

Bari, 7th November 2024

To the kind attention of the Head of Committee of the Ph.D. course in Chemistry of the Brno University of Technology, Faculty of Chemistry, Institute of Chemistry and Technology of Environmental Protection,

Enclosed please find the following **Review Report** for the Ph.D. thesis „**Synthesis and application of nature-inspired materials for photocatalytic processes**“ by the student **Ing. Lucia Ivanová**

- The Ph.D. thesis by Ing. Lucia Ivanová entitled „Synthesis and application of nature-inspired materials for photocatalytic processes“ is focused on the design, synthesis, characterization and application of flavin-type molecular materials suitable for hydrogen photogeneration and cancer photodynamic therapy, both topics being of great current interest for worldwide academic and industrial communities. Hence the topicality of the research themes is clear and evident. In particular, the thesis deals with the synthesis of some interesting alloxazine and lumazine derivatives and it further investigates the efficiency of six butyl substituted target molecules (four lumazine flavins L-Ph-Bu, L-Py-Bu, L-Th-Bu, and L-BrTh-Bu and two alloxazines A-phe-bu & A-Pyr-bu) as photosensitizers in hydrogen evolution reactions. A detailed comparison of several literature reported approaches for the synthesis is presented and a number of new *N,N'*-alkyl-substituted flavin derivatives have been synthesized using an elegant method. The reaction conditions were also optimized to improve yields. Four water-soluble tetraethylene glycol (TEG) substituted alloxazines were also synthesized and tested on human foreskin BJ cells and human fibrosarcoma tumour cell line HT-1080 cells for possible application in PDT cancer therapies.
- The aims set at the beginning of the thesis have been addressed. Indeed, the first aim was to design and synthesize nature inspired molecular organic photosensitizers and evaluate their applicability in light driven processes. A total of eleven alloxazine and lumazine target molecules were therefore prepared and experimental parameters such as reaction temperature, time and ratio of reagents were optimized at various points during the preparation of the precursors as well as of the final molecules. Photophysical characterization of the molecules was successfully carried out. The hydrogen evolution experiments were performed on the butyl substituted derivatives and their efficacies were optimized by a detailed study and compared with those of other standard compounds reported in the literature. The cell-viability for four other water soluble *N,N'*-TEG substituted alloxazine derivatives were also studied for their possible application in cancer therapy.
- Regarding the reported results, the thesis shows a detailed comparative overview of the literature-reported synthetic methods and derivatization procedures for alloxazine and lumazine compounds. A well-designed synthetic method was optimized starting from a method previously reported in the literature by the research group where this thesis was carried out. Among the synthesized molecules, eight products have been reported for the first time. Some low final yields observed were due to the complexity of molecular structures together with repetitive and rigorous purification procedures. Overall, four new *N, N'*-butyl substituted lumazine derivatives and four new water soluble *N,N'*-tetraethylene glycol (TEG) substituted alloxazines were prepared and characterized. The best performances and photostability in hydrogen evolution processes were observed for A-Pyr-Bu and they were found to be fairly competitive with those of other suitable materials reported in the literature for efficient hydrogen evolution.

Moreover, among water soluble alloxazine derivatives with polar tetraethylene glycol side chains, A-Pyr-TEG and A-Phe-TEG molecules were able to show better toxicity for cancer cells compared to healthy cells. Thus, the author has provided good rationale for future further investigation as potential agents in PDT.

- Regarding the specific contribution of the PhD student to the presented thesis, the primary role of Ing Lucia Ivanová in achieving the report results is clear. Except for DFT calculations, the student has carried out most of the research work reported therein, including the synthesis, the photophysical characterization and investigation of molecules suitability as photosensitizers in hydrogen evolution processes and PDT experiments.
- The implication of the results for further development and discovery in the field of small molecular organic photosensitizers is also clearly rational. The lower energy demand of the flavin derivatives in the photocatalytic experiments makes them suitable candidates to be used in-vivo. The sufficient photostability, ability to operate under lower energy irradiation and reproducibility of A-Pyr-Bu makes it a novel photocatalytic system for hydrogen production. The poor performance observed for the synthesized lumazines are likely due to the overlap of frontier molecular orbital and slowed down effective charge separation in the excited state. The implications of the results are relevant in the sense that they provide the right direction to proceed to structural modifications. The inference made by the author in this direction concerning future molecular design engaging bathochromic shift to enhance the performance and utilize longer visible-light wavelengths are thus thoughtful. The structural similarity of the *N, N'*-TEG substituted alloxazines together with their water solubility and fair cell viability at low concentration are also promising for future application in PDT and cancer therapy. A continuation of the present work in the examination and identification of the radical species formed by electron paramagnetic resonance method to confirm activity for PDT and to evaluate the cytotoxicity upon blue light irradiation can be relevant.
- Regarding the language level, English writing is very good and comprehensible. The experimental methods are described in detail. All data presented for the synthesis and characterization of compounds are in order. The references added are to my knowledge proper and just. The results and summary are clear, and the clarity of the figures is satisfactory. Overall, the presentation is successful in delivering useful information to the reader.
- The results reported in the Ph.D. thesis have been also published in the research article *ACS Omega* 2024, 9, 5, 5534-5540, with Ing. Lucia Ivanová as the first author, this confirming both her primary role and the novelty of the research work. As further evidence of the quality of results, a patent application has been achieved (PUV 2022-503 "*Method of Producing Hydrogen from Water by a Photocatalytic Process using Metal-free Organic Molecules*"). The synthesis of water soluble alloxazine derivatives with TEG groups, as well as their photophysical and cell-viability analysis are to my knowledge original and yet to be published.
- About the results reported in thesis, the following questions and comments are provided:
 1. Which structural modifications does the student expect to be suitable to improve the photostability of the flavine-type molecules for future synthetic work?
 2. Which kind of novel noble metal free co-catalysts are expected to improve the efficiency of flavin type molecules in hydrogen evolution processes?
 3. Can the student provide a possible reason why A-Pyr-TEG and A-Phe-TEG molecules are more effective than the other analogues in reducing cell viability in cancer cells compared to healthy cells? Is it possible that the presence of more extended polycyclic aromatic moieties in these compounds

with respect to the other ones may generate aggregates or micellar structures and the observed cytotoxicity may be a result of this phenomenon?

In summary, the Ph.D. student has clearly shown creative skills in her research work, demonstrating to be successful in achieving interesting and promising results. Her thesis meets standard requirements for PhD thesis and, in my opinion, **Ing. Lucia Ivanová fully deserves recommendation to be awarded the title of “Ph.D.”**

Kind regards,

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