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Review report of the Ph.D. Thesis by Sandra Kozieł entitled:

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

The doctoral dissertation by M.Sc. Sandra Kozieł was prepared and written in an international scientific team supervised by dr hab. Alina Bieńko, WrU Prof.; dr Urszula Komarnicka (auxiliary supervisor) from the Faculty of Chemistry, University of Wrocław (Poland) and Prof. Stefano Caramori from the Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara (Italy).

The research was focused on the structural characteristics of new bioactive organometallic iridium(III) compounds containing aminomethylphosphines derived from fluoroquinolone antibiotics: norfloxacin (HCp), norfloxacin (HNr), and lomefloxacin (HSf). Implementation of such a topic, which requires extensive knowledge in chemistry, biology, and toxicology, in the doctoral dissertation corresponds with contemporary world research trends concerning developing new pharmacological solutions in tackling neoplastic diseases. The subject of the study is valid as cancer still constitutes a serious health problem worldwide, mainly due to high toxicity and poor selectivity of the currently used oncological drugs which additionally cause a wide range of undesirable side effects. Obviously, modern inorganic chemotherapeutic agents, cisplatin, carboplatin, or oxaliplatin, have several limitations such as side effects, a narrow spectrum of action, and intrinsic or acquired resistance of cells towards drugs. As we are constantly facing the problem of cancer on the global scale, there is an urgent need to design new cytostatic drugs characterized by lower toxicity and higher selectivity against neoplastic cells.

The reviewed doctoral dissertation is based on three subject-related scientific articles published within the years 2020-22 in *Dalton Transaction* (S1), *Inorganic Chemistry Frontiers* (S2), *Pharmaceuticals* (S3), and one original work submitted in *Inorganic Chemistry Frontiers* (S4) which can be considered as a presentation of further research perspectives. The above mentioned Ph.D. student is the first author of the three publications (IF ~ 17), so her contribution to the preparation of the published papers is significant. From the formal point of view, the doctoral dissertation, which is a guide to the published articles, is a 77 pages long typescript which includes a summary in Polish (3 pages), a summary in English (2 pages), a table of contents, a list of abbreviations, introduction, objectives, experimental part, results and discussion, resume, and a list of 128 literature positions. In addition, at the end of the doctoral dissertation, the updated Curriculum Vitae of the Ph.D. student presenting her scientific activity was enclosed.

The introduction is divided into three sections in which the Author presents the current state of research on Ir(III) compounds. Subsequently, M.Sc. Sandra Kozieł characterized fluoroquinolones - gyrase inhibitors - that act bactericidally by inhibiting the DNA synthesis in bacterial cells. Unfortunately, their use causes several side effects, e.g. damage to the musculoskeletal system (tendons, muscles, joints), disturbance of the CNS (central nervous system) functioning, or induction of photosensitizing reactions, prevalently the photoallergic or phototoxic ones. Despite these drawbacks, their antibacterial and anticancer potential is significant. This fact encourages scientists to attempt to reduce the undesirable effects of fluoroquinolones by obtaining less toxic derivatives, *e.g.* by including a phosphine moiety. The need to extend knowledge in this field motivated M.Sc. Kozieł to undertake research, the main goal of which was to develop a method for synthesizing and isolating organometallic iridium(III) inorganic compounds with phosphine derivatives of fluoroquinolones and to determine their physicochemical and biological properties in the context of their potential use in anticancer therapy.

Implementing the above goal required the performance of an effective ligand complexation reaction with iridium(III) ions, which resulted in obtaining three new compounds containing phosphine ligands in the coordination sphere with a fluoroquinolone fragment.

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The most extensive chapter, *Results and Discussion*, begins with the description of the synthesis and the physicochemical characteristics of the cyclopentadienyl Ir(III) complexes without (IrPOH) and with the antibiotic fragment (IrPCp, IrPLm, IrPNr). The mode of coordination of the discussed complexes was determined using various techniques (IR, NMR, UV-Vis, X-Ray, MS, CV (S1-S3)). Among the individual achievements made in this part of the work, the Ph.D. student should be congratulated on obtaining all the compounds in single crystals suitable for crystallographic studies (S1, S2), which unambiguously confirmed the proposed structure of Ir(III) complexes.

It is worth emphasizing that the experimental data concerning the study of the physicochemical properties of Ir(III) complexes are presented with clarity and reliability, and documented in the form of NMR, UV-Vis, IR, MS spectra, voltamperograms, tables, and drawings of crystal structures included directly in the dissertation as well as in the supplementary materials of the published articles (S1-S4). The presented study clearly shows the excellent proficiency and skillfulness with which the author uses various spectroscopic methods, which, in my opinion, proves that the Ph.D. student is well prepared.

In the next stage of the doctoral dissertation, the Ph.D. student focuses on biological research on Ir(III) complexes concerning: ***i)*** determination of the cytotoxic potential of the obtained Ir(III) complexes against five human neoplastic lines (metastatic human melanoma (WM2664), human lung adenocarcinoma (A549), human breast adenocarcinoma (MCF7), human pancreatic/duct carcinoma (PANC-1), human prostate carcinoma (DU-145)); ***ii)*** establishment of the mode of action, e.g. the cell cycle study, reactive oxygen species generation, or the determination of the type of interaction with CT-DNA and selected proteins (albumin and apo-transferrin); ***iii)*** use of the Pluronic®P123 copolymer as a nanocarrier for the selected inorganic complexes to overcome their poor solubility and to prevent the acquisition of resistance.

From the extensive experimental material presented in the doctoral dissertation, the following undoubted achievements can be mentioned: ***i)*** presentation of the physicochemical characteristics of 3 new organometallic Ir(III) complexes, ***ii)*** numerous successful studies aimed at determining the mechanism responsible for the cytotoxic activity of Ir(III) complexes, ***iii)*** indication of the encapsulation of the active iridium(III) complex in micelles

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as a method improving its the availability in a cancer cell, ***iv)*** a thorough analysis of the experimental results, which not only enriched bioinorganic chemistry with new knowledge but allowed opening interesting future research perspectives (S4).

Regarding the linguistic aspect, the work was very well written. Its graphic and editorial layout should also be considered a strong point. Nevertheless, in this type of dissertation, it is impossible to avoid some editorial mistakes I only mention.

When the text is read, some questions for discussion and clarification may arise:

1) What are the values of log*P* of the obtained Ir(III) complexes?

2) What was the reason for the selection of neoplastic cell lines?

3) How does the Ph.D. student assess the safety of fluoroquinolones and their phosphine derivatives? What is the current state of knowledge on this subject?

Summing up my review, I can conclude that M.Sc. Sandra Kozieł demonstrated her skills in the application of modern spectroscopic techniques, analytical techniques, and biological experiments. The appropriate selection allowed her to attempt to explain the cytotoxic mechanism of the activity of organometallic Ir(III) compounds. The financial support granted by the National Science Center as part of the Preludium (no 2020/37/N/ST4/02698) and the possibility to conduct research in an international team played the key role in the performance of interdisciplinary research on a global scientific level.

Summing up, I can state that the scientific publications in the doctoral dissertation of the Ph.D student, Sandra Kozieł, constitute an original contribution to the development of inorganic chemotherapeutic agents. They were published by prestigious publishers with a high reputation in the scientific community, finding recognition among reviewers and editors who set high requirements at the stage of their publication. It is worth mentioning that the Ph.D. student's research interests were not limited to the subject of the doctoral dissertation, as evidenced by multi-author scientific publications (6 published, IF ~ 29), which S. Kozieł is a co-author of. The doctoral student also successfully presented the results of her research at six national and international conferences, receiving the award for the best poster presentation during the 4th National Forum of Inorganic Chemistry in Toruń, Poland. In addition, for an outstanding scientific activity, she obtained, among others, a fellowship from the Minister of Science and Higher Education for the best Ph.D. students, a P.L. Sosabowski fellowship,

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and a fellowship from the President of Wrocław for achievements in the field of interdisciplinary research.

In conclusion, I state that the doctoral dissertation by M.Sc. Sandra Kozieł fully meets the requirements for doctoral dissertations described in the *Law on Higher Education and Science Act* (Dz. U. z 2020 r. pos. 85 as amended) and I would like to place my positive recommendation to the Chemical Sciences Discipline Council of the University of Wrocław concerning Sandra Kozieł's admission to further stages of the doctoral degree procedures. Moreover, taking into account the high substantive value of the dissertation and its significant contribution to the development of coordination and bioinorganic chemistry presented in detail in the review, I appeal to the High Scientific Chemical Sciences Discipline Council at the University of Wrocław for its distinction.

Hałopmła

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