferrin was also determined. These proteins may have a key influence on the transport and metabolism of synthesized iridium compounds in the body. The analysis was performed by quenching the characteristic fluorescence of both proteins due to their mixing with iridium complexes. In addition, several selected compounds were tested for their ability to interact with DNA using the spectrometry of the disappearance of the characteristic fluorescence of the ethidium bromide (EB; intercalation), 4′,6-diamidino-2-phenylindole (DAPI; binding to a minor groove), and methyl green (MG; binding to a major groove) complex with calf thymus DNA (CT DNA).
- Gel electrophoresis of the pBR322 plasmid was used to determine the ability of the compounds to induce single- and/or double-strand damage of the DNA and to confirm the generation of particular types of ROS involved in plasmid degradation.

The author independently conducted all chemical tests and took an active part in biological research with the use of cell cultures. This is evidenced by research internships in the above-mentioned centers.

4. Results and discussion

Using synthetic strategies to achieve the aim of the dissertation, a new coordination framework was obtained which was fully characterized in the papers **S1** - **S3**. For this reason, my report will present the most important achievements and results.

This guide is divided into two parts. The first one presents the synthesis and physicochemical characteristics of mononuclear iridium(III) complexes, while the second one presents their biological properties together with an attempt to explain the mechanism of their action.

4.1. Synthesis and physicochemical characteristics of mononuclear Ir(III) complexes

In the first step of my work, four phosphines were synthesized Ph_2PCH_2Nr(**PNr**) [55], Ph_2PCH_2Cp (**PCp**) [54], Ph_2PCH_2Lm [57] (**PLm**) (**Fig. 11**, page 22), Ph_2PCH_2OH (**POH**) [58]. These starting ligands were used to obtain three mononuclear iridium(III) complexes: Ir(η^5-Cp*)Cl_2PNr (**IrPNr**), Ir(η^5-Cp*)Cl_2PCp (**IrPCp**), [Ir(η^5-Cp*)Cl_2PLm (**IrPLm**) (**Fig. 14**). In order to understand the biological role of antibiotics in the resulting complexes, a phosphine-iridium(III) compound was also synthesized without fluoroquinolones: Ir(η^5-Cp*)$Cl_2PPh_2CH_2OH$ (**IrPOH, Fig. 14)**).

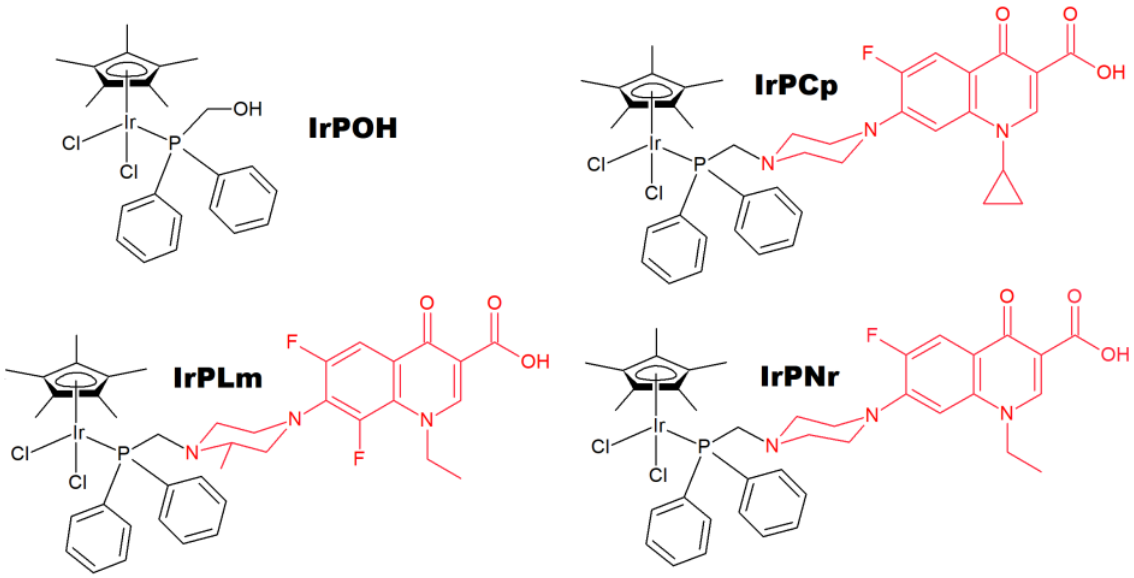
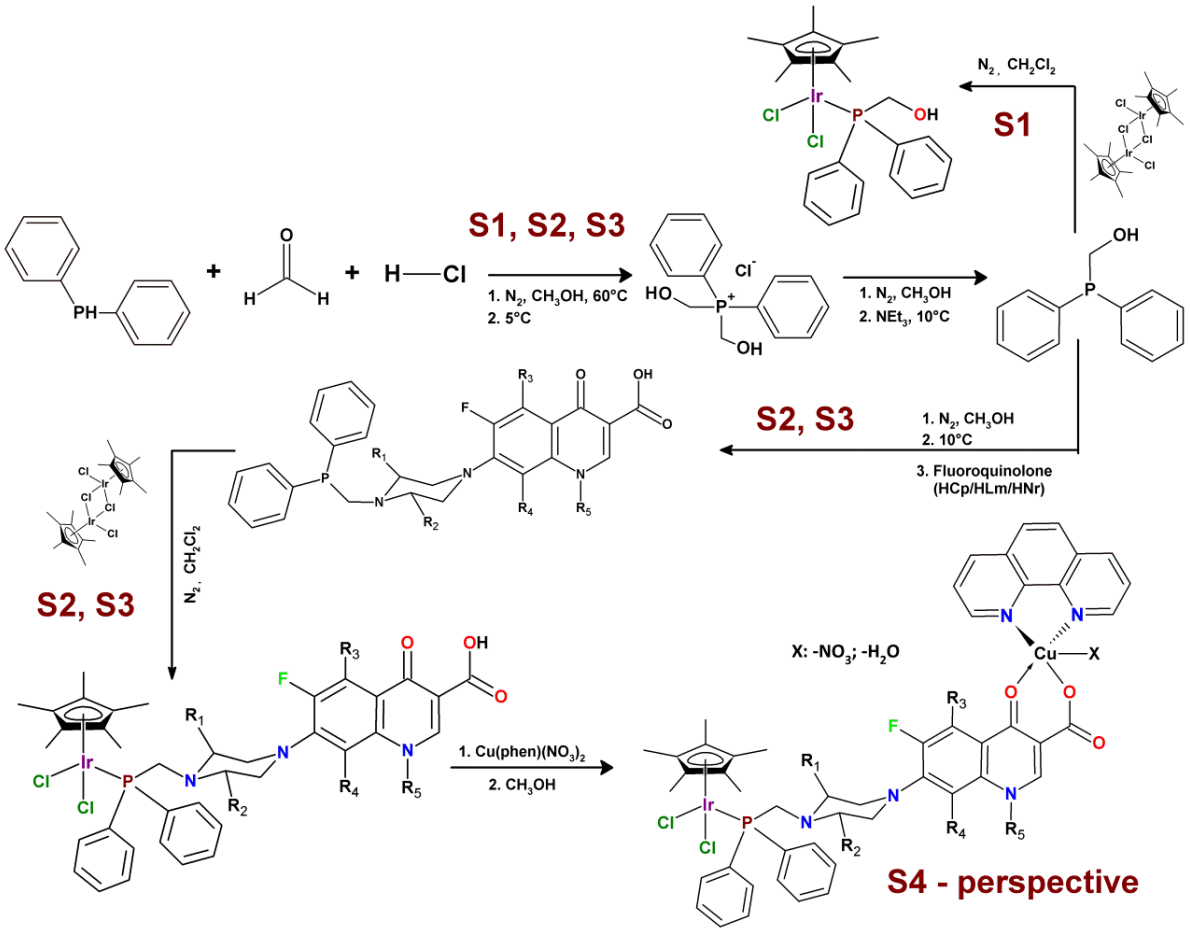


Figure 14 Coordination scheme of iridium(III) ion with pentamethylcyclopentadiene and phosphine ligand.

4.1.1. Synthesis of mononuclear Ir(III) complexes [S1,S2, S3]

All syntheses were carried out under a nitrogen environment using Schlenk techniques. The complexes and phosphines derived from fluoroquinolones are soluble in dimethyl sulfoxide, chloroform, and dichloromethane. Additionally, the iridium(III) complexes are insoluble in water but become highly water-soluble when a small amount of DMSO (1-2%) is added. The synthesized homonuclear Ir(III) complexes are stable in the solid-state.



Substituent	Ciprofloxacin (PCp)	Lomefloxacin (PLm)	Norfloxacin (PNr)
R_1	H	CH_3	H
R_2	H	H	H
R_3	H	H	H
R_4	H	F	H
R_5	△	⌊	⌊

Scheme 1 Schematic view of the compounds and synthetic routes.

4.1.2. Analysis of structures in solution [S1, S2]

The techniques such as NMR, UV-Vis and mass spectrometry allowed us to confirm the mononuclear structure of the discussed Ir(III) complexes in solution under the atmospheric oxygen.

A characteristic signal from the phosphorus atom was observed on all the $^{31}P\{^{1}H\}$ NMR spectra of the examined compounds **IrPOH**, **IrPNr**, **IrPLm** and **IrPCp** (**Fig. 15**). The lack of other signals in the phosphorus spectrum proves that the obtained systems are free of phosphine derivatives (*e.g.* phosphine oxides).

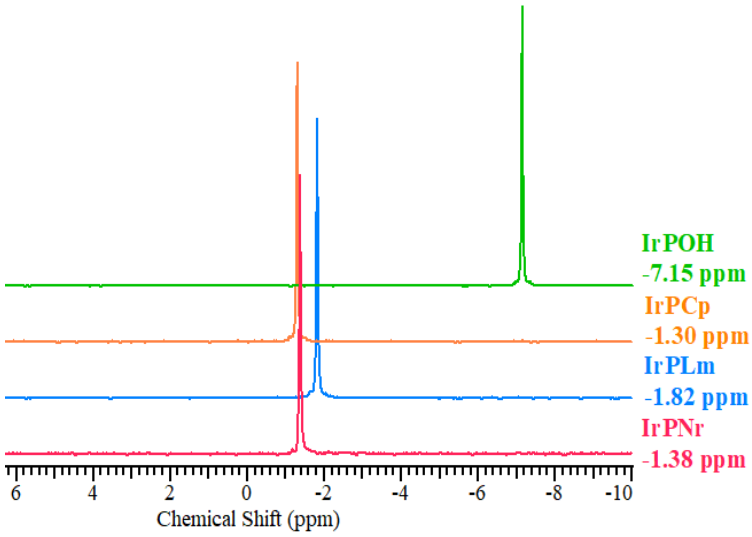


Figure 15 Comparison of $^{31}P\{^{1}H\}$ NMR spectra for mononuclear Ir(III) complexes.

Literature and experimental data indicate that the signal of the uncoordinated aminomethylphosphane is in the negative part of the spectrum (**POH**: -11.46 ppm; **PCp**: −27.4 ppm; **PLm**: −27.4 ppm; **PNr**: −27.5 ppm) [54, 55, 57, 58]. Coordination of phosphine to iridium(III) ion caused a downfield shift of the signal originating from the phosphorus atom (**IrPOH**: -7.15; **IrPCp**: −1.30 ppm; **IrPLm**: −1.82 ppm; **IrPNr**: −1.38 ppm, (**Fig. 15**). Most likely, this may be due to an increase in the screening effect (known as the Shielding effect) resulting from the formation of iridium(III) inorganic compounds. It is also noted that the singlet characteristic for **IrPOH** at -7.15 ppm is shifted by about 5.5 ppm towards the higher fields, after the addition of a fluoroquinolone molecule to the PPh_2CH_2- motif, which is undoubtedly related to the change of the substituent in the vicinity of the phosphorus atom. The coordination process also initiates changes in the $^{13}C\{^{1}H\}$ spectra as well as in the ^{1}H spectra. The data obtained from the $^{13}C\{^{1}H\}$ and ^{1}H NMR spectra indicate that the biggest changes are observed for atoms close to the coordination. Namely, the H^1 proton of the

Ph_2PCH_2- group is undergoing an upfield shift regardless of the type of substituent attached to the piperazine ring.

The ESI(+)MS spectra (recorded in the positive ion modality) show the corresponding peaks of molecular ions $[M+H]^+$ by the neutral character of these compounds only for complexes with phosphine derivatives from fluoroquinolones. Fewer peaks corresponding to $[M-Cl]^+$ and $[M-2Cl]^+$ ions were also recorded, indicating that chloride groups were easily displaced under the appropriate conditions. In the case of the **IrPOH** complex, abundant $[M+Na]^+$ sodiated ions were detected at m/z 637, however $[M - Cl]^+$ ions dominated at m/z 579. In the ESI(+)MS spectra no peaks corresponding to the loss of the phosphine ligands and the arene ring were observed. These results indicate strong metal-ligand and metal-arene bonds.

As the Me-Cl (Me: metal) bond in this type of complex is often subject to hydrolysis, it was decided to measure their stability using UV-Vis spectroscopy. The electronic spectra (recorded over 72 hours) of all mononuclear iridium(III) complexes were measured in a cellular medium (DMEM with 2% DMSO), due to the poor solubility of these complexes. During the 72-hour incubation, significant changes in the intensity and shape of the characteristic absorption band (MLCT) were observed only for **IrPOH**. The attachment of the fluoroquinolone motif to the starting phosphine ligands (PPh_2CH_2-) changed the electronic nature of the final complexes and most importantly increased their stability in solution. These complexes did not dissociate or decompose under the model conditions. The stability of complexes with phosphines derived from fluoroquinolones was also confirmed by ^{1}H NMR (**Fig. 16** shows a selected **IrPCp** complex). As in the case of the UV-Vis spectra, no additional peaks were found in the NMR spectra during the 48 h incubation with the compounds.

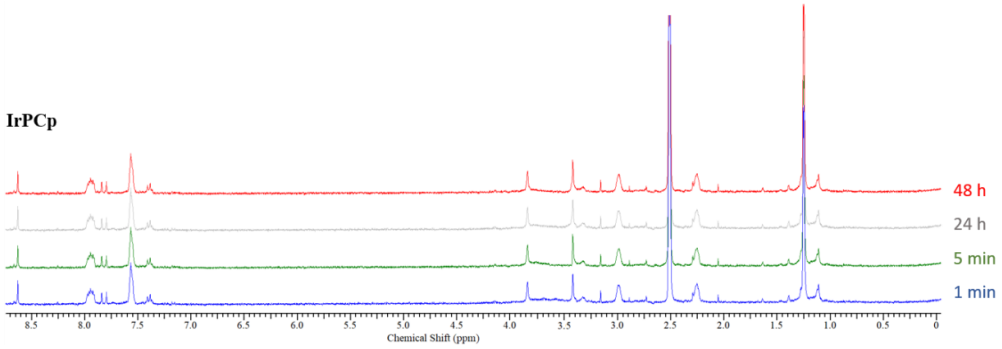


Figure 16 Time-dependent ^{1}H NMR spectroscopic stability study for **IrPCp** in the mixed of 80% DMSO-d_6 and 20% D_2O for48 h of the experiment.

Therefore, in order to confirm the hydrolysis of the **IrPOH** complex, experiments have been performed using ^{1}H NMR spectroscopy in the presence of NaCl in different concentrations (C_{NaCl} = 4, 23 and 104 mM), which reflect the chloride concentrations in the cell nucleus, cytoplasm and blood plasma, respectively. Along with NaCl concentrations increased, the decrease of aqua complex (Ir-OD_2/OD) simultaneously with the increase of chloro-complex (Ir-Cl_2) was observed (**Fig. 17)**.

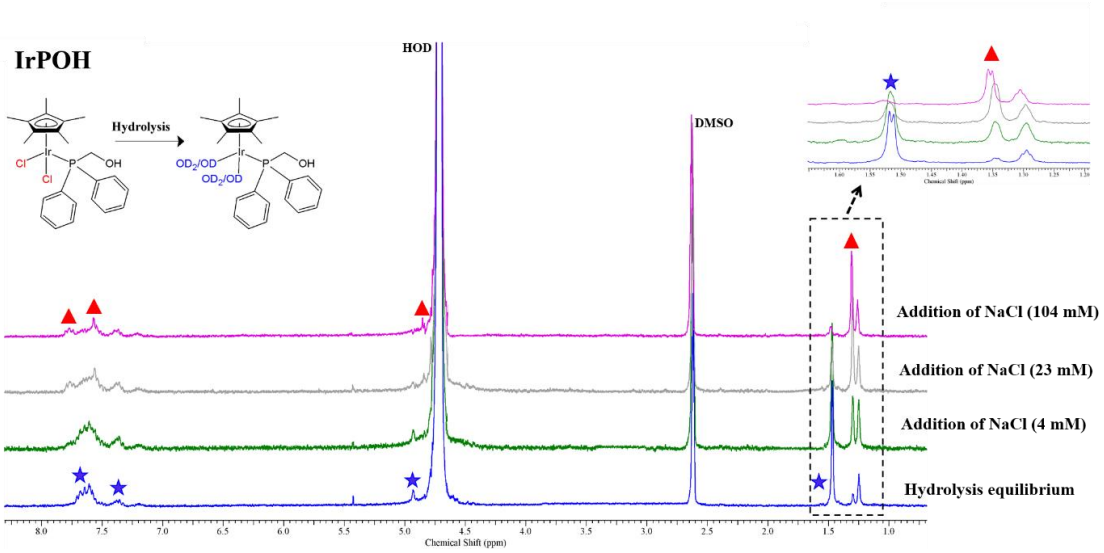


Figure 17 Confirmation of hydrolysis of **IrPOH**. From the bottom: ^{1}H NMR spectrum of an equilibrium solution of **IrPOH** (1 mM) in a mixture of 20% DMSO-d_6 and 80% D_2O (v/v) at 298 K. The spectra were measured 10 min after the addition of: 4 mM NaCl; 23 mM NaCl; 104 Mm NaCl to the equilibrium solution of **IrPOH**.

The analysis of the NMR spectra showed that at a concentration of 104 mM [Cl^-] there was still a small amount (approx. 10%) of the aqueous form. These results illustrate that Ir^{III} complex at biologically relevant chloride concentrations exists as aqua form (Ir-OH_2) [107]. Hydrolysis is a common mechanism of metal drug activation by displacing weakly bound σ-donor ligands by water molecules [108]. However, inside cells, where chloride ion concentration is lower, hydrolysis can be inhibited. On the other hand, metal complexes can also interact with many targets inside the cell, which can also be reflected in their hydrolysis. One of the reasons may be the interaction of metal complexes by direct substitution of chloride by nucleobases (DNA) [44, 108, 109].

As rapid hydrolysis complexes sometimes lead to decrease cytotoxic activity due to rapid inactivation by side reactions before achieving the intended goals. The complexes formed during hydrolysis containing water molecules in an aqueous solution in their structure (Ir-OH_2)

react more easily with nucleobases than chloride complexes (Ir-Cl). So, it has been also decided to investigate the hydrolysis rate of the **IrPOH** complex using UV-Vis spectroscopy (**Fig. 18**).

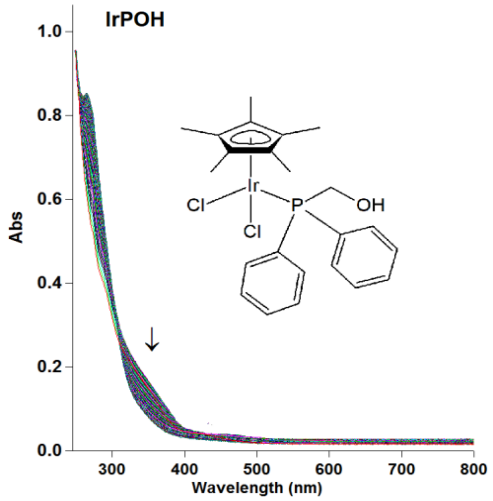


Figure 18 UV/Vis spectra of **IrPOH** in 20 % DMSO/80% H_2O (v/v) solution over 24 h.

The spectral characteristics of mononuclear iridium(III) complexes indicate the presence of an absorption band at high energies (<300 nm) in the UV-Vis spectra. These absorption bands are attributed to the ligand-centered (LC) $^1\pi$-π^* transitions of both the phosphine ligands (POH and FQ). In the range of 300 to 400 nm, metal-to-ligand charge transfer (MLCT) transitions are observed [110]. Changes in the band intensity were noted within 24 h of the experiment (**Fig. 18**). The dependence on the time of Ir-OH_2 formation for the **IrPOH** complex was fitted to the pseudo-first-order kinetics. The value of the hydrolysis rate constant was $4.57 \cdot 10^{-4}$ min^{-1}, and the half-lives of hydrolysis were 1515.2 min. Comparing the half-lives of the **IrPOH** complex with other complexes containing the iridium ion, it can be concluded that hydrolysis occurs relatively quickly (in minutes) [14, 111]. For example, this value for the compound $[Ir(H_2O)_6]^{3+}$ was over 300 years [112], while trans-$[IrCl_4(DMSO)(Im)]$ [ImH] (DMSO=dimethylsulfoxide, ImH=imidazole) compound was neutral to hydrolysis and showed no anti-tumor activity (NAMI-A iridium analog) [113].

Additionally, it is worth mentioning that complexes with phosphine conjugates of fluoroquinolones show intense fluorescence in solution in contrast to **IrPOH**. The fluorescent properties of the complexes (**IrPCp**, **IrPNr**, **IrPLm**) can provide useful information on the distribution, accumulation, and uptake of anti-cancer drugs in living cells or organisms. The maximum emission band for the second generation antibiotics (**PCp**, **PNr** and **PLm**) is around 420 nm, and it is in agreement with literature data [54, 55, 57]. After coordinating the phosphine ligands of fluoroquinolones to the iridium(III) ions, a redshift was observed in the emission spectra, showing purple emission at a maximum wavelength of around 440 nm (**Fig. 19**).

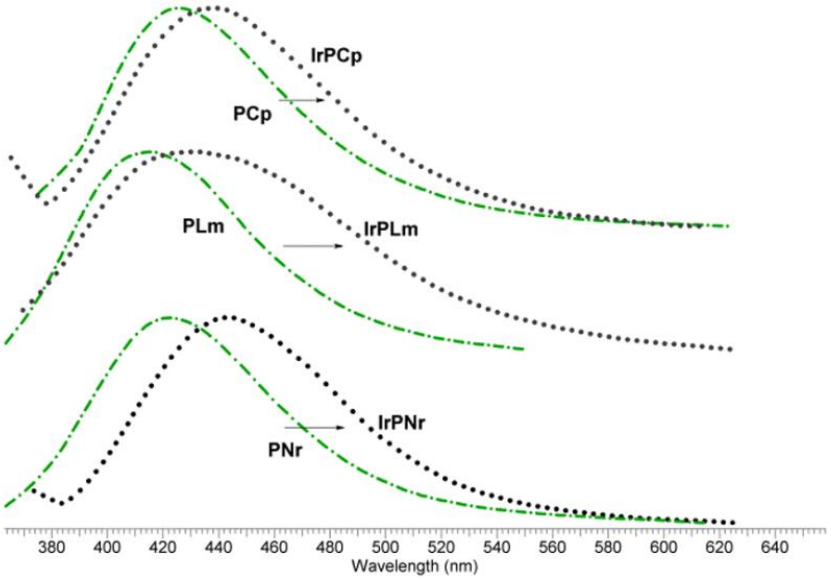


Figure 19 Normalized emission spectra for Ir(III) complexes and the corresponding phosphine ligands; λex = 340 nm, 298 K.

To obtain qualitative information about the course of the electrochemical reaction, cyclic voltammetry (CV) was performed for all examined complexes. It will also allow us to understand the redox activity of the studied complexes through the production of reactive oxygen species in cancer cells. This fact is due to the reduction or oxidation of metal centers or the ligands surrounding them, or by their interaction with various biomolecules in the redox pathways [108].

Cyclic voltammograms for the **IrPOH** complex showed one irreversible oxidation peak at 1.5-1.7 V attributed to the phosphine ligand. In the case of complexes containing the fluoroquinolone motif (**IrPCp**, **IrPLm**,**IrPNr**) two irreversible oxidation peaks at about 1.0 V and 1.25 V are observed, which are attributable to the phosphine ligand and the iridium(III) ion. Moreover, for all investigated complexes, one quasi-reversible reduction peak of about -0.55 V is observed, which is attributed to the phosphine ligand (**Fig. 20**). In addition, both the cathode peak and the anode peak were shifted only slightly as the scan speed increased. This shows that the redox process occurs through controlled diffusion. Using cyclic voltammetry in conjunction with absorption spectroscopy, we can determine the electron transfer reactions that occur without changes in the stereochemistry of the complexes. The production of ROS in the examined systems is related to the oxidation of iridium(III) ions to iridium(IV) ions.

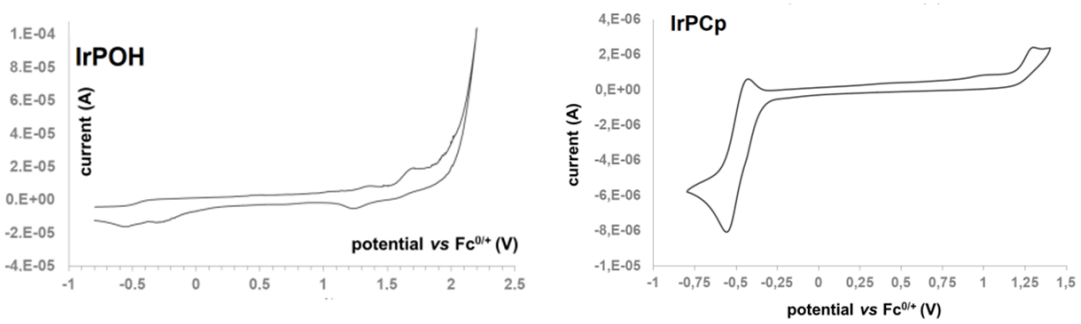


Figure 20 Cyclic voltammograms of **IrPOH** and **IrPCp** (1 mM), recorded with 0.1 M tetrabutyl ammonium perchlorate (TBAP) as supporting electrolyte in DMF solution. Scan rates (10 mVs^{-1}). The potentials were referenced to the $Fc^{0/+}$ redox coupe.

4.1.3.Analysis of structures in solid state [S1, S2]

In the process of crystallization by slow solvent evaporation, single crystals of all four coordination compounds were obtained (**Fig. 21**). All the complexes were structurally identified by single-crystal X-ray diffraction analysis. Details concerning crystal data and refinement are given in publications **S1** and **S2**. Despite similar molecular structures, the presented derivatives crystallize in different space groups, additionally, in each case, there is only one molecule in the asymmetric unit.

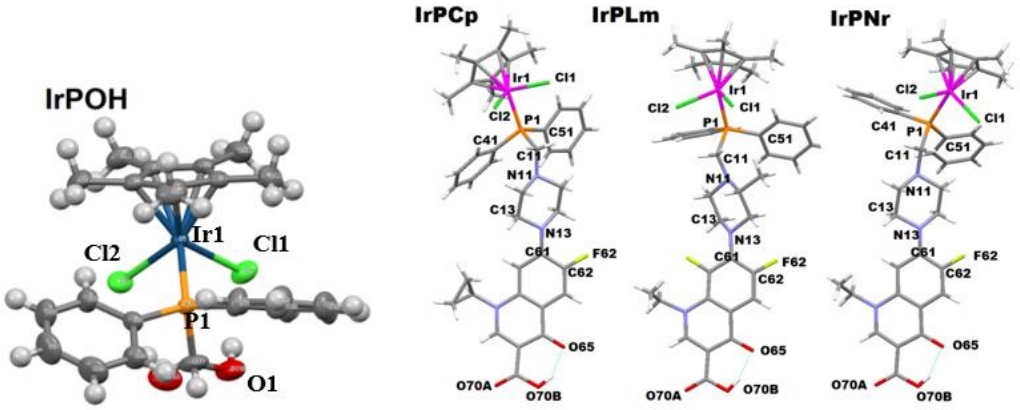


Figure 21 The monocrystal structures of the complex molecules **IrPOH**, **IrPCp**,**IrPLm** and **IrPNr**. The solvent molecules are omitted for clarity.

Interpretation of the crystallographic data obtained for the complexes confirms (similarly to the studies of the complex solutions) that the central iridium(III) ion is coordinated by the cyclopentadienyl moiety, phosphorous atoms from ligands and two terminal chloride anions.

The mononuclear iridium(III) complexes adopt half-sandwich pseudo-octahedral “three-leg piano-stool” geometry. The bond lengths and angles are within the range typical of iridium(III) complex compounds with cyclopentadienyl moiety and phosphine ligands. The bond lengths P1-Ir1 in all structures were found to be on an average value of 2.3 Å, whereas distances between P1-C11 are in the range of 1.810-1.865 Å.

In the case of the complex without the fluoroquinolone motif (**IrPOH**), the strongest hydrogen bond form between the hydroxyl group (-OH) acting as a donor and the chlorine atom (-Cl) acting as an acceptor. This intramolecular interaction gives rise to the S_1^1 motif (6).

The arrangement of molecules in complexes containing fluoroquinolone derivatives in the crystal structure of the discussed compounds is determined by the orientation of the piperazine ring and fluoroquinolone moiety. In each examined molecule, intramolecular hydrogen bonds between hydroxyl groups (O70B–H70B) and oxygen atoms from carbonyl groups (O65=C65) are observed. Among all the obtained single crystals, only in one crystal structure - **IrPCp·CHCl$_3$**, the intermolecular π-ring stacking in the unit cell is observed. A fragment of the heterocyclic moiety of fluoroquinolone (*i.e.* 6-membered rings) is involved in a strong π–π interaction with symmetry related to the other fragment of the fluoroquinolone moiety a neighboring ligand in the unit cell. The arrangement of π – π in the structure of this complex is a face to face pattern, with a centroid (N67 – C67 – C66 – C65 – C64 – C68) – centroid (C69 – C68 – C64– C63 – C62 – C61) a distance of value 3.652 Å. Moreover, the chloroform molecule represented in the **IrPCp** structure is located in the peripheral part of the examined molecule. Such an arrangement allows the π - π interactions in the **IrPCp** molecule (**Fig. 22)**.

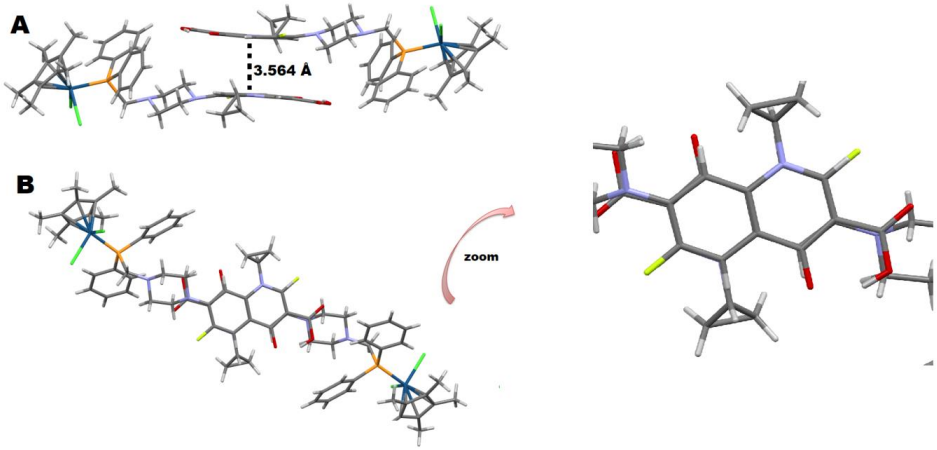


Figure 22 The packing in the crystal **IrPCp·CHCl$_3$** showing π-stacking interaction between the fluoroquinolone rings **(A)** and face-to-face pattern of the π–π stacking in this complex **(B)**.

The conformation of the fluoroquinolone motif of examined compounds can be defined in terms of torsion angle, defining the orientation of the piperazine ring and antibiotics moiety (**IrPCp**: C13 – N13 – C61 – C62 −165.92°; **IrPLm**: C13 – N13 – C61 – C62 117.48° **IrPNr**: C14 – N13 – C61 – C62 −165.97°). The comparative analysis of the data obtained for the **IrPLm** complex with the data for the **IrPCp** and **IrPNr** complexes showed that the values of the torsion angle described above are significantly different. As in the **IrPCp** and **IrPNr** complexes, the solvent molecules are placed differently positioned in the molecule and the fluorine atoms do not interact with them. The situation is different in the case of the **IrPLm** complex, where weak interaction of the fluorine atom with the solvent molecule was observed.

4.2. Biological properties analysis [S1, S2, S3]

In order to characterize the biological properties of the obtained compounds, *in vitro* cytotoxic activity was determined towards five selected cancer cell lines: metastatic human melanoma (WM2664), human lung adenocarcinoma (A549), human breast adenocarcinoma (MCF7), human pancreatic/duct carcinoma (PANC-1), human prostate carcinoma (DU-145) and one normal which was human embryonic kidney (HEK293T) cell line. Moreover, in order to increase the cellular accumulation of metal complexes and to control their uptake only to neoplastic cells as compared to normal cells, which would further reduce the systemic toxicity of the complexes, the synthesized compounds were encapsulated in micelles. Mechanistic studies were performed for the most active compounds. Additionally, *in vitro* cytotoxicity assays within multicellular tumor spheroids (3D) has been also performed. Cytotoxicity was assessed on the basis of the IC_{50} value (drug concentration required to inhibit the growth of 50% of cells). As the table shows (**Tab. 1**), the studies were carried out in two different approaches - after 24 h and 24 h + 48 h of incubation of compounds with cancer cells. Cell lines were also treated with cisplatin (reference drug) in the same concentration range as the synthesized complexes. This approach not only provides more accurate information on their cytotoxicity, but also allows the rate of their entry into the cell to be estimated.

Table 1 Values of IC_{50} [μM] (concentration of a drug required to inhibit the growth of 50 % of the cells) for WM2661, A549, MCF7, PANC-1, DU-145, HEK293T cells after 24 h and 24 h + 48 h treatment with the studied compounds and cisplatin as reference

	IC_{50} [μM] ± SD;24h					
	A549	MCF7	PANC-1	DU-145	WM2664	HEK293T
IrPOH	45.8± 1.6	>1000	35.5±1.1	31.5±0.4	>1000	754.2±10.1
IrPCp	68.8±1.7	65.7±2.8	43.0±1.3	11.8±1.1	*	42.1±2.1
IrPLm	69.7±4.1	64.5±2.2	48.1±4.3	11.3±0.9	*	46.0±1.6
IrPNr	71.4±1.6	68.8±5.3	42.6±8.1	12.9±2.1	*	41.8±1.7
cisplatin	>100	51.9±4.6	>100	>100	2.63±0.6	21.0±1.8
	IC_{50} [μM] ± SD; 72h (24h + 48h)					
IrPOH	22.5±1.3	>1000	139.1±2.	52.4 ± 0.9	>1000	834.2±11.8
IrPCp	29.5±0.7	35.0±0.9	8.7±0.3	4.8±0.1	*	28.5±1.5
IrPLm	27.4±1.7	33.7±3.8	8.1±1.1	5.1±0.4	*	28.0±1.7
IrPNr	29.1±1.2	31.7±0.3	8.3±1.3	5.5±0.2	*	21.4±1.2
cisplatin	71.7±3.7	17.7±8.6	74.5±2.3	65.5±3.6	8.29±0.4	10.3±2.1

* no available data

The organometallic Ir(III) complexes showed significant and differentiated cytotoxic activity against all the tested cell lines. Comparing these data with the literature data, it is worth noting that all complexes were characterized by higher cytotoxicity than phosphine ligands without and with fluoroquinolone motifs (**POH**, **PCp**, **PNr**, **PLm**), regardless of the cell type or incubation time [54, 55, 57, 58]. The activity of Ir(III) complexes for all tested cell lines was much better after 24 h of incubation time and 48 h of regeneration time (24 h + 48 h) than after 24 h of the experiment (without recovery time – extra 48 h without complexes). This is a very good result because the cytotoxic changes initiated in the cells during the 24-hour incubation cannot be repaired by the cells. Most likely, their repair systems to minimize toxicity are not sufficient, which may result in resistance breakdown. What is worth emphasizing, the introduction of the fluoroquinolone motif in complexes significantly increased the antitumor cytotoxicity of the final compounds against the lung, breast and melanoma cell line. Several recent reports suggest that fluoroquinolones have the potential to be anticancer agents [61, 62, 72], which is consistent with our results. Moreover, it can be observed that prostate cancer (DU-145) and pancreatic/duct carcinoma (PANC-1) cells were the most sensitive cell line to the mononuclear iridium(III) complexes even with both experimental approaches. Among all tested Ir(III) complexes, the **IrPCp** complex showed the most significant antitumor activity *in vitro* with an IC_{50} value of 4.8 μM for DU-145 in 24 h + 48 h approach.

Drug encapsulation has revolutionized drug delivery research, especially in the treatment of cancer. This is because nano-systems promote drug retention in tissues, protect against

chemical and biological degradation, reduce non-specific side effects and toxicity of the encapsulated drug, and increase cellular uptake. Encapsulation also eliminates the need to dissolve a lipophilic drug in organic solvents such as DMSO, which can be toxic to human health. For this purpose, we also encapsulated metal compounds into micelles (**Fig. 23**). Detailed characteristics of the prepared nanoformulations can be found in the manuscript of publications **S2**.

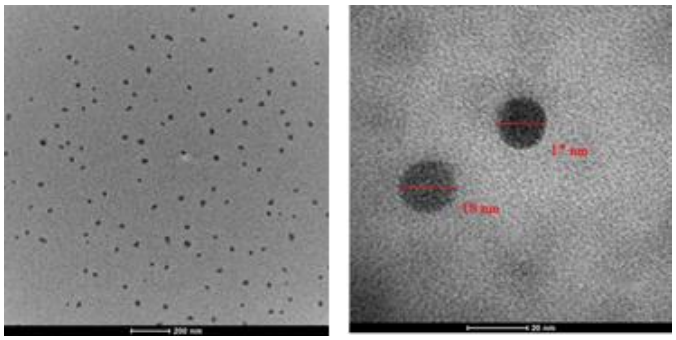


Figure 23 TEM images of Pluronic P-123 formulation with encapsulated **IrPCp** complex (**IrPCp_M**).

It is worth noting that the obtained IC_{50} values for the complex encapsulated inside micelles (**IrPCp_M**) are an order of magnitude lower than for the corresponding complexes in case A549 cells (line most resistant to non-encapsulated Ir(III) compounds). In addition, in the case of the most sensitive line - prostate cancer (DU-145) – the anticancer effect was about 3 times higher for **IrPCp_M** (**Tab. 2**). From these data, it is concluded that the encapsulation of compounds in micelle increased drug accumulation in the tumor.

Therefore, IrPCp was selected as the one with the best activity for further mechanistic studies.

Table 2 IC_{50} (μM) values of the investigated compounds toward

	IC_{50} [μM] ± SD; 24h	
	A549	DU-145
IrPCp	68.8 ± 1.7	11.8 ± 1.1
IrPCp_M	8.9 ± 0.7	4.1 ± 0.6
Cisplatin	>100	>100

Since conventional 2D cell cultures cannot mimic the complexity and heterogeneity of clinical tumors, three-dimensional (3D) spheroids where prospered. Compared to classic adherent culture, spheroids can provide a microenvironment that more closely mimics the cellular interactions observed in tumor tissues *e.g.* cell-cell or a cell-intercellular substance [114]. The therapeutic potential of Ir(III) complex **IrPCp_M** towards 3D A549 and DU-145

spheroidal culture was detected by fluorescence staining of live and dead cells (**Fig. 24**). As illustrated, mainly live cells (green) and a relatively small number of dead cells (red)were observed in the control spheroids (without studied complex). In contrast, a high percentage of dead cells, especially in the inner core, were observed in spheroids treated with the compound **IrPCp_M**. When DU-145 spheroids were treated with **IrPCp_M**, the structural integrity of spheroids was even destroyed at the increased concentration of the studied compound. It means that complex is highly cytotoxic.

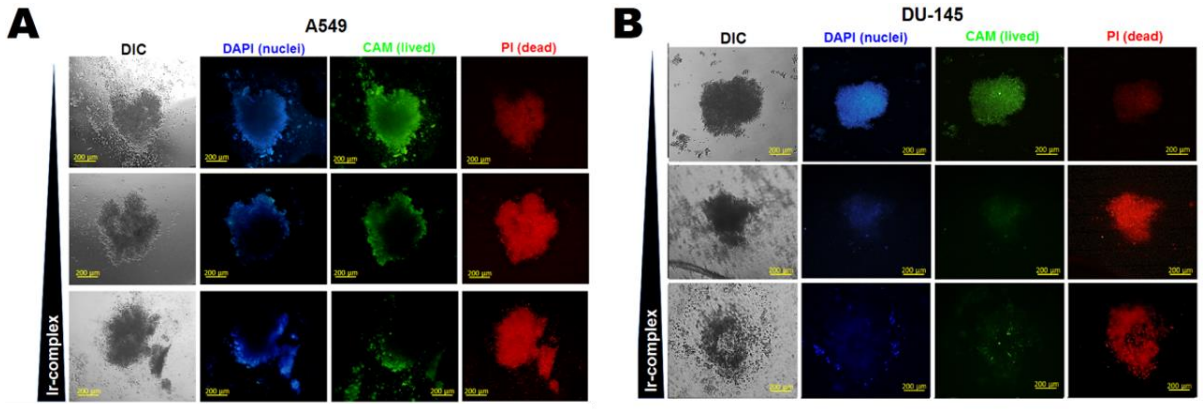


Figure 24 (A) A549 and **(B)** DU-145 spheroids after treatment with increasing concentration of **IrPCp_M** complex. DAPI: 4′,6-diamidino-2-phenylindole, CAM: calcein AM, PI: propidium iodide, Hoechst 33342.

Since many aspects of the inhibitory effects of iridium complexes on cancer are still unknown, attempts have also been made to approximate their mode of action. For precise determination of the mode of observed cell death, flow cytometry was applied. Data analysis proved that treatment of the cancer breast and lung cells with **IrPCp** complex resulted in the vast majority of the population of apoptotic cells appearing, opposite to necrotic ones. It is worth mentioning that inducing apoptosis has become very important in cancer drug research and is a more desirable process of cell death than necrosis [115, 116]. This is because it does not lead to the inflammatory process that accompanies necrosis [117].

Additionally, it was proven that **IrPCp** complex probably induced G2/M phase arrest. However, in the case of line A549, the G0/G1 phase population was still observed. Many metal complex cytotoxic agents inhibit the proliferation of cancer cells, causing the G0-, S-, or G2/M-phase arrest cell cycle. The G2 checkpoint ensures the propagation of error-free copies of the genome to each daughter cell by preventing cells from entering mitosis in the event of DNA damage. Whereas, the S phase of the cell division cycle represents the period during which cells

replicate their DNA. The arrest of the cell cycle by the compounds in this phase could result in an inhibition of DNA replication [118].

Moreover, a study of the mitochondrial membrane potential was performed along with the determination of caspase 3/7 activation. Mitochondria play a key role in apoptotic cell death, and a reduction in their membrane potential (MMP) usually triggers a cascade of executive caspases [81]. After 24 hours of incubation with **IrPCp** in lung cells (A549) it was observed that the investigated complex significantly decreased the mitochondrial membrane potential. In addition, the study clearly showed that this complex activated caspase-7 and caspase-3 simultaneously. In contrast, in prostate cells (DU145), caspase-3/7 activation decreased, possibly indicating activation of necrotic cell death instead of apoptosis.

Localization studies revealed that Ir(III) complexes can be accumulated inside the whole cancer cells (**Fig. 25**), so it has been decided to check if the complex can interact with DNA. Several well-known DNA-binding dyes [ethidium bromide (EB; intercalation), 4′,6-diamidino-2-phenylindole (DAPI; binding to a minor groove), and methyl green (MG; binding to a major groove)] were used to study the mode of drug-DNA interactions.

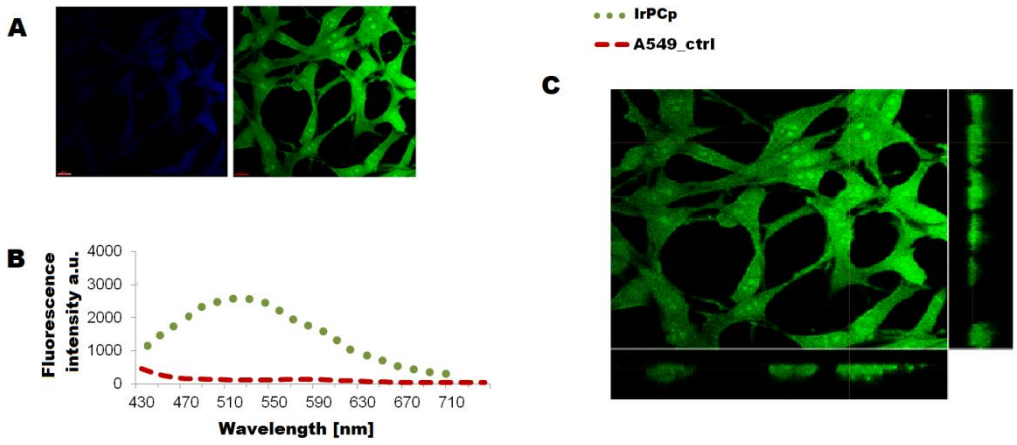


Figure 25 Selected images of A549 cells obtained by confocal microscopy (magnification 60.00×, ex = 358 nm) after treatment with **IrPCp** (c = 1 μM) for 4 h **(A, C)**. Emission spectra of cells after treatment with **IrPCp** together with the reference spectra of control untreated **(B)**.

It was found that our complexes exhibited multimodal DNA interaction with predominance of minor groove binding (**Fig. 26**). It is worth noting that the trend in the ability to interact with CT DNA was different than in the case of previously reported complexes based on identical phosphine ligands [57, 82]. Previously reported compounds interacted with CT DNA mainly true by intercalation. This tendency suggests that the type of metal ions

influences the type and intensity of intercalation with DNA. Additionally, circular dichroism (CD) was used to confirm how the complex interacts with CT DNA. After titration of CT DNA with the studied **IrPCp**, the signals in the 250-300 nm range changed slightly. This means that the compound prefers to bind in a minor groove, consistent with the results obtained above. This slight change also means that the **IrPCp** did not affect the DNA helix.

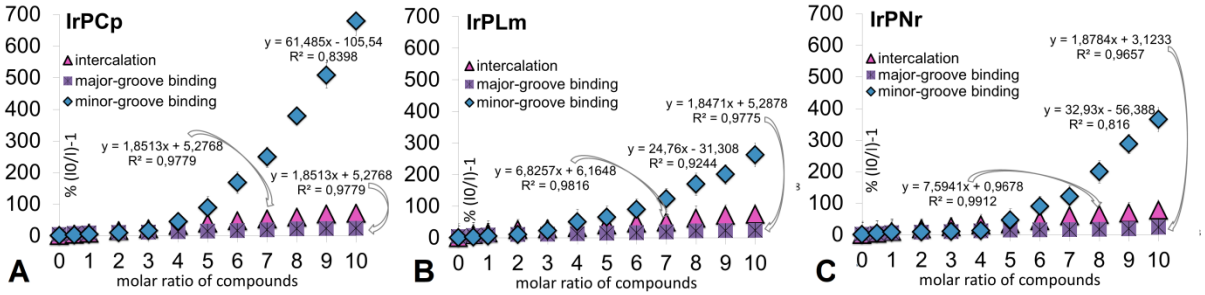


Figure 26 Stern–Volmer plots of the CT DNA-EB, CT DNA-DAPI and CT DNA-MG system quenched by examined complexe: **(A) IrPCp; (B) IrPLm; (C) IrPNr** (on the left) (I0 and I-intensity of CT DNA-EB or DAPI or MG in the absence and the presence of increasing concentration [mM] of the compounds.

What is important and worth to emphasize **IrPCp** did not cause a double-strand cleavage of DNA even at very high concentrations (**Fig. 27**). This experimental evidence suggests a different mechanism of action than targeting DNA - a mechanism typical of Pt(II) drugs, mainly based on damage to nucleic acids, e.g. intercalation. This hypothesis is also confirmed by the results obtained by fluorescence spectroscopy and circular dichroism.

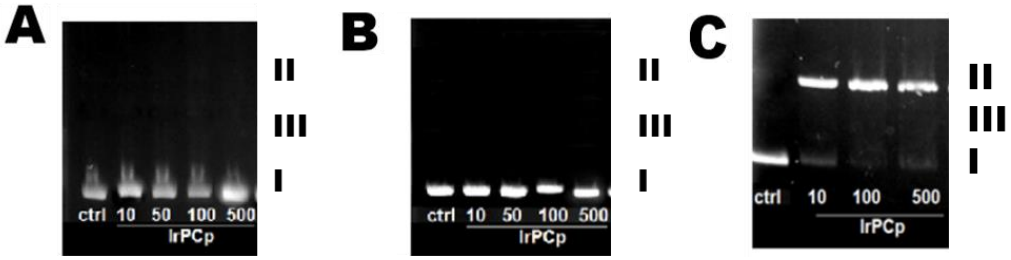


Figure 27 Agarose gel electrophoresis of pBR322 plasmid cleavage by different concentration (10-500uM) of **IrPCp** in the 10 % DMF solution, ctrl: plasmid—control. **(A)** 1 h of incubation; **(B)** 4 h of incubation; **(C)** 24 h of incubation.Forms of plasmid DNA:superhelical (form I); relaxed/nicked (form II) and linear (form III) forms.

The level of intracellular reactive oxygen species generated by the test compound was measured. The formation of reactive oxygen species (ROS) was monitored using fluorescence

spectroscopy and a non-fluorescent 2′,7′-dichlorodihydrofluorescein diacetate ($DCFH_2$-DA) probe as a detector. The complex with the ciprofloxacin derivative showed the ability to induce ROS production in DU-145 tumor cells at a much higher level than the ligand alone and the positive control. Moreover, even after 72 hours, increased production of ROS was observed.

To confirm the type of reactive oxygen species involved in the degradation of the plasmid, an experiment was carried out with hydrogen peroxide and with the use of DMSO (effective scavenger of hydroxyl radical: $^{\bullet}OH$), SOD (effective scavenger of superoxide radical anion: $O2^{\bullet-}$), and NaN_3 (effective scavenger of singlet oxygen: $^1O_2$). The **IrPCp** complex in the presence of H_2O_2 caused marked changes in the structure of the plasmid, resulting in increased amounts of form II (relaxed/nicked form). After the addition of a radical inhibitor (DMSO), a clear inhibition of DNA damage was observed, suggesting the participation of the $^{\bullet}OH$ in the cleavage process. Moreover, also, a slight inhibition of DNA cleavage was observed in other cases, *i.e.* NaN_3 and SOD, confirming the presence of $^1O_2$, $O_2^{\bullet-}$ respectively.

Using cyclic voltammetry in conjunction with absorption spectroscopy, it can be stated that ROS generation in the studied systems is related to the oxidation of iridium(III) ions to iridium(VI). The analysis of my research allowed me to hypothesize that ROS are involved in the mechanism of cytotoxic action. It was also proven that the studied iridium(III) complex (**IrPCp**) is responsible for DNA damage through a ROS-dependent mechanism involving hydroxyl radical, singlet oxygen and superoxide anion radical.

Another factor extremely important for understanding the activity of the examined compound is knowledge about the transport processes of the studied compound into the cell. The binding of biologically active substances to these proteins may lead to the loss or increase in their activity or may allow the transport of the compound in the body [119]. For example, albumin tends to accumulate in cancer tissue and inflamed tissues [120]. The expression of transferrin is significantly increased in neoplastic cells and often correlates with the tumor stage which makes it an attractive natural carrier for anti-cancer chemotherapeutic agents [121]. Therefore, it was also decided to determine the ability of the complex to bind human albumin serum (HSA) and apo-Transferrin (apo-Tf) using spectroscopic methods combined with theoretical calculations such as molecular docking. For all the complexes containing the fluoroquinolone motif, it was noticed that with increasing concentrations, the intensity of the emission band of human albumin (HSA) at a wavelength of 342 nm gradually decreased. At the same time, an additional fluorescence band appeared with an emission maximum at 425 nm, which is due to the fluorescence from the starting complexes or the new system of HSA-Ir(III) complex. These changes indicate a strong interaction of complexes with albumin,

additionally increased polarization in the area surrounding the Trp residue and/or energy transfer between the investigated complexes and human albumin. The binding strength to albumin can be represented in the following order: **IrPLm**> **IrPCp**> **IrPNr**. This phenomenon may be related to the presence of additional fluorine atoms in the quinolone moiety and the methyl group attached to the piperazine ring. Additionally, the studied compound was found to bind to HSA tryptophan residues at the site I (subdomain II A) (**Fig. 28**). Molecular docking confirmed that the investigated complexes bind to HSA, where the Ir(III) core is located at the entrance to the binding pocket and the fluoroquinolone motif is buried deep in the binding site. In addition, hydrogen bond formation was observed between the fluoroquinolone motif and the residue Arg218. Other hydrogen bonds have also been found which may exist between the complex carboxylate group and the His242 residue in the case of the **IrPNr** and **IrPLm** complexes or Arg257 in **IrPCp**.

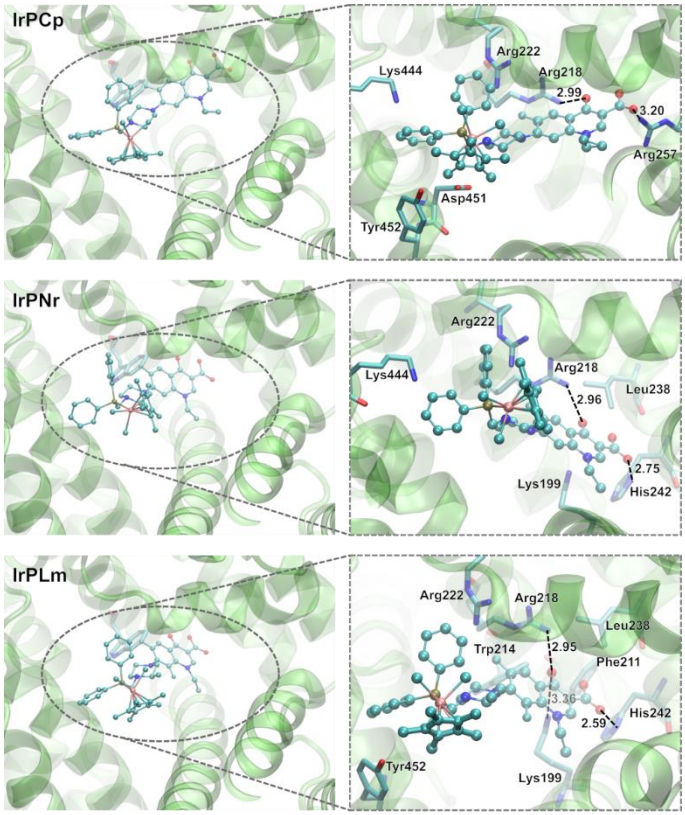


Figure 28 Binding mode of HSA-phosphino iridium(III) complexes.

When investigating the interaction of the synthesized compounds with apo-transferrin a decrease in the fluorescence intensity was also noticed (at 343 nm). This suggests that these compounds may bind to apo-transferrin and disturb the tryptophan microenvironment. The strength of apo-Tf fluorescence quenching by compounds can be presented in the following order: **IrPCp**> **IrPNr**> **IrPLm**. However, the binding strength of the complexes with

transferrin was lower than with albumin. This suggests that different moieties in the structure of the complexes and different interactions are responsible for the quenching of the emissions of these two proteins. Molecular docking shows that all complexes bind to all four possible apo-Tf binding sites containing tyrosine or tryptophan residues and they are located between the residues Tyr317 and Tyr647 in the apo-transferrin molecule (**Fig. 29**). The **IrPCp** and **IrPLm** complexes form three hydrogen bonds with the residues Tyr317, Arg677 and Asp240. The **IrPNr** complex exhibits a separate binding mode and binds to the Lys401 residue *via* a hydrogen bond mediated by an oxygen-containing fluoroquinolone moiety.

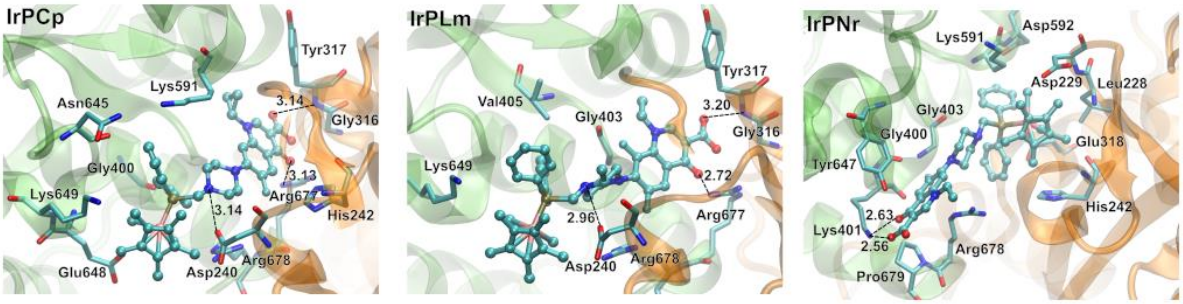


Figure 29 Binding mode of apo-Tf-complexes within the #2 binding pocket.

5. Concluding Remarks

The aim of the studies presented in this thesis was to design and synthesize monometallic iridium(III) complexes with phosphine ligands derived from fluoroquinolone antibiotics possessing potential anticancer activity.

To achieve the assumed research goals of this work, three iridium(III) complexes with aminomethyl(diphenyl)phosphines derived from fluoroquinolones (ciprofloxacin: PCp (PPh_2CH_2Cp), lomefloxacin: PLm (PPh_2CH_2Lm), and norfloxacin: PNr (PPh_2CH_2Nr)) were synthesized (**IrPCp**, **IrPLm** and **IrPNr**) as well as with phosphine without antibiotic motif (**IrPOH**). Physicochemical properties of these obtained compounds in solution and solid-state were determined using elemental analysis, mass spectrometry, cyclic voltamperometry, and spectroscopic methods (NMR, IR, UV-Vis, fluorescence). These studies allowed us to obtain information about the fluorescent properties, structure, purity, and stability of the Ir(III) complexes in aqueous solutions as well as in the presence of atmospheric oxygen. For all systems, crystal structures were determined using X-ray diffraction. In these complexes, the central iridium(III) ion is coordinated by a cyclopentadienyl moiety, phosphorus atoms from the ligands and two terminal chloride ligands.

To determine their anticancer properties and the initial mechanism of their action a series of biological tests were also carried out. The *in vitro* cytotoxic activity of all synthesized compounds was determined against healthy and cancer cells (determination of IC_{50} using the MTT test): human breast adenocarcinoma (MCF7), human lung adenocarcinoma (A549), mouse colon carcinoma (CT26), human pancreatic/duct carcinoma (PANC-1), (human prostate carcinoma (DU145), metastatic human melanoma (WM2664), human embryonic kidney (HEK293T). This study allowed selecting the compound with the best effect and attempting to determine its mechanism of cytotoxic action. For this purpose: ***(i)*** metal uptake and intracellular localization were determined using ICP-MS, confocal microscopy, and commercially used tests; ***(ii)*** the type of cell death using flow cytometry and commercially used kits; ***(iii)*** the cell cycle and apoptosis-related proteins e.g. caspase-3/7; ***(iv)*** mitochondrial membrane potential; ***(v)*** cellular ROS generation us; ***(vi)*** interaction complexes with DNA and serum proteins by gel electrophoresis, fluorescence spectroscopy, circular dichroism; ***(vii)*** drug cytotoxicity analysis on 3D tumor spheroids.

The results of these studies allowed for the formulation of a number of conclusions:

- Homonuclear Ir(III) complexes containing the fluoroquinolone motif are stable in an aqueous solution.
- The **IrPOH** complex hydrolyzes in an aqueous solution. This property is biologically important, as $M–OH_2$ water complexes are often more reactive than the corresponding complexes
- In the case of all complexes, two bands are observed in the UV-Vis spectra: the first being the result of MLCT transitions and the second is attributed to the spin allowed ligand centered (LC) $^1\pi$-π^* transitions of both the phosphine ligands.
- In all complexes, irrespective of the type of phosphine ligand, the iridium(III) ion adopts the half-sandwich pseudo-octahedral “three-leg piano-stool” geometry.
- In the aqueous solutions of the complexes containing the fluoroquinolone motif, intense luminescence is observed as a result of the transitions inside the quinolone fragment.
- Prostate carcinoma cancer cells (DU-145) and human pancreatic/duct carcinoma were the most sensitive cell lines to Ir^{III} complexes.
- Introduction of the fluoroquinolone motif in complexes significantly increased the antitumor cytotoxicity of the final compounds against the lung, breast, and melanoma cell line.

- Of all the synthesized compounds, the **IrPCp** complex showed the best anticancer properties.
- ICP-MS analysis revealed efficient iridium accumulation with increasing time, which was assessed to be the highest in the case of prostate cells.
- The investigated complexes presumably induce G2/M phase arrest, along with caspase-3/7 activation accompanied by a decrease in mitochondrial membrane potential.
- Precise cytometric analysis provided clear evidence for the predominance of apoptosis in the induced cell death.
- Inorganic compounds exhibited multimodal DNA interaction with a predominance of minor groove binding.
- Investigated iridium(III) complexes are responsible for DNA damage *via* a ROS-dependent mechanism involving hydroxyl radical, singlet oxygen and superoxide anion radical.
- Additionally, the test compound was found to bind to HSA tryptophan residues at site I (subdomain II A) and bind to all four possible apo-Tf binding sites containing tyrosine or tryptophan residues.
- Enclosure of compounds in micelles (**IrPCp_M**) improved the effective accumulation of drugs in human lung adenocarcinoma and human prostate cancer.
- In addition, it also increased the cytotoxicity of the A548 and DU-145 tumor cells by an order of magnitude.

6. The most important achievements

- Synthesis of four new iridium(III) complexes containing phosphine ligands with/without a fluoroquinolone motif.
- Characterization of the physicochemical properties of all compounds in a solid as well as in a solution: (*i*) complexes structure and their geometry, (*ii*) the stability of compounds in aqueous solutions in the presence of oxygen, (*iii*) the ability to hydrolyze in aqua solutions (*iv*) luminescent properties, (*v*) electrochemical potential.
- The cytotoxicity of all compounds was tested *in vitro* against the five most common cancer cell lines: lung, prostate, pancreatic, breast, and skin as well as one normal, human embryonic kidney. Based on these results, **IrPCp** complex was characterized by much higher cytotoxicity than cisplatin simultaneously being less toxic to healthy cells.
- The mode of cytotoxic action of homonuclear Ir(III) complexes has been proposed.

- Encapsulation of inorganic compounds in Pluronic P-123 micelles to overcome low solubility, severe side effects associated with the systemic cytotoxicity, and acquired cancer cell resistance.

The chosen method of modifying the structure of antibiotics by phosphine motif attachment allowed obtaining complexes with unique anticancer properties, where the mechanism of action was initially proposed in this work. In the era of constant and frightening reports (WHO 2020 "WHO Cancer Report 2020") on the number of cases and deaths caused by neoplastic diseases. In addition, the currently used anticancer drugs are not sufficiently selective. They cause a number of side effects such as diarrhea, anemia, hair loss, damage to the heart, kidneys, bladder, lungs, and nervous system, and many more. All of these facts show how important it is to develop a system that selectively kills cancer.

7. Perspectives [S4]

Date describing physicochemical and biological properties of Ir(III)-Cu(II) complexes (Ir(η^5-Cp*)Cl_2PCp-Cu(phen) – **IrPCpCu**; Ir(η^5-Cp*)Cl_2PLm-Cu(phen) **– IrPLmCu**; Ir(η^5-Cp*)Cl_2PCp-Cu(phen) **– IrPNrCu**) have been presented here to demonstrate possible use and evolution of Ir(III) complexes with phosphines derived from fluoroquinolones (**Fig. 30)**. In addition, a potential platform for dual drug delivery using magnetic nanoparticle systems has been proposed.

These issues are carried out by me as part of a NCN research project, PRELUDIUM which I am the leader.

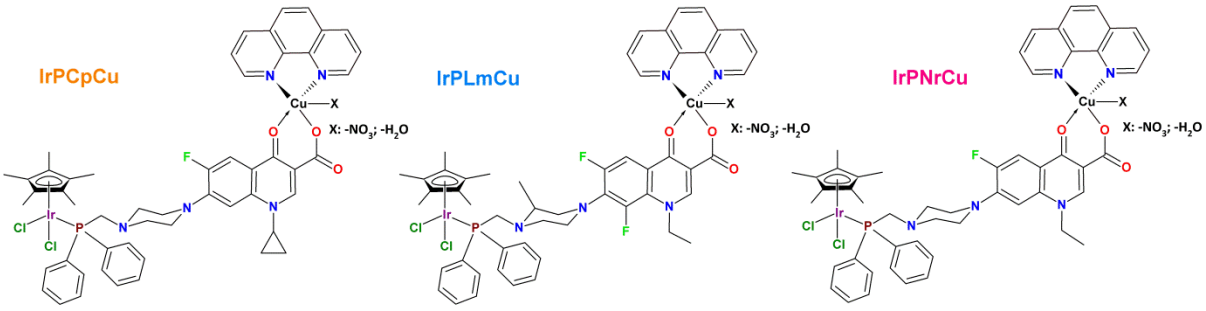


Figure 30 Structure of heteronuclear iridium(III)-copper(II) complexes.

7.1. Modification of biological activity and characteristic of heteronuclear Ir(III) / Cu(II) complexes

To increase the selectivity towards neoplastic cells and to reduce the toxicity leading to side effects, further modifications of the obtained complexes were performed. One of the strategies adopted to overcome these limitations, scientists to find inspiration in the activity of novel heteronuclear complexes [122 -124]. The presence of two different metals in one molecule can improve their activity as anti-cancer agents due to interactions between different metals with multiple biological targets or through improved chemical-physical properties of the resulting compound [124]. Therefore, it was decided to attach a copper(II) ion to the homonuclear complexes (IrPCp, IrPNr, IrPLm). It was proved that through many processes, such as DNA damage or generation of ROS, Cu(II) complexes could effectively induce cancer cells death [83, 125]. In addition, the superiority of these substances is also represented by the fact that Cu(II) ions are already present in the human body limiting the possibility of excessive immunological system response is low [125]. Most importantly, the introduction of a transition metal, such as copper(II), can also give the obtained complexes unique magnetic properties [126].

Analysis of structures in solution

All heteronuclear Ir(III)/Cu(II) complexes were characterized by mass spectrometry recorded in the positive ion modality and infrared spectroscopy. For the **IrPNrCu** and **IrPLmCu** complexes, the corresponding molecular ion was detected, consistent with the expected isotopic distribution for $[M]^+$ or $[M+H]^+$. Only the complex with ciprofloxacin **(IrPCpCu**) did not show the corresponding $[M]^+$ molecular ion peaks. The analysis of MS spectra showed that in all the types of complexes mentioned, chloride groups were easily displaced. As a result, adducts with solvent molecules, *e.g.* H_2O or CH_3OH, were observed.

In the IR spectra of fluoroquinolones, very strong bands around 1720 cm^{-1} were observed, corresponding to the stretching vibrations of the C=O group of the carboxyl group (-COOH), while in the case of complexes they were very weak or not observed in the ATR spectra. Additionally, in the FT-IR spectra of heteronuclear Ir^{III}/Cu^{II} complexes, two characteristic bands were observed around 1630 and 1335 cm^{-1}, corresponding to antisymmetric stretching vibrations (υCOO^-) which may be a marker of the coordination model. A model for the binding

of phosphine fluoroquinolone ligands to the Cu^{2+} ion was also determined by the difference in the υCOO^- bands described above, which is in the range 285-339 cm^{-1}.

Coordination of Cu(II) ions to Ir(III) complexes leads to a bathochromic shift of the maximum wavelength of the peak emission (**Fig. 31**). At room temperature, a structureless emission curve can be identified with a maximum centered at 450 nm relating to all the solutions of Ir(III)/Cu(II) complexes. These emission patterns were obtained upon photoexcitation at a specific wavelength of 340nm.

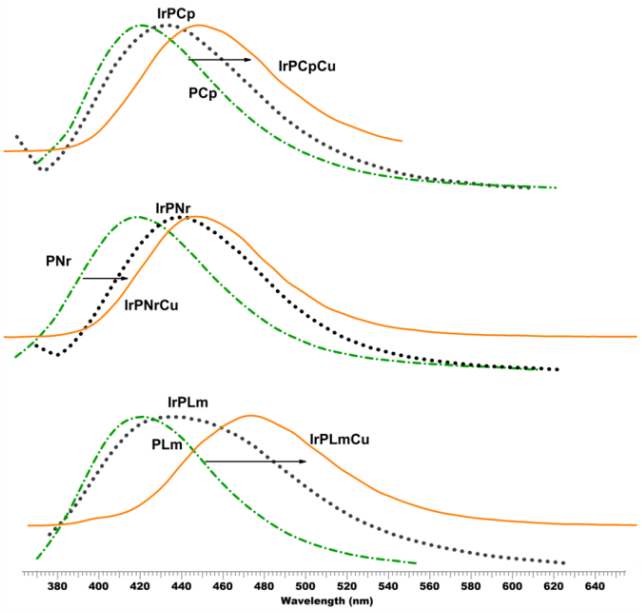


Figure 31 Normalized emission spectra for heteronuclear Ir(III)/Cu(II) complexes, homonuclear Ir(III) complexes and the corresponding phosphine ligands; λex = 340 nm, 298 K.

In addition, cyclic voltammetry (CV) was performed to thoroughly understand the redox activity of the investigated heteronuclear Ir(III)/Cu(II) complexes. The cyclic voltammograms of the complexes exhibit two irreversible oxidation peaks at around - 1.6 V and -1.1 V which are assigned to the phosphine ligands and iridium(III) ion. One quasi-reversible reduction peak with $E_{1/2}$ of ca. 0 V is observed for all complexes and refers to the Cu(II)/(I) redox process.

Analysis of structures in solid

The coordination geometry of the iridium(III) ion in all heteronuclear complexes is the same as that of the Ir(III) heteronuclear complexes. The complexes **IrPNrCu·(NO_3)·1.75(CH_4O)·0.75(H_2O)** and **IrPCpCu·(NO_3)·2.75(H_2O)** crystallize in the triclinic crystal system, in the P1 space group. Whereas, **IrPLmCu·(NO_3)·1.3(H_2O)·1.95(CH_4O)** crystallizes in the P*bcn* space group (orthorhombic system) and contains the 1D metal-organic polymer assembled from copper(II) centers, iridium(III) complex linkers, phenanthroline molecule and OH- ligands (**Fig. 32**).

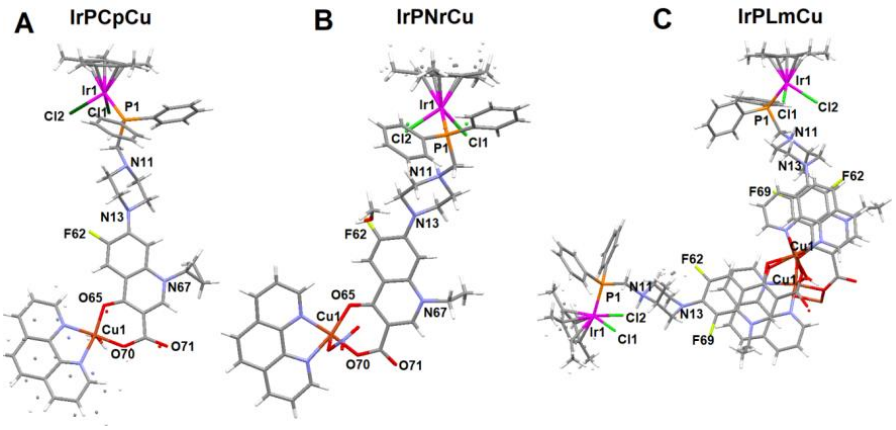


Figure 32 The monocrystal structures of the complex molecules **IrPCpCu**, **IrPLmCu** and **IrPNrCu**. The solvent molecules are omitted for clarity.

In the **IrPCpCu** and **IrPNrCu** complexes Cu(II) ion adopt a distorted square-pyramidal coordination geometry, where the copper ion is coordinated *via* two nitrogen atoms (from phenanthroline ligand) and IrPNr or IrPCp complex *via* deprotonated carboxylate and pyridone oxygen atoms. In the case of **IrPLmCu**, the packing analysis showed that the Cu(II) ion adopts an octahedral distorted geometry, and is coordinated by four oxygen atoms (two carboxylates, one pyridine oxygen, and one from hydroxy group) and two nitrogen atoms from phenanthroline ligand (**Fig. 33**).

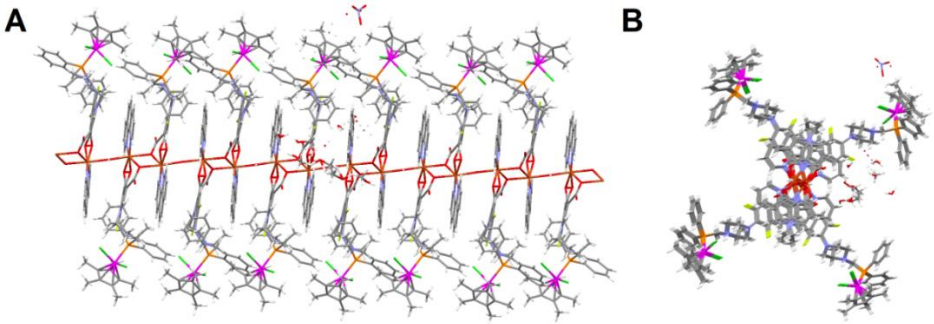


Figure 33 A perspective view (**A** and **B**) of the 1D polymer chain in the crystal structure of **IrPLmCu**.

The presence of copper(II) ion in Cu(II) – Ir(III) heterometallic complex was also confirmed by magnetic measurement and EPR spectroscopy. Magnetic data were acquired with the help of the SQUID magnetometer (MPMS, Quantum Design) at the applied field of B_0 = 0.5 T and, after correction to the underlying diamagnetism, transformed to the temperature dependence of the $\chi_M T$ product (or effective magnetic moment, (**Figures 34** – left). The field dependence of the magnetization per formula unit $M = M_{mol}/N_A\mu_B$ at the constant temperature is shown in figure 34 – right.

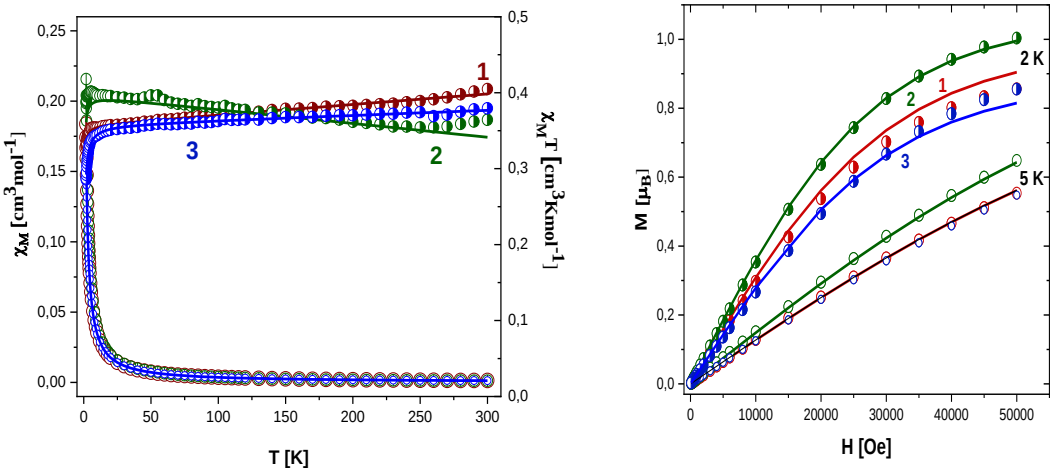


Figure 34 Left - thermal dependencies of $\chi_M T$ (half-open circles) and χ_M (open circles) for **1 - IrPCpCu**, **2 - IrPNrCu**, **3 -IrPLmCu;** right - magnetization as a function of magnetic field at 2.00 K (half-open circles) and 5.00 K (open circles) for**1 - IrPCpCu**, **2 - IrPNrCu** , **3 - IrPLmCu.** The solid lines (on all graphs) are calculated using the HDVV spin Hamiltonian and PHI programme.

All examine complexes magnetically behave as a mononuclear unit with S_{Cu} = 1/2 because Ir(III) ions are diamagnetic. $\chi_M T$ vs T graph indicates the antiferromagnetic nature of exchange interaction for complex **IrPCpCu** and **IrPLmCu** and weak ferromagnetic coupling in compounds **IrPNrCu**. The magnetization data at T = 2.0 and BDC = 5.0 T saturates to M = $M_{mol}/(N_A\mu_B)$ = 0.86 μB (**IrPCpCu**, **IrPLmCu**) and 1.00 (**IrPNrCu**) . Theoretical calculation of these magnetic data using the proper Hamiltonian (for an alternating Ising chain for polymeric complex **IrPLmCu** or Heisenberg-Dirac-Van Vleck for **IrPCpCu** and **IrPNrCu**) confirm the nature and strength of observed magnetic interaction.

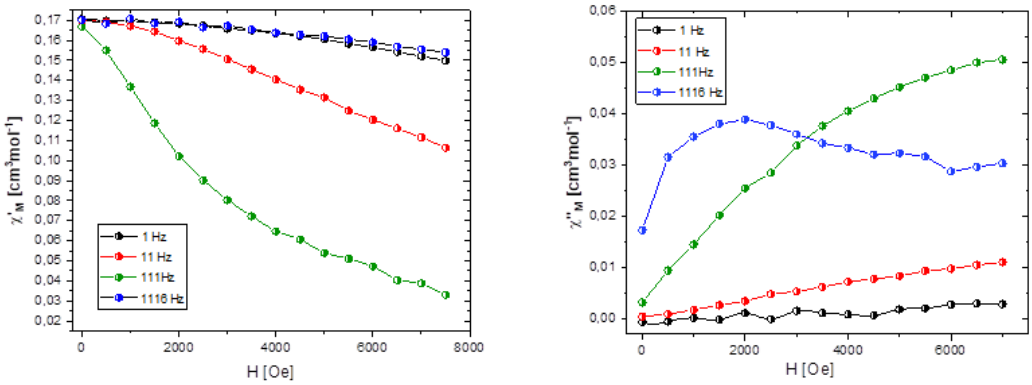


Figure 35 Field dependencies of the AC susceptibility components for 2 at T = 2.0 K for a set of frequencies of the AC field. Lines are a guide for the eye.

New information was obtained from the AC susceptibility measurements. The in-phase ($\chi M'$) and out-of-phase ($\chi M''$) components (**Fig. 35**) exhibit small frequency dependences with the application of an external field of 0.2 T, indicative of the possibility of slow relaxation of magnetization, although the maxima in χ'' are missing. Using this data we cannot suggest SMM or SIM behavior. However, the relaxation process for Cu(II) ions is very rare due to the absence of a barrier to spin reversal: the axial zero-field splitting parameter D is undefined. The presence of a relaxation process in complex **IrPNrCu** can be a result of geometry around Cu(II) ions. Though the D parameter cannot be assigned to mononuclear copper(II) complexes, these are well-known as anisotropic systems showing at least two distinct $g_z \neq g_x$ values well seen in the EPR spectra of an axial type. Thus, even in the absence of the zero-field splitting, there exists a magnetic anisotropy. The results of magnetic studies in the alternating field (AC) indicate a significant role and usefulness of phosphine ligands in the functionalization of organic ligands leading to modification of the geometry of the metal coordination sphere and, consequently, the magnitude of anisotropy. This fact may mark an important path toward obtaining multifunctional materials.

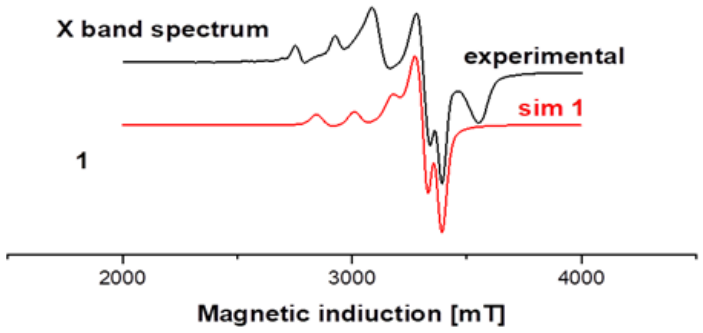


Figure 36 EPR frozen solution spectra (at 77 K) of **IrPCpCu** in DMSO solvent together with the theoretical spectrum calculated.

The polycrystalline EPR spectra of the magnetically concentrated samples and also in the frozen solution confirm the axial symmetry with d_{x2-y2} ground state, where the geometry can correspond to an elongated octahedral, a square pyramidal or a square planar (**Fig. 36.** – an exemplary spectrum for one of the examined complexes). The frozen solution EPR spectra exhibit a well-defined resolution of hyperfine splitting of parallel orientation resulting from the interaction of an unpaired electron with copper nuclei (I = 3/2). The spin Hamiltonian parameters are obtained by computer simulation of the experimental spectra with $g_x = g_y = g_\perp = 2.065$, $g_z = g_k = 2.211$ and $A_k = 164$ G for **IrPCpCu**, $g_x = 2.069$, $g_y = 2.073$, $g_z = g_k = 2.215$ and $A_k = 113$ G for **IrPNrCu**, $g_x = g_y = g_\perp = 2.098$, $g_z = g_k = 2.289$ and $A_k = 163$ G for **IrPLmCu.**

Biological properties

The comparison of the IC_{50} values determined for homo- and heteronuclear complexes (**Tab. 3**) showed that the presence of the second metal caused a significant increase in cytotoxic activity against MCF7 and A549 cell lines, and most importantly a reduction in toxicity against normal cell lines (HEK293T). Additionally, what was remarkable, despite the addition of a second metal with phenanthroline - which is known to be toxic - there was a significant reduction in toxicity to normal cell lines by more than 40 times than cisplatin. This suggests that the introduction of a second metal is an effective method of minimizing toxicity to healthy cells and may bring into play different properties of the resulting compound. Interestingly, human lung adenocarcinoma (A549) was the most sensitive cell line to heteronuclear Ir^{III}/Cu^{II}complexes even in the case of both experimental approaches (24 h and 24 h + 48 h treatment with the examined compounds). *In vitro* cytotoxicity assays were also carried out within multicellular tumor spheroids and efficient anticancer action on these 3D assemblies was demonstrated.

Table 3 Values of IC_{50} [μM] (concentration of a drug required to inhibit the growth of 50% of the cells) for WM2661, A549, MCF7, PANC-1, DU-145, HEK293T cells after 24 h and 24 h + 48 h treatment with the studied compounds and cisplatin as reference

IC_{50} [μM] ± SD;24 h					
	A549	MCF7	DU-145	WM2664	HEK293T
IrPCpCu	35.5 ± 5.6E-03	35.3± 6.5	12.8 ± 2,7E-07	12.8 ± 2,7E-07	786.8 ± 11.2
IrPLmCu	31.6 ± 7.6E-03	24.2 ± 7.22	14.2 ± 2.4E-03	10.1 ± 2.2E-03	775.8 ± 15.7
IrPNrCu	11.2 ± 7.8E-03	29.97 ± 0.67	10.8 ± 1.9E-04	9.9 ± 3.8E-03	756.8 ± 5.7
cisplatin	>100	51.9±4.6	>100	2.63±0.6	21.0±1.8
IC_{50} [μM] ± SD; 72 h (24 h + 48 h)					
IrPCpCu	42.4 ± 7.3E-05	>1000	125.7 ± 3.4	137.1 ± 2.2	886.8 ± 12.7
IrPLmCu	36.0 ± 2.2E-02	>1000	126.2 ± 4.4	229.3 ± 25.9	822.8 ± 12.3
IrPNrCu	36.6 ± 2.8E-03	>1000	122.7 ± 5.4	155.1 ± 3.2	856.8 ± 15.9
cisplatin	71.7±3.7	17.7±8.6	65.5±3.6	8.29±0.4	10.3±2.1

In order to increase the cellular accumulation of heteronuclear complexes and to control their uptake only to neoplastic cells, bypassing healthy cells - as was the case with homonuclear complexes - we encapsulated compounds in liposomes. In the case of DU145 line, it can be observed as a 10-fold decreasein cytotoxicity (**Tab. 4**).

Table 4 IC_{50} (μg/ml and μM) values of the investigated compounds toward the selected cancer cell lines for 24 h.

	HaCaT	A549	DU145
L	180.47±0.108 μg/mL	21,18±0,1430 μg/mL	12,56 ± 0,1746 μg/mL
IrPCpCu	200.68±2.081 μM	9.47±0.261 μM	1.34±0.051 μM
cisplatin	32.20±0.881 μM	>100	>100

In addition, the accumulation of liposome compounds was confirmed by ICP-MS and a confocal microscope. For all tumor cell lines, a significant increase in iridium accumulation was detected after incubation with compounds as compared to normal control cells. Furthermore, analyses of the cross-sectional images, nuclei specific probe Hoechst33342 (blue) and intensity of the emission bands of the cancer cells proved clearly that the compounds penetrate into the tumor cells. This analysis proves that the heteronuclear complexes accumulate in the nucleus. A precise cytometric analysis revealed a predominance of apoptosis over the other types of cell death. Furthermore, the investigated nanoformulation may induce changes in the cell cycle leading to S phase arrest in a dose-dependent manner.

7.2. Magnetic drug targeting – *nanoformulation*

A promising approach to drug delivery is to target drugs magnetically, *e.g.* with an applied magnetic field, taking advantage of the fact that the drug delivery vehicle has a strong magnetic moment. For this purpose, it was decided to use the well-known magnetic properties of magnetite (Fe_3O_4) particles, which, after being minimized to nanometric structures, are characterized by the phenomenon of superparamagnetism. In practice, this phenomenon consists in imparting magnetic properties to nanomaterials after applying an external magnetic field and their extinction after removing the source of the magnetic field. The fragmentation of the material causes the separation of magnetic domains, which significantly reduces the value of the coercivity and makes them applicable in the human body. The reason for the use of iron oxides as magnetic nano molecules in biological applications is the fact that iron oxide nanoparticles show low cytotoxicity, unlike other ferromagnetic materials. Moreover, they have an easily functionalized material surface, which allows for high biocompatibility with biological material. Nevertheless, the magnet geometry and the tumor-magnet distance are critical to the efficient delivery of magnetic compounds.

In the first stage, it was chosen one complex **IrPOH (**Ir(η^5-Cp*)$Cl_2PPh_2CH_2OH$**)**, which presents high cytotoxicity to the A549 human lung adenocarcinoma. This compound was surrounded by a polymeric micelle doped with a properly selected amount of Fe_3O_4 particles with a grain size of 10 mm. The presence of magnetite nanoparticles in examine material was confirmed by magnetic measurements (DC) using a superconducting quantum interference device (SQUID) within a magnetic field of 0 – 5 T (**Fig. 37**) at 2 and 300 K. The results clearly show the superparamagnetic behavior of nanoparticles. The magnetization vs magnetic field curve indicates a nonlinear variation at all measuring temperature; 2, 5, and 300K (**Fig. 37).**

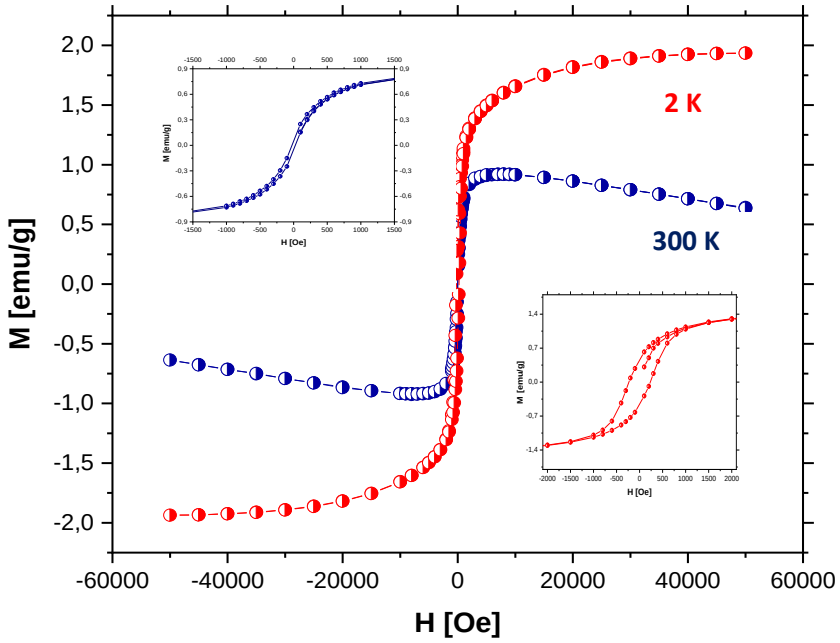


Figure 37 Field H dependence of magnetization M of **IrPOH - M** at 2 K (red circle) and 300 K (blue circle).

This sample is saturated at the low magnetic field with the Ms saturation magnetization value (obtained as an extrapolation to the zero-field from the high field area in M (H)) of 2.1 EMU/g (at 2 K) and 1.2 EMU/g (at 300 K). The former is significantly lower than the saturation magnetization of bulk magnetite (Ms bulk = 98 EMU/g) [127] which may be the result of the final particle size effect and high surface area to volume ratio, the spin deflection effect at the grain boundary or the presence of other materials in the examined species that may lead to a reduction of the effective magnetic moment [128]. At 300 K, the coercivity (28.3 Oe) and remanence values (0.2 EMU/g) are not discernible, indicating a superparamagnetic

behavior while at 2 K the value of coercivity (251 Oe) and remanence (1.9 EMU/g) showing a ferrimagnetic behavior.

The obtained core/shell structures are the starting point for further modifications aimed at giving the magnetic nanoparticles appropriate functions and properties. Moreover, such intelligent materials can deliver drugs directly to the diseased tissue thanks to the magnetic field - thanks to this solution; we can avoid systemic toxicity as the drug will be delivered only to the diseased area.

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Curriculum Vitae

Education

- **2018 – present:** Ph.D. student (4th year) Faculty of Chemistry,University of Wroclaw, Poland
- **2018 – present:** Ph.D. student (4th year)Department of Chemical and Pharmaceutical Sciences at the University of Ferrara, Italy
- **2016-2018:** University of Wroclaw, Faculty of Chemistry: Research Group of Structural Applications of Electron Paramagnetic Resonance Spectroscopy, **MA Environmental Chemistry; Title of master thesis:** "Because it takes three to tango..." drug-linker-carrier. Synthesis and physicochemical and biological properties study of phosphine $P(p\text{-}OCH_3Ph)_2CH_2OH$ and its Cu(I) complex
- **2012-2016** Wroclaw University of Science and Technology, Faculty of Chemistry: Department of Analytical Chemistry and Chemical Metallurgy, **Chemistry BEng; Title of engineer's thesis**: Non-chromatographic approach to the arsenic speciation - application of the HG technique

Publication

Total publications: 9

Total IF: 45.98

Average IF: 5.11

Publications constituting the basis of this doctoral dissertation (Total impact factor: 16.822)

1. Urszula K. Komarnicka[a], **Sandra Kozieł[a]**, Agnieszka Skórska-Stania, Agnieszka Kyzioł, FrancescoTisato, Synthesis, physicochemical characterization and preliminary *in vitro* antitumor activity of phosphino Ru(II) and Ir(III) complexes, ***Dalton Transactions***, 2022, DOI: 10.1039/D2DT01055K; IF: 4.39; **back cover,** a – first author
2. **Kozieł Sandra**, Komarnicka Urszula K., Ziółkowska Aleksandra, Skórska-Stania Agnieszka, Pucelik Barbara, Płotek Michał, Sebastian Victor, Bieńko Alina, Stochel Grażyna, Kyzioł Agnieszka, Anticancer potency of novel organometallic Ir(III) complexes with phosphine derivatives of fluoroquinolones encapsulated in polymeric

micelles; ***Inorganic Chemistry Frontiers***, 2020, 7, 3386-3401; IF: 6.569; DOI:10.1039/d0qi00538j

3. **Kozieł Sandra***, Lesiów Monika Katarzyna, Wojtala Daria, Dyguda-Kazimierowicz Edyta, Bieńko Dariusz, Komarnicka Urszula Katarzyna, Interaction between DNA, albumin and apo-transferrin and iridium(III) complexes with phosphines derived from fluoroquinolones as a potent anticancer drug; ***Pharmaceuticals***, 2021, 14, 685/1-685/25; IF: 5.863; DOI:10.3390/ph14070685, * - corresponding author
4. Liposome-mediated delivery of magnetic iridium-copper complexes with phosphine derived from fluoroquinolones for prostate carcinoma treatment, ***Inorganic Chemistry Frontiers***, submitted

Others publications (Total impact factor:29.152)

1. Komarnicka Urszula K., Niorettini Alessandro, **Kozieł Sandra**, Pucelik Barbara, Barzowska Agata, Wojtala Daria, Ziółkowska Aleksandra, Lesiów Monika, Kyzioł Agnieszka, Caramori Stefano, Porchia Marina, Bieńko Alina, Two out of Three Musketeers Fight against Cancer: Synthesis, Physicochemical, and Biological Properties of Phosphino Cu^{I}, Ru^{II}, Ir^{III} Complexes, ***Pharmaceuticals***, 2022, 15, 169/1-169/22, IF: 5.863; DOI: 10.3390/ph15020169,
2. Wojciechowska Agnieszka, Bregier-Jarzębowska Romualda, Komarnicka Urszula K., **Kozieł Sandra**, Szuster-Ciesielska Agnieszka, Sztandera-Tymoczek Monika, Jarząb Anna, Staszak Zbigniew, Witkowska Danuta, Bojarska-Junak Agnieszka, Jezierska Julia, Isothiocyanate L−argininato copper(II) complexes : solution structure, DNA interaction, anticancer and antimicrobial activity, ***Chemico-Biological Interactions***, 2021, 348, 109636/1-109636/12, IF: 5.192; DOI: 10.1016/j.cbi.2021.109636
3. Świtlicka Anna, Machura Barbara, Bieńko Alina, **Kozieł Sandra**, Bieńko Dariusz C., Rajnák Cyril, Boča Roman, Ozarowski Andrew, Ozerov Mykhaylo, Non-traditional thermal behavior of Co(II) coordination networks showing slow magnetic relaxation.***Inorganic Chemistry Frontiers***, 2021, 8, 4356-4366, IF: 6.569; DOI: 10.1039/d1qi00667c,
4. Guz-Regner Katarzyna, Komarnicka Urszula Katarzyna, Futoma-Kołoch Bożena, Wernecki Maciej, Cal Magdalena, **Kozieł Sandra**, Ziółkowska Aleksandra, Bugla-Płoskońska Gabriela, Antibacterial activity and action mode of Cu(I) and Cu(II) complexes with phosphines derived from fluoroquinolone against clinical and

multidrug-resistant bacterial strains, ***Journal of Inorganic Biochemistry***, 2020, 210, 111124/1-111124/11, IF: 4.155; DOI: 10.1016/j.jinorgbio.2020.111124

5. Komarnicka Urszula K., **Kozieł Sandra**, Zabierowski Piotr, Kruszyński Rafał, Lesiów Monika K., Tisato Francesco, Porchia Marina, Kyzioł Agnieszka, Copper(I) complexes with phosphines P(p-OCH_3-Ph)$_2CH_2OH$ and P(p-OCH_3-Ph)$_2CH_2$SarGly: synthesis, multimodal DNA interactions, and prooxidative and in vitro antiproliferative activity, ***Journal of Inorganic Biochemistry***, 2020, 203, 110926/1-110926/14, IF: 4.155; DOI: 10.1016/j.jinorgbio.2019.110926
6. Komarnicka Urszula K., **Kozieł Sandra**, Starosta Radosław, Kyzioł Agnieszka, Selective Cu(I) complex with phosphine-peptide (SarGly) conjugate contra breast cancer: synthesis, spectroscopic characterization and insight into cytotoxic action, Journal of ***Inorganic Biochemistry***, 2018, 186, 162-175, IF: 3.224; DOI: 10.1016/j.jinorgbio.2018.06.009

Lider in research project

- **14.01.2021 – 13.01.2024 Preludium 19 NCN** (2020/37/N/ST4/02698): Homo- and heterometallic phosphine ruthenium and iridium complexes - design, synthesis, bioactivity and magnetic-nanoformulation as a potential platform for dual-targeted drug delivery. **Amount: 195 600 PLN**
- **19.11.2018 – 30.10.2019 MNiSW**(nr decyzji 4478/E-344/M/2018): Przeprowadzanie badań dotyczących fosfinowych kompleksów rutenu(II) i irydu(III) jako potencjalnych leków przeciwnowotworowych. **Amount: 14 000 PLN**

Co-investigator in research project

- **1.11.2018 – 7.11.2021 SONATA 12 NCN** (2016/23/D/ST5/00269): Do copper(I) complexes with conjugates of phosphine-peptide carriers can cause selective cancer cells death? Synthesis, physicochemical and biological properties (start: 2017-07-12). **Amount: 452 800 PLN**

Conference contributions (posters)

- *Poster:* **Sandra Kozieł**, Agnieszka Skórska-Stania, Barbara Pucelik, Victor Sebastiane, Alina Bieńko, Grażyna Stochel, Agnieszka Kyzioł, Urszula K. Komarnicka „Aktywność przeciwnowotworowa nowych kompleksów irydu(III) zawierających fosfinowe pochodne fluorochinolonów zamkniętych w polimerowych micelach" IV Ogólnopolskie Forum Chemii Nieorganicznej, Wydział Chemii Uniwersytetu Mikołaja Kopernika w Toruniu, 7-9.09.2021
- *Oral presentation*: **Sandra Kozieł**, Agnieszka Skórska-Stania, Barbara Pucelik, Victor Sebastiane, Alina Bieńko, Grażyna Stochel, Agnieszka Kyzioł, Urszula K. Komarnicka: „Anticancer potency of novel organometallic Ir(III) complexes with phosphines derived from fluoroquinolones encapsulated in polymeric micelles". International Symposium on Thermodynamics of Metal Complexes ISMEC 2021, Białystok, Polska, 16-18.06.2021
- *Flash talk and poster*: **Sandra Kozieł**, Agnieszka Kyzioł, Alina Bieńko, Urszula K. Komarnicka "Novel piano-stool Ru(II) and Ir(III) complexes containing aminomethylphosphanes based on fluoroquinolones". ISABC 2019 – 15th International Symposium on Applied Bioinorganic Chemistry; 2-5 June 2019, Nara, Japan
- *Oral presentation*: **Sandra Kozieł** "Kompleksy miedzi(I) z fosfinowym koniugatem dipeptydu Sar-Gly-OH zawierającym motyw(p-$OCH_3Ph)_2CH_2OH$. Multimodalne interakcje DNA, aktywność prooksydacyjna i cytotoksyczna". Young scientists conference. Analysis of the issue, analysis of results - presentation of a young scientist; 21 May 2019, Rzeszów, Poland
- *Poster*: **Sandra Kozieł**, Agnieszka Kyzioł, Alina Bieńko, Urszula K. Komarnicka "Nowe kompleksy Ru(II) i Ir(III) z aminometylofosfinowymi pochodnymi fluorochinolonów". Young scientists conference. Analysis of the issue, analysis of results - presentation of a young scientist; 21 May 2019, Rzeszów, Poland
- *Poster*: **Sandra Kozieł**, Radosław Starosta, Agnieszka Kyzioł, Urszula K. Komarnicka "Kompleksy miedzi(I) z fosfinowym koniugatem dipeptydu (SarGlyOH) w walce z rakiem piersi - charakterystyka fizykochemiczna i biologiczna". 61. Zjazd Naukowy Polskiego Towarzystwa Chemicznego, 17-21 September 2018, Krakow, Poland

Awards and Fellowships

- Finalist in the "Young Talent" competition in the category of scientific success, organized by “Dolnośląski Klub Kaptiału” (**2022,** Wrocław, Poland)
- Jan Mozrzymas scholarship in the field of interdisciplinary research conducted by the Wrocław Academic Center and the city of Wrocław **(2021/2022)**
- Award for a distinction in a poster session: „Aktywność przeciwnowotworowa nowych kompleksów irydu(III) zawierających fosfinowe pochodne fluorochinolonów zamkniętych w polimerowych micelach” IV Ogólnopolskie Forum Chemii Nieorganicznej, Wydział Chemii Uniwersytetu Mikołaja Kopernika w Toruniu, **7-9.09.2021**
- Nomination for the award: Scientist of the Future 2021 in the category: Science for a better life in the future (**2021**, Wrocław, Poland)
- Scholarship of the Rector of the University of Wrocław for the best PhD students (**2018-2022**)
- Scholarships of Ministry of Higher Education and Science for the best PhD students (pro-quality scholarship, **2018-2022**)
- Piotr Ludwik Sosabowski Scientific Scholarship (**2019/2020**)

Intership

- **2018 - 2022** Italy, doctoral studies at the University of Ferrara, Department of Chemical, Pharmaceutical and Agricultural Sciences (collaboration with Professor Stefano Caramori)
- **2018 - 2022** Poland, Jagiellonian University, Krakow; Department of Inorganic Chemistry (total 9 months, collaboration with Professor Grażyna Stochel and Dr Agnieszka Kyzioł)
- **2021 - 2022** Poland, University of Wroclaw, Poland; Institute of Genetics and Microbiology, Department of Microbiology (total 2 months, collaboration with Professor Gabriela Bugla-Płoskońska and Dr Katarzyna Guz- Regner)
- **1 April – 31 October, 2020** Poland, internship at Colgate-Palmolive Services (Poland) sp.z o.o. in Świdnica
- **7 November – 5 December 2018** Italy, Institute of Condensed Matter Chemistry and Technologies for Energy (ICMATE) in Padua (collaboration with Marina Porchia and Francesco Tisato)

Training

- **09 - 18 April 2018** Poland, Wrocław University of Science and Technology European standardization of cosmetic products.
- **15 - 20 January 2018** Poland, Wrocław University of Science and Technology Participation in team projects of biological chemistry conducted by Professor Marcin Drąg
- **12 - 13 October 2017** Poland, Wroclaw Technology Park Accreditation of analytical laboratories using alternative determination methods
- **26 June - 08 July 2017** Poland, Wrocław University of Science and Technology Gas chromatography

Organizational activities

- **2020 – present:** Chemistry teacher in Dialogue Of Culture Primary School Etz Chaim
- **2019 – present:** An active member of the „Stowarzyszenie Nauczycieli Przedmiotów Przyrodniczych (PSNPP)"
- **2019 – present:** Laboratory classes for elementary school and high school students at the University of Wrocław
- **2020 – present:** Co-managementsocial media of the Faculty of Chemistry, University of Wrocław (instagram, facebook, website)
- **2021 – 2022** Organizing an auction for the Great Orchestra of Christmas Aid (WOŚP): "a day in the life of a mad chemist"
- **2019-2020:** Assistance in organizing the "Dolnośląski Festiwal Nauki" at the Faculty of Chemistry and at the Faculty of Law, Administration and Economics of the University of Wrocław
- **1.10.2019 - 30.09.2020:** Chairwoman of the council of doctoral students at the Faculty of Chemistry, University of Wrocław.
- **2015 - 2020:** Member of the Students' Scientific Circle of Forensics at the Department of Forensics at the Faculty of Law, Administration and Economics of the University of Wrocław.

Dyplom

DOLNOŚLĄSKI KLUB KAPITAŁU MMXV

LOWER SILESIA CAPITAL CLUB

KAPITUŁA KONKURSU MŁODE TALENTY
NOMINUJE DO NAGRODY

MŁODE TALENTY

PANIĄ

Sandrę Kozieł

ZA WYBITNY SUKCES
NAUKOWY

KAPITUŁA VI EDYCJI KONKURSU MŁODE TALENTY
• WROCŁAW 2022 •

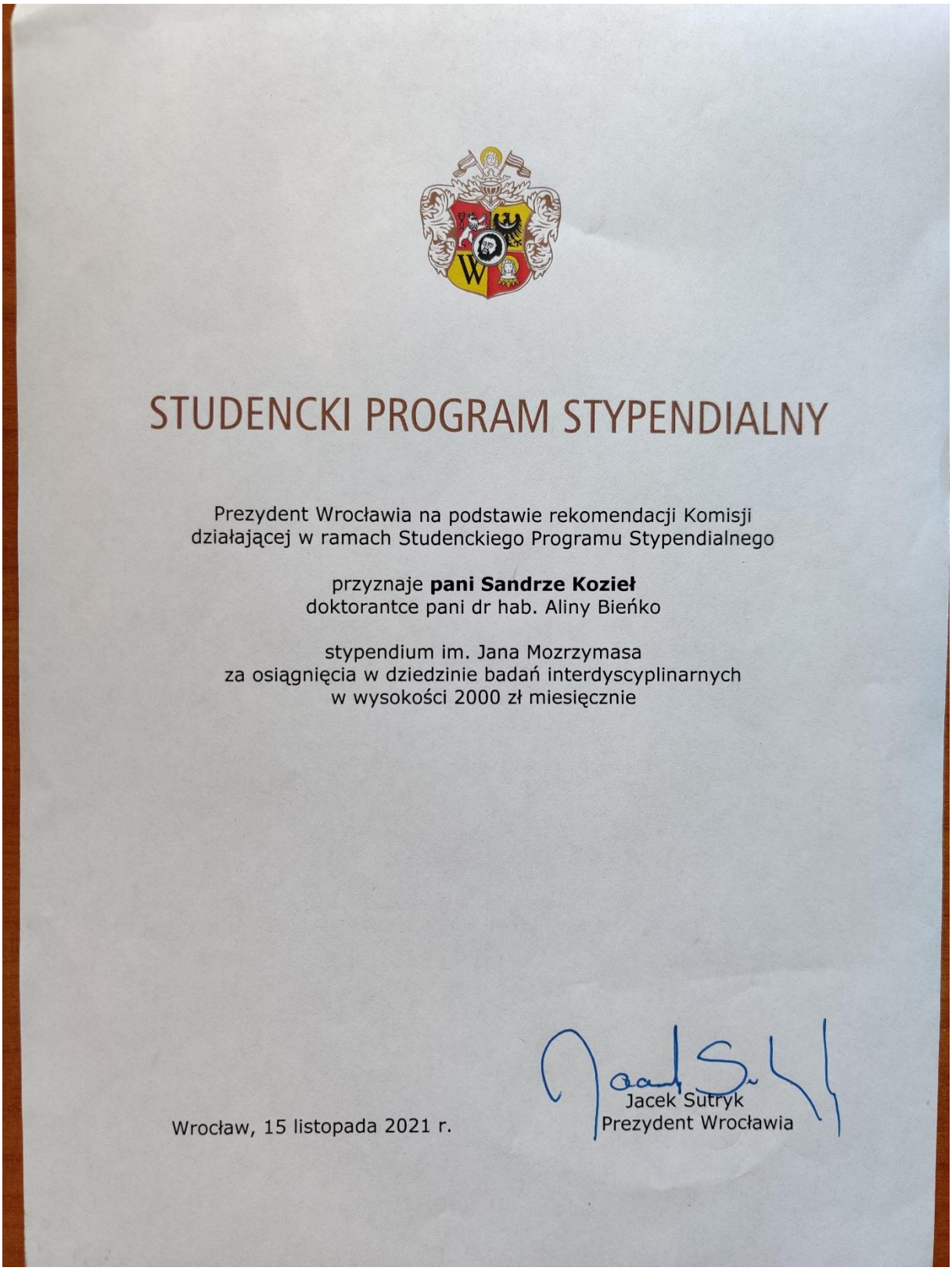
prof. dr hab. Waldemar Banasiak, prof. dr hab. Marek Bojarski, prof. dr hab. inż. Jarosław Bosy, Piotr Chęciński, Marcin Chludziński, dr Janusz Cymanek, prof. dr hab. Szymon Dragan, prof. dr hab. Bogusław Fiedor, dr Stanisław Han, Rafał Holanowski, prof. dr hab. Andrzej Kaleta, prof. dr hab. Krystian Kiełb, prof. dr hab. Roman Kołacz, Tomasz Kurzewski, prof. dr hab. inż. Cezary Madryas, Andrzej Panas, płk dr hab. Piotr Płonka, Roman Potocki, Cezary Przybylski, Andrzej Przybyło, prof. dr hab. Wojciech Pukocz,prof. dr hab. Andrzej Rokita, Janusz Rybak, Jacek Sutryk, prof. dr hab. Tadeusz Szulc, Robert Ślawski, prof. dr hab. inż. Tadeusz Trziszka, prof. dr hab. inż. Arkadiusz Wójs

Prezydent
Dolnośląskiego Klubu Kapitału

Janusz Rybak

Przewodniczący
Kapituły Konkursu Młode Talenty

prof. dr hab. Bogusław Fiedor



STUDENCKI PROGRAM STYPENDIALNY

Prezydent Wrocławia na podstawie rekomendacji Komisji
działającej w ramach Studenckiego Programu Stypendialnego

przyznaje **pani Sandrze Kozieł**
doktorantce pani dr hab. Aliny Bieńko

stypendium im. Jana Mozrzymasa
za osiągnięcia w dziedzinie badań interdyscyplinarnych
w wysokości 2000 zł miesięcznie

Wrocław, 15 listopada 2021 r.

Jacek Sutryk
Prezydent Wrocławia