Faculty
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A REVIEW

on the PhD thesis of Sandra Koziel (Department of Chemistry, University of Wroclaw) entitled:

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

The PhD thesis has 257 pages in total, consisting of 68 pages of the thesis itself, supported by various attachments, including three published papers and one submitted manuscript. The 68 pages of the thesis include 37 figures, 1 scheme, 4 tables and 128 references. The aim of this thesis is defined as design and synthesis of new half-sandwich iridium(III) dichlorido complexes involving phosphane derivatives of various fluoroquinolones as bioactive P-donor ligands, and encapsulation of these new Ir(III) compounds into various nanoformulations for drug delivery.

The student prepared a series of four mononuclear complexes of the general formula $[Ir(\eta^5\text{-}Cp^*)Cl_2L]$, where L symbolizes a monodentate P-donor ligand, specifically (diphenylphosphanyl)methylene derivatives of fluoroquinolone-based antibiotics ciprofloxacin, norfloxacin and lomefloxacin, or (diphenylphosphanyl)methanol used for comparative purposes; Cp* = pentamethylcyclopentadienyl. These complexes were thoroughly characterized (including a single-crystal X-ray analysis and multinuclear NMR) and studied for their in-solution stability in the presence of water. The complexes were tested for their anticancer activity at selected human cancer and non-cancerous cell lines. Further, several processes connected with the mechanism of action of the new complexes were studied.

Similar chemical and biological studies were performed for a series of dinuclear heterobimetallic Ir–Cu complexes bearing a {Cu(II)(phen)} moiety, which is bidentate coordinated through the quinolone part of the $[Ir(\eta^5\text{-}Cp^*)Cl_2L]$ compounds, used this time as metalloligands. In addition to the synthesis of complexes, various nanoformulations were prepared as carriers of new Ir and Ir–Cu complexes for targeted drug delivery. The prepared hybrid micelles (without or with superparamagnetic magnetite) were also bioanalysed for their anticancer activity.

This PhD thesis is of a good quality with many new and interesting results. Especially I appreciate very much that the author paid an attention to the solution behaviour (stability) of the complexes in the presence of water, which is a very basis for new compounds planned to be studied for their biological activity (but often overlooked in the literature). For fluoroquinolone-based complexes, an introduction of bioactive substituents (i.e. fluoroquinolone) to the phosphane ligand enhanced the resulting cytotoxic activity of the studied Ir complexes, which also showed a predominance over cisplatin with some differences in their MoA. Further improvement of biological activity was reached by encapsulation of complexes in and pluronic-123-based micelles. Although a relevance of the obtained results was clearly proved by peer reviews in highly-rated scientific journals, I have several comments and questions on the student, as follows:

1) There are a lot of mistakes in using IUPAC recommendations for nomenclature (e.g. chloride ligand, phosphine) and formula writing (square brackets for complexes, order of ligands). This has to be used correctly within a defence talk.
2) It is claimed in Summary (page 7) that "... iridium compounds are probably the most promising group of potential medicine." Could it be supported somehow (examples of compounds? references? compounds in clinics/clinical trials?)?
3) Figure 1 - three of four structural formulas are wrong.
4) Introduction - I miss some information for widely studied Ru and Pd compounds (with representatives in clinical trials), which represent more important platinum metals in the field of medicinal chemistry than is iridium. Could you provide us with some examples?
5) Further for Introduction - although the student prepared and studied Ir dichlorido complexes (i.e. a RAPTA type), heterobimetallic Ir-Cu complexes and nanosystems biofunctionalized by Ir complexes, she did not mention these types of compounds/formulations in Introduction of her thesis. Would it be possible to show some examples to illustrate novelty and pharmacological prospective of her compounds/formulations?
6) Figure 5 and ref. 43,44 - did Sadler and co-workers really use benzoquinoline as a C,N-ligand in the cited papers?
7) Mass spectra contained the $[M-Cl]^+$ and $[M-2Cl]^+$ species - how did the second one reached a +1 charge? Similarly, this comment can be applied for the $[M]^+$ and $[M+H]^+$ species for dinuclear complexes?
8) Figure 17 (104 mM NaCl) - together with the rest of hydrolysate (ca. 1.5 ppm) we can see two Cp* signals at ca. 1.25 ppm, but only one is expected for the initial complex - any explanation? Did you try ^{31}P NMR stability studies?
9) Do the data in Table 2 really proved higher accumulation of complexes, as claimed above this table?
10) Page 44 - it is claimed here that "... 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. ..." Does it mean that anticancer effect of Pt drugs is based on their intercalation to DNA?

11) Page 56 - the discussion does not correspond to the data in Table 3 (and Table 1), unless the exposure time is specified. In particular, it is claimed that activity against MCF7 and A549 cells is higher for Ir-Cu complexes than for Ir ones. This, however, is not true at the 24+48 h exposure time. Further, A549 cells were not the most sensitive at both exposure times, as stated by the student. In addition, using of e.g. E-03 for SD is a bit confusing since it is not clear if it belongs to the SD or IC_{50} values.
12) In connection with comment 11 - how would the student explain the several fold decrease of cytotoxicity at some cell lines tested at 24 h 24+48 h time levels?
13) Table 4 - define what is L and IrPCpCu. In the case IrPCpCu is free complex, how would you explain a difference in its cytotoxicity (see Tables 3 and 4)?
14) The student, as a synthetic chemist, should specify her contribution to the used biological experimental methods.

Minor points:

- missing references in Summary (page 7–8)
- HeLa - missing tissue specification (e.g. page 10)
- missing negative charge of phosphane ligands in formulas of dinuclear compounds
- missing units, spaces
- C for concentration
- A548 (page 49)
- 10 mm size of NPs (page 58)

In total, the student proved in her thesis all the skills generally required for a PhD student in chemistry. She did a good literature research with many relevant references from well-known international scientific journals, and an adequate number of new compounds was prepared, characterized and bioanalysed for their anticancer activity. The student also prepared advanced dinuclear heterobimetallic Ir–Cu complexes and micellar formulations for passive and targeted (magnetic) drug-delivery. In connection, the student is an author (the first author in all cases) of four scientific publications relevant to her PhD thesis, and six other papers, clearly showing that she became an important part of the research team during her PhD study. Although some mistakes can be found in the PhD thesis and I also raised some comments, I can claim here that the aims of this PhD thesis were successfully achieved and the results of the PhD study of Sandra Koziel represent a new knowledge in a scientific area of bioinorganic chemistry. The syntheses and performed experiments are well-described and adequately interpreted and discussed within the thesis and attached papers. It also seems that the PhD student contributed to most theoretical and experimental aspects of her thesis.

Guided by my assessment of the achievements of Mrs. Sandra Koziel, I express the opinion that her PhD thesis fully meets the requirements for doctoral dissertations in Poland set out in the Act of July 3, 2018 and the Regulation of the Minister of Science and

Higher Education of 19 January 2018, and I hereby request admission of Mrs. Sandra Koziel to further stages of his doctoral dissertation.

Taking into account the wide scope of the research carried out, the difficulty and topicality of the topic, the comprehensiveness of interdisciplinary research as well as the substantive value of the results obtained, which were largely published in prestigious scientific journals, I would like to ask the Chemical Sciences Discipline Council of the University of Wroclaw to award this PhD thesis.

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