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THESIS REPORT

on the dissertation presented by **Sharonova Tatiana Valerievna**,
entitled "**On the prospects of using human carbonic anhydrase inhibitors in anticancer therapy**"

submitted for the degree of Candidate of Chemical Sciences in specialization 1.4.16. Medicinal chemistry

The thesis of Sharonova Tatiana Valerievna is focused to the design synthesis and evaluation of human carbonics anhydrase (hCA) inhibitors as potential anticancer agents. Isoforms IX and XII are expressed in hypoxic solid tumors regulating the microenvironment and promoting the survival and proliferation of cancer cells. Since hCA IX and XII isoforms take part in almost all stages of tumorigenesis, these enzymes are considered a sufficiently validated target for the therapy of certain types of tumors. Consequently, the inhibition of hCA IX and XII can be considered as a novel and rational approach for the pharmacotherapy of solid tumors as monotherapy or in combination with other drugs.
The research strategy was first investigating hCA IX and XII inhibitors alone, then developing dual acting compounds with hCA and thioredoxin inhibitory activity and finally to test hCA IX and XII inhibitors in combination with the EGFR inhibitor gefitinib. The results of this work was summerized in the 114-page dissertation having 3 main chapters. In the first chapter the candidate summarized the background information available on the targets including target biology its validation in preclinical and clinical settings. This chapter reviews the published hCA IX and XII inhibitors and discusses hybrid compounds with hCA inhibitory activity. Finally, attempts developing drug combinations with hCA IX and XII inhibitors were summarized. The second chapter contains the key results of the thesis work including (i) the synthesis, enzyme inhibition and antiproliferative activity of of compounds prepared by carbenoid NH- insertion and the Wolff rearrangement as individual agents, (ii) design, synthesis and biological testing of dual acting hCA IX and XII and thioredoxin reductase inhibitors, and (iii) combination studies of hCA IX and XII with gefitinib. Chapter 3 contains all the relevant experimental data, while the thesis is finished by Conclusions and References. The results of the thesis work were published in 6 scientific articles in international peer-reviewed journals and were also presented at multiple national conferences.

Based on the careful evaluation of the dissertation the following key results of the thesis work are identified:

1. New synthetic approaches were developed for the preparation of benzenesulfonamide-containing scaffolds equipped with diverse substituents
2. The inhibitory effect of benzenesulfonamide derivatives has been investigated against various isoforms of human carbonic anhydrase

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3. The antiproliferative effects of human carbonic anhydrase inhibitors was evaluated and correlated with their inhibitory properties against IX and XII isoforms
4. Dual acting compounds against human carbonic anhydrase and thioredoxin reductase were designed, synthesized and their antiproliferative activity was tested
5. hCA inhibitors of were tested in combination with gefitinib against cancer cell lines evaluating their combined effect on the migration of cancer cells

The practical significance of the research is of a high value and the developed hCA inhibitors might be considered as useful tools in oncology research. Their standalone activity and their combination with TrxR1 inhibitors (pharmacophore linking) or gefitinib (fix dose) represent promising options for the pharmacotherapy of oncology indications. However, there are several remarks on the research:

1. No rational approach has been applied for the design of hCA IX and XII inhibitors. I accept that isoforms IX and XII are highly similar and therefore it is not feasible to find isoform selective compounds. Other isoforms, however, are considerably different and therefore considering a number of selective hCA IX and XII inhibitors in a ligand based approach would be rational.
2. How did the candidate identified malonate containing, monocarbonyl and acetamide type compounds as potential hCA inhibitors? How do these compounds compare to known hCA IX and XII inhibitors?
3. The synthesis of diazodicarbonyl compounds were realized in one step according to the previously developed «SAFE» (‘sulfonyl azide free’) protocol, which implies the use of an aqueous solution of sodium azide, 3-carboxybenzenesulfonyl chloride and K2CO3 to generate carboxybenzenesulfonylazide in situ. How does the candidate comment the safety conditions of this procedure? Isolation of the diazocarbonyl starting materials might be problematic. How were these compounds isolated? Did the candidate find any safety concern?
4. Diazo compounds are frequently prepared by flow synthesis that provide a safe and efficient alternative to batch reactions. Did the candidate consider this option?
5. Table 2.1 shows the inhibitory activity of the synthesized compounds against different hCA isoforms. There were few compounds (2.15b, 2.16f, 2.16j and 2.17d) that showed superior activity relative to the positive control AAZ. What about the selectivities? Considering that IX vs XII and II vs III are less important: what are the selectivities critical? How did the best compounds perform?

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6. Despite of their promising enzyme inhibitory activity, compounds 2.15, 2.16, 2.17 and 2.18 showed limited efficacy in the cell based antiproliferative assay. The candidate performed a large scale analysis on the published compounds to understand the criteria selecting compounds for cell based in vitro testing. The conclusion was that candidate compounds should be tested primarily against cancer cells and next they can be profiled against different hCA isoforms. Unfortunately, this approach does not provide a rational way selecting compounds for screening. Was there any difference between the physico-chemical profile of cell active and cell inactive compounds?
7. Addressing hCA IX and XII with reversible and TrxR1 with covalent mechanism by a single molecule seems to be interesting. If the compound binds covalently to TrxR1 then only the hCA isoforms in close proximity can be blocked. Where is TrxR1 localized and how does it relate to hCA localisation?
8. Furthermore, different potencies might complicate this situation since the hCA IX and XII inhibitory potency of the compounds is in the low nanomolar, while the TrxR1 blocking activity is in the low micromolar range. Therefore, it is not clear why the 2.28, 2.46 and 2.47 compounds were used against carbonic anhydrase at a concentration of 100 µM, and the inhibitors of thioredoxyreductase (2.48-2.50) at 1 µM concentration.

Despite the above-mentioned remarks which do not spoil the key results and the general positive impression made by this research, the thesis should be evaluated positively. The thesis fulfils the requirements of the St. Petersburg State University and after a successful defence it would be a solid basis for the degree of Candidate of Chemical Sciences in specialization Medicinal chemistry.

Kind regards,

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