CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRALOVE

Department Department of Organic And Bioorganic Chemistry

Study program: Pharmacy

Opinion of the Opponent of the Diploma Thesis

Year of the defense: 2024

Student:	**Dominika Pinterova**
Thesis Tutor:	PharmDr. Lukas Opalka, Ph.D.
Consultant:	PharmDr. Lukas Gorecki, Ph.D
Opponent:	Dr. rer. nat. Mgr. Ing. Tomas Hodik
Thesis title:	**Synthesis of chemosensitisers for the treatment of glioblastoma multiforme**

Scope of work, number of 64 pages, 11 figures, 2 tables, 70 citations

Evaluation of the work:

a)	Processing of the theoretical part:	Good
b)	The complexity of the methods used:	Very good
c)	Preparation of the methodological part (clarity, comprehensibility):	Good
d)	The quality of the experimental data obtained:	Very good
e)	Processing of results (clarity):	Good
f)	Evaluation of results, including statistical analysis:	Good
g)	Discussion of results:	Good
h)	Clarity, conciseness, and adequacy of conclusions:	Good
i)	Meeting the objectives of the work:	Excellent
j)	Quantity and up to date of references:	Excellent
k)	Language level (stylistic and grammatical level):	Excellent
l)	Formal level of the work (text structure, graphic design):	Failed

I recommend the thesis for recognition as a rigorous thesis ☐

Comments on the evaluation:

Domika Pinterová's thesis was written under the formal supervision of Dr. Lukáš Opálka (Faculty of Pharmacy, Charles University) and was completed under the supervision of Dr. Lukáš Górecký and Assoc. Prof. Jan Korábečný (Biomedical Research Centre). The thesis deals with the very urgent topic of potential pharmacotherapy of the most aggressive type of malignant brain tumour, i.e. glioblastoma multiforme (GBM).

The thesis counts as 64 pages divided into 5 chapters as usual for this type of work. The introductory part defines glioblastoma tumour and offers the state of the art of its current treatment approaches and attems in its improvement. Although the introductory part is supported by 56 references, the random checking of most of them revealed their irrelevance to the describing paragraph and did not support the information as stated in the thesis. E.g. Page 14 ref. 29, there are only a few sentences about Temozolomide (TMZ) that do not include the information that over 90% of methylated nucleobases are repaired via the BER pathway. I looked for it myself and found different information (see here: Trivedi RN, Almeida KH, Fornsaglio JL, Schamus S, Sobol RW. The role of base excision repair in the sensitivity

and resistance to temozolomide-mediated cell death. Cancer Res. 2005;65(14):6394-6400. doi:10.1158/0008-5472.CAN-05-0715). Another example, page 15, ref. 30, here i was completely unable to double-check the information as stated in the thesis.

Later, the introductory subchapter "Exploiting synthetic lethality" defines its concepts of which the designed drugs in this thesis arrised. The concept is described within 4 short sentences which resulted in its low clarity and improper description. Unfortunatelly, the improper references were confirmed in this paragraph too.

The aims of the work are clearly stated and are based on structural similarity of designed compounds to known potent chemotherapeutics, namely ceralasertib and elimusertib. The Results and discussion section explains the design of the new compounds, unfortunately without the references expected here. The chemical part includes the synthesis of 14 new potent compounds prepared solely by the student and starts from simple and commercially available starting materials. The discussion of this part is unusual in the field of synthetic chemistry. The schemes used here are unclear with many errors that a proper proofreading would have caught. Althought the synthetic part should be the main part of the work, it is described in less than 7 pages (including schemes) and lacks detailed information about the actual work and results, such as optimization of reactions, yields, whether everything went as described in the literature, etc. Since all reactions are analogous to the previously described procedures, it seems that everything went well, but in the conclusion we find the statement that the synthetic pathways were optimized.
Here, i would like to highlight some (not all that have been found) of the errors found in the body of the text -

- Figure 7 shows concentrated HCl solution althoug 10wt-% solution was used.

- Product 6/7 in Figure 7 is methyl ester instead of ethyl ester.

- Page 26, the reductive amination was run in MeOH or DCM?

- Page 26, what does 21% sodium ethoxide in EtOH mean? What concentration is meant? It would be much better to use molarity instead of undefined percentage.

- Page 27, overall yields are unclear to me how that was calculated. I got different numbers.

- IUPAC nomenclature is not usually followed in the whole thesis. e.g. methanesulfonyl group should be methylsulfonyl, the indicated hydrogen should be with italic, etc.

- Figure 9, alkylation of dimethyl malonate shows the reaction under the presence of NaH only. However, it was run once with NaH (product 18) and once with potassium carbonate depending (product 17) on the substrate. Why did you use different bases?

- Later in the experimental part (product 18), the information about the NaH concentration is missing. Or did you work with a neat NaH?

- Step C in Figure 9 is not aromatisation as stated on page 30.

Without any doubts, there was a lot of synthetic work done and Ms. Pinterova has proven her ability to work in the lab. Unfortunately, these errors significantly impair the quality of the work.

The biological part was carried out in collaboration with other colleagues and revealed a great potential of the prepared compounds, especially DP-6, which showed comparable activity to the clinical candidate ceralasertib. The following discussion of the results is very brief and formally incorrect since Table 1 is not commented and Figure 11 (wrongly labelled as Figure 14 in the text) contains much more information than is provided in the text. It indicates that this part was highly likely written in hurry or with less effort to discuss the collected results e.g. the structural effect of the novel compounds on its activity.

The experimental part is probably written according to the standards in their lab. However, it would have been better to collect more analytical data for all new compounds (e.g. melting points for solids) and to provide at least NMR spectra in an appendix. Among many typos and other formal errors, references in this section are also mixed. E.g. Ref. 67 describes cyanation reaction and not pyrazole formation. Ref. 68 focuses on the bromination with NBS instead of the cyanation etc. The experimental part includes statistical analysis of the biological testing without any justifying comment or discussion.

Questions and comments to student:

Some of the questions were pronounced above, here I have attached another one.

Interestingly, the cyclization reaction yielding products 8 and 9 gave the single regioisomers with very good yields (77% and 81%, respectively). How did you control or confirm the regioselectivity? (It is known from the literature that it usually proceedswith low selectivity only.)

The submitted thesis contains undoubtedly a sufficient quantity of synthetical work done by Dominika Pinterova although its written part, which was reviewed, would need to be reworked. However, the faculty of pharmacy either the department of organic and bioorganic chemistry does not specify the minimum either optimal requirements for this type of work, therefore there is no other way than to recommend this work for defense.

Evaluation of the thesis: Good		**For the defense:** **Recommend**
In Hradec Kralove	15. září 2024	signature of the opponent