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Reviewer's Report on the Habilitation Thesis

Design, Synthesis and Biological Evaluation of Novel Hsp90 Inhibitors with Reduced Toxicity

by dr. Russell Richard Anthony Kitson

The habilitation thesis under review was submitted to Faculty of Pharmacy in Hradec Králové, Charles University. The thesis systematically examines the relationship between the structure and activity of low molecular weight inhibitors targeting heat shock protein 90 (HSP90). HSP90 plays a crucial role in maintaining protein homeostasis. As a molecular chaperone, it assists in the folding, stabilization and activation of various proteins, including oncoproteins critical for cancer progression. Consequently, HSP90 has emerged as an attractive therapeutic target, with inhibitors being developed for various indications, notably cancer treatment. However, the story is not simple and straightforward. Development of promising candidates, including 17-AAG, has been hampered by concerns about financial viability after patent expiry. In addition, clinical trials of HSP90 inhibitors have often yielded disappointing results, mainly due to significant drug toxicity. Nevertheless, the recent approval of the first HSP90 inhibitor, pimitespib, in Japan for the treatment of chemotherapy-refractory GIST is a significant milestone.

Concurrently, numerous research groups have focused on synthesizing analogs of geldanamycin and other HSP90 inhibitors to enhance their stability, solubility, potency, and reduce toxicity. Strategies to improve therapeutic efficacy and minimize toxicity include structural optimization, combination therapies and targeted delivery to tumor sites. Dr. Kitson has contributed significantly to this area, particularly in designing and synthesizing derivatives of geldanamycin and radicicol. The essence of the author's work involves rational designing novel derivatives, synthesizing and characterizing them chemically, and assessing their biological efficacy.

The first section of the thesis outlines the investigation of geldanamycin analogs. Compounds were designed to elucidate the structure-activity relationship (SAR) of the quinone moiety, hypothesized to contribute to compound toxicity via redox reactions or conjugation with biological nucleophiles. Specifically, analogs of 17-AAD substituted at position 19 were synthesized, characterized, and evaluated across various models, including recombinant HSP90 binding assays (ITC and cocrystal structures), cancer cell lines (effects on client proteins like RAF, AKT or CDK4), a neuroblastoma cell line model for Parkinson's disease (observed induction of other HSPs like HSP70 and HSP27) and a cellular model of HIV gene expression and replication (loss of potency compared to parental geldanamycin or 17-AAD).

The subsequent results focus on modifications of another HSP90 inhibitor, radicicol, to assess metabolic stability and the impact of removing reactive moieties and altering macrocycle size on

biological activity. Although these compounds generally exhibited reduced affinity to HSP90, the rationale behind the modifications was sound, the experimental procedures meticulous, and the findings contributed to understanding their binding characteristics to the target chaperone.
Dr. Kitson's research, forming the core of this thesis, has resulted in seven original papers and one review, all published in prestigious journals and widely cited (288 citations as of February 2024), indicating significant interest from the scientific community. The average citation rate is 36 citations per publication, underlining the author's expertise in organic and medicinal chemistry and providing a solid basis for a habilitation in pharmaceutical chemistry.
Regarding the formal organization of the habilitation thesis, it primarily comprises the author's published works. The arrangement may seem unconventional, with an extensive review article directly placed in Chapter 1 followed by shorter commentary in Chapter 2 and the full-length articles in Chapter 3. This structure reflects the author's preference, but the content is clear and sufficient.
Despite the brevity of Chapter 2, there are some formal errors. In particular, there is a case of misnumbering, where Table 1 and Figure 5 on page 37 are incorrectly labelled; however, the correct numbers are likely to be Table 2 and Figure 6.

Questions:

1. HSP90 inhibitors are typically categorized into three classes: N-terminal domain inhibitors, C-terminal domain inhibitors and isoform-selective inhibitors. However, some consider this classification unsystematic. Which of these classes encompasses compounds that directly disrupt the interaction between HSP90 and its co-chaperones? Are there other clearly distinguishable classes?
2. What are the most significant common pharmacophores and toxicophores for at least some of these classes?
3. Given that most of the author's papers are relatively dated, I recommend summarizing recent advancements in clinical HSP90 inhibitor development during the defense, particularly focusing on pharmacological parameters influencing efficacy and adverse effects.

Conclusion
The originality of the text of the habilitation thesis was confirmed using the online TURNITIN system, which yielded results supporting its authenticity. In my opinion, Dr. Kitson's thesis submitted to Charles University meets the requirements for a habilitation thesis in pharmaceutical chemistry.

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