GDAŃSK UNIVERSITY OF TECHNOLOGY | FACULTY OF CHEMISTRY

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Dr. Eng. Łukasz Ponikiewski
Department of Inorganic Chemistry
Faculty of Chemistry
Narutowicza Str.11/12
80-233 Gdańsk
Email: lukasz.ponikiewski@pg.edu.pl

Gdańsk, 15 July 2022

Reviewer report on the PhD thesis submitted by MSc Sandra Kozieł entitled: „Iridium(III) complexes with phosphine-fluoroquinolone conjugates - design, synthesis, bioactivity and nanoformulation as a potential platform for drug delivery".

I. Introductory information.

During the evaluation of the doctoral thesis of MSc Sandra Kozieł, I was guided by Art. 187 of the Act of 20 July 2018 "Law on higher education and science" Dz. U. from 2021 year position 478, 1630. The content of the article:

1. Rozprawa doktorska prezentuje ogólną wiedzę teoretyczną kandydata w dyscyplinie albo dyscyplinach oraz umiejętność samodzielnego prowadzenia pracy naukowej lub artystycznej.
2. Przedmiotem rozprawy doktorskiej jest oryginalne rozwiązanie problemu naukowego, oryginalne rozwiązanie w zakresie zastosowania wyników własnych badań naukowych w sferze gospodarczej lub społecznej albo oryginalne dokonanie artystyczne.
3. Rozprawę doktorską może stanowić praca pisemna, w tym monografia naukowa, zbiór opublikowanych i powiązanych tematycznie artykułów naukowych, praca projektowa, konstrukcyjna, technologiczna, wdrożeniowa lub artystyczna, a także samodzielna i wyodrębniona część pracy zbiorowej.
4. Do rozprawy doktorskiej dołącza się streszczenie w języku angielskim, a do rozprawy doktorskiej przygotowanej w języku obcym również streszczenie w języku polskim. W przypadku gdy rozprawa doktorska nie jest pracą pisemną, dołącza się opis w językach polskim i angielskim.

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II. The form of doctoral dissertation.

The presented doctoral dissertation is based on three thematically related scientific articles. These publications are describe in summarized form which composes of: introduction, aims of this work, experimental section, results and discussion, concluding remarks and the most important achievements. Additionally, the PhD student added also a chapter concerning on perspectives in relation to the results presented in the work and being a continuation of the research contained in the first three publications.

The dissertation was accompanied by the statements of the co-authors which indicate a significant participation of the PhD student in formulating the concept of the work together with the auxiliary supervisor Dr. Urszula Komarnicka. At each stage of the research, the PhD student independently synthesized and characterized chemical compounds that were the basis for research related to anti-cancer activity. It is worth emphasizing that candidate independently planned the research concept and select methods leading to solving the problem in the third publication (the last one). Some of the declarations of other co-authors indicate the auxiliary nature of their research, necessary for the full characterization of obtained compounds and the conducted research. This includes measurements such as:

- X-ray measurements performed by Dr. Agnieszka Skórska-Stania from Jagiellonian Univeristy of Kraków;
- Mass spectroscopy measurements performed by Dr. Francesco Tisato from ISMATE-CNR;
- TEM images of Plytronic P-123 formulation with the encapsulated IrPCp complex performed by Prof. Victor Sebastian from Department of Chemical Engineering, Aragon Institute of Material and Nanoscience (INMA), University of Zaragoza;
- DFT calculation performed by Dr. Eng. Edyta Dyguda-Kazimierowicz from Advanced Materials Engineering and Modelling Group and Dr. Dariusza Bieńko from Department of Physical and Quantum Chemistry from Wroclaw University of Technology;

The part of the statements of the co-authors indicates that the MSc Sandra Kozieł benefited from substantive support of Dr. Monika Lesiów, Dr. Alina Bieńko from University of Wrocław and Dr. Barbara Pucelik, Dr. Michał Płotek, Dr. Agnieszka Kyzioł and Prof. Grażyna Stochel from Jagiellonian University.

The PhD student is the first author in all publications and in one of them is also the corresponding author together with Dr. Urszula Komarnicka. It should be noted that all articles are published in valued journals (one for 100 and two for 140 points according to the Ministerial list of Journals). Moreover, the position of the first author in publications indicates on the large contribution to the performed research, whereas the position of the corresponding author indicates on the large contribution to the final version of the manuscript as well as participation in discussions with reviewers.

III. Description of the subject.

The cancer is one of the problem still faced by modern medicine. In view of the fact that most of the current platinum ion-based drugs on the market show a limited spectrum of action and

high toxicity it is necessary to search for new anti-cancer drugs. One alternative is to employ new transition metal complexes (Cu, Ru, Pd or Ir), following the example of the already known platinum compounds. Additionally, it is very common to use previously known drugs, such as antibiotics, and combine them with transition metals. This solution provide them new properties, often including anti-cancer ones. An additional factor which is now being consider is targeted in way of serving and regulation of drug release. Therefore, very often nanostructures (micelles, liposomes) are used as transport systems.
Sandra Kozieł's PhD researches were carried out in simultaneously in the Department of Chemistry in the University of Wrocław and in the Department of Chemical, Pharmaceutical and Agricultural Science in the University of Ferrara under the supervision of Dr. Alina Bieńko and Prof. Stefano Caramori. Additionally, Dr. Urszula Komarnicka from University of Wrocław was auxiliary supervisor. Subjects of the collected publications and the aim of the doctoral dissertation were the two main trends:

- syntheses of organometallic iridium(III) complexes with phosphines derived from fluoroquinolone antibiotics possessing anti-cancer potential;
- anti-cancer activity studies of obtained compounds and design of methods for their selective delivery using encapsulation in nanoformulations;

The conducted research are continuation of previous research carried out by Dr. Bieńko and Dr. Komarnicka.

IV. Comments on the components of the work.

a) Introduction

The introduction is divided into several subsection, which makes it very transparent. In the subsection: "Iridium compounds as anticancer agents" the literature reports related to iridium(III) complexes have been described. This chapter reveals very well the influence of ligands in Ir(III) complexes on their biological activity. The next subsections contain the characterization of fluoroquinolone compounds, modification of these molecules (also with phosphines) and description of compounds with antibacterial activity and in terms of increasing their activity, not only antibacterial but also anti-cancer. The aim of the doctoral dissertation is a logical consequence of the data provided in introduction.
I have the following comments and questions regarding the chapters:

- *I do not fully understand the formula $[(\eta^5\text{-}Cp^*)Ir(L^\wedge L')Cl]^{0/+}$ containing N^N- (e.g.1,10-phenanthroline (phen)) (exactly L^L'), would not it be easier to write $[(\eta^5\text{-}Cp^*)Ir(L,L')Cl]^{0/+}$, N,N- (e.g.1,10-phenanthroline (phen)?*
- *does the counterion (-) in ionic compounds Ir(III/IV) has an impact on their biological activity?;*
- *Figure 7: the description of -i-Pr is not very common. I suggest the description in form of i*Pr *or* ^{i}Pr;
- *I would also suggest using subscript for the numbers instead of reduced font (e.g. CH_3);*
- *The author stated in the description of "Fluoroquinolones" that complexes of metals with quinolones exhibit anti-cancer properties. Immediately after this it is added that*

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the overuse of antibiotics leads to a significant increase in bacterial resistance to the drugs. Does it mean that it has an indirect effect on the reduction of the anti-cancer activity of metal complexes with these compounds as well?

- *there are also a few minor mistakes in this part, the most noticeable ones are: "Phosphinesdervied" or "aminomethylphosphaneswith";*

b) Aims of this Work and Experimental section

The research work of MSc Sandra Kozieł is very well motivated from a scientific point of view. Already in the introduction, the PhD student showed what important issue did she set herself as a research problem. Therefore, the MSc Sandra Kozieł decided to synthesize the new iridium(III) complexes with phosphine-fluoroquinolone systems and to examine the biological activity *in vitro* against several cancer cell lines and one normal cell line (human embryonic kidney, HEK293T). The planned biological studies required, first of all, to obtain Ir(III) complexes in pure form, as well as accurate characterization of their structures. This was achieved by X-ray, elemental analysis, MS spectroscopy, UV-Vis and IR spectroscopy. The determination of the cytotoxicity of the obtained compounds towards 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) and the action mechanisms were great challenge.

Both chapters, the aim of this work and Experimental section are described very clearly, the processes or issues are in the bulleted form which is readable for the recipient.

c) Results and discussion (discussion of the articles included in the dissertation)

I do not understand why the articles in the dissertation are not numbered chronologically according to the years of publication. Does this mean that subsequent study was not a continuation of those already performed, or it was carried out simultaneously??

At the beginning of the chapter, the MSc Sandra Kozieł described the synthesis of four Ir(III) complexes, $Ir(\eta^5\text{-}Cp^*)Cl_2PNr$ (IrPNr), $Ir(\eta^5\text{-}Cp^*)Cl_2PCp$ (IrPCp), $[Ir(\eta^5\text{-}Cp^*)Cl_2PLm$ (IrPLm) and $Ir(\eta^5\text{-}Cp^*)Cl_2PPh_2CH_2OH$ (IrPOH) and their characterization (which is presented on the next few pages - NMR, ESI-MS, UV-Vis and X-ray spectroscopy). In addition, cyclic voltammetry (CV) was performed for all examined complexes in order to provide qualitative information about the course of the electrochemical reactions. In the following work, the PhD student describes the biological properties of obtained compounds. The *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, the encapsulation processes were performed for all Ir(III) complexes including the IC_{50} parameters testing. The chapter also describes the mechanisms of drug actions on cancer cells.

Remarks and comments regarding this chapter:

- *Due to the fact that I am a synthetic chemist, please explain what is the acceptable ratio of IC_{50} for cancer cells vs. IC_{50} for healthy cells for a compound to be considered promising as a future drug? I the case of compound IrPCp the ratio is 4.8 [μM] and 28.5 [μM,] respectively;*
- *Are you considering the synthesis of compounds with substituents other than Ph groups on the phosphorus atom? and hence whether the electron density on the phosphorus atom will change?;*
- *If the hydrolysis process was studied, was there no exchange of protons observed from the CH_2OH group to the Na^+ ion?;*
- *The dissertation describes the encapsulation processes for all Ir(III) compounds. Why, then, there is only the IC_{50} parameters for two cancer cell lines and two encapsulated Ir(III) complexes described in the dissertation? It means that the encapsulation processes did not improve IC_{50} parameters in the other cancer cell lines? Will changing the encapsulation of compound have an impact?*
- *On the page 33 is described "Me-Cl (Me: metal)". Please note that in the scientific literature the abbreviation Me means methyl group. Perhaps it would be better to use simply M.*
- *Why the proton from -OH group in IrPOH complex was not observed in the ^{1}H-NMR spectrum? Very often it is a very broad signal with low intensity. However, why was there no visible correlation between methylene carbon atom and this proton on the $^{13}C/^{1}H$- HMBC spectrum?*
- *In the publication S2 was also described the complex IrPSf. Why it was not described in the dissertation?*
- *Regarding crystallographic data, please note that some characters in space groups are written in italics, e.g., $P2_1$* rather than P 2_1, or *$P2_1/n$* rather than P 21/n;
- *What is the reason for the large "Largest diff. peak" values for X-ray measurements of the compounds IrPCp, IrPLm, and IrPNr? Is it a matter of not dissolved disorder or these are densities localized close to the metal atom?*
- *In the publication S1 the molecular structure does not possess labeled atoms which is quite difficult for the observer. I am full of admiration that this passed in this journal;*

d) Conclusions

The conclusions consist of two chapters entitled: "Concluding Remarks" and "The most important achievements". As at the beginning of the work, the achievements and conclusions are presented here point by point. I personally think it could be combined together, but that is just my feeling. I do not really understand the perspectives chapter. On the one hand, I understand that the PhD student was trying to show that she had an idea for further research and to boast about the research she had already done, but this could have been done in one, top two pages at most, but not eight.

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e) References

The bibliography includes 128 items of literature and fully represents the current state of knowledge, both on the topic of compound synthesis with anticancer properties, as well as on biological studies of these compounds.

V. Other comments and final conclusion.

All publications of MSc Sandra Kozieł (S1, S2 and S3) describe innovative ideas implemented by the candidate and her supervisors. A major advantage is that the PhD student dealt with a rather important problem, whose mainstream was the search for new compounds with anticancer properties. Importantly, the research were not only limited to compound syntheses and test their anti-cancer properties, but also showed the mechanism of compound actions on cancer cells. As part of the research, the PhD student synthesized new Ir(III) compounds and investigated their anti-cancer properties showing that for the complexes studied, the IC_{50} index is much better than for currently using platinum compounds. These biological studies were conducted for 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. Each of the publications submitted for evaluation is a complete set of studies in which the participation of PhD student was very important. MSc Sandra Kozieł also largly participated in manuscript preparations.

The candidate not only had the good fortune to learn how to conduct scientific research in a dynamic scientific group, but also became an important part of it. The total publishing output of MSc Sandra Kozieł includes 9 publications in JCR journals and 6 communications at scientific conferences, both national and international. The PhD student participated in the implementation of research projects and was also the manager of two projects. Moreover, the PhD student can boast of awards and scholarships, such as:

- scholarship of the Rector of the University of Wrocław for the best PhD students;

or

- scholarships of Ministry of Higher Education and Science for the best PhD students;

I find that the work presented to me for evaluation meets the requirements of Art.187. of the Act of July 20, 2018. "Law on Higher Education and Science", which means that it is solution to an original scientific problem, and I request that MSc Sandra Kozieł be allowed to proceed to the further stages of the doctoral protocol.

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Dr. Eng. Łukasz Ponikiewski
Department of Inorganic Chemistry
Faculty of Chemistry
Narutowicza Str.11/12
80-233 Gdańsk
Email: lukasz.ponikiewski@pg.edu.pl

Gdańsk, 15 July 2022

Application for distinction

the doctoral dissertation of MSc Sandry Kozieł entitled: "Iridium(III) complexes with phosphine-fluoroquinolone conjugates - design, synthesis, bioactivity and nanoformulation as a potential platform for drug delivery".

I submit a request for the distinction of Sandra's dissertation due to the innovative research carried out during PhD studies. The research include synthesis, characterization, as well as, most importantly, biological studies targeting anticancer properties. Based on the statements, I conclude that participation of MSc Sandra Kozieł was significant at all stages in the conducted research. The research that were performed in the course of the doctoral thesis may have applications, if not directly then perhaps indirectly in the battle against cancer.

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