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R E P O R T

on the Ph.D. Thesis of candidate Sharonova Tatiana Valerievna, entitled "On the prospects of using human carbonic anhydrase inhibitors in anticancer therapy" submitted for the Scientific specialty 1.4.16. Medicinal chemistry

The Ph.D. Thesis by Sharonova Tatiana Valerievna contributes to the global efforts to find the best anticancer approach, particularly for hard-to-treat solid tumors of epithelial origin – resistant carcinomas. Ph.D. candidate focused her work on the development of human carbonic anhydrase inhibitors (hCA) against the isoforms IX and XII which are highly relevant targets in cancer. This is a high topic in cancer research because inhibiting hCA IX and XII can suppress tumor growth and cancer metastasis. Even though a huge number of hCA inhibitors have been presented in the literature over the past decades as promising anticancer agents, only SLC-0111 has reached clinical trials as monotherapy. Later, it was recognized as an anti-metastatic agent valuable in combination with the clinically approved drug - gemcitabine.

Ph.D. candidate synthesized a series of benzenesulfonamides with diverse molecular periphery using the diazo chemistry approach. She evaluated their inhibitory properties against 4 isoforms of hCA and tested their anticancer potential. She observed the apparent discrepancy between the ability of some highly active hCA inhibitors to block the catalytic function of the enzyme and their effect on cancer cells. Therefore, she conducted a comprehensive critical analysis of the literature data on hCA inhibitors to find the correlation between their inhibitory effect on hCA IX and XII and anticancer efficacy in hypoxic conditions. Further, she synthesized a hybrid compound containing two pharmacophore elements responsible for inhibiting hCA and thioredoxin reductase and studied its anticancer effect. Previously, it was found that the hCA gene family is synthetically lethal with a group of genes encoding enzymes responsible for resistance to oxidative stress, particularly those with selenocysteine reactive oxygen species neutralization group such as thioredoxin and thioredoxin reductase.

Besides investigating novel hCA inhibitors as monotherapeutic agents, she selected two hCA inhibitors for combination studies with the EGFR inhibitor – gefitinib. The rationale for this lies in the fact that hCA inhibitors are effective adjuvants for other anticancer agents in combination therapy and that the anticancer activity of a hybrid molecule based on gefitinib and sulfonamide pharmacophore responsible for hCA inhibition was previously reported. Although the research was conducted using only cancer cell lines of different origins (breast, lung, and pancreatic carcinoma), it represents a solid foundation for further in-depth preclinical studies of herein presented hCA inhibitors.

Therefore, the Ph.D. candidate successfully defended all postulated provisions: (i) synthesis of primary benzenesulfonamides with diverse molecular periphery using diazo chemistry approach; (ii) inhibitory profile of the obtained primary benzenesulfonamides concerning various hCA isoforms; (iii)

potential of the obtained primary benzenesulfonamides as individual anticancer agents; (iv) results of critical analysis of data on the biological activity of previously described inhibitors of human carbonic anhydrase IX/XII performed to establish a possible correlation between the value of their inhibitory action and antiproliferative properties on cancer cells; (v) antiproliferative effect of hCA inhibitors obtained in the present study in combination with antitumor drug - gefitinib; (vi) synthesis and investigation of antiproliferative action of a hybrid compound containing pharmacophore elements are responsible for inhibition of hCA and thioredoxin reductase.

The Ph.D. candidate showed considerable knowledge of the topic literature. This was evident from the Literature overview and the design of the conducted studies that brought utterly new light on accumulated findings in the hCA inhibitors investigation. She emphasized some of the most relevant results in the up-to-date literature: (i) the ability of SLC-0111 to enhance the efficacy of temozolomide against glioblastoma; (ii) synergistic effects of the combination of SLC-0111 and gemcitabine; (iii) synergistic effects of the combination of SLC-0111 and sunitinib; (iv) development of hybrid molecules containing dihydroartemisinin and various types of hCA IX/XII inhibitors in their structure; (v) development of a hybrid molecule based on gefitinib and sulfonamide pharmacophore; (vi) development of P-pg and hCA IX/XII inhibitor hybrid compounds.

Among the most valuable findings of this Thesis according to my expertise as a biologist and cancer researcher, I would like to mention:

- Hybrid molecule 2.58 containing a sulfonamide fragment and a Michael acceptor fragment induced concentration-dependent cytotoxicity in pancreatic adenocarcinoma cells PANC-1 with IC50 value 1.8 ± 0.4 μM.
- The combinations of hCA inhibitors 2.16d and 2.19 (25 μM and 50 μM) with gefitinib (10 μM) suppressed the growth of lung adenocarcinoma cells A549 and affected the migratory potential of A549 cells in hypoxic conditions. Importantly, these effects were more pronounced in combined treatment when compared to single treatments.

This scientifically sound Ph.D. Thesis provides important findings in the field of medicinal chemistry by creating new ideas for the reliable development of efficient therapeutic approaches. The Thesis structure, presented publications, and profound multidisciplinary knowledge are reliable indicators of the Ph.D. candidate's serious and comprehensive approach to an ambitious endeavor.

In my opinion, this Ph.D. Thesis is in accordance with the high-quality standards, and hence, the Ph.D. candidate Sharonova Tatiana Valerievna deserves to be granted a Degree of Candidate of Sciences - Scientific specialty 1.4.16. Medicinal chemistry.

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