Memorial Sloan Kettering
Cancer Center

February 14, 2024

Re: Review of Dr. Russel Kitson Habilitation Thesis

To whom it may concern:

Dr. Kitson's research, submitted as part of his Habilitation thesis, comprises a series of insightful studies in the field of synthetic and organic chemistry. These contributions significantly advance our understanding of HSP90 inhibitors and their potential implications for therapeutic development.

Dr. Kitson has submitted for evaluation as part of his Habilitation thesis work performed during his postdoctoral studies at the University of Nottingham, UK (2010-2014, supported financially by the Parkinson's Disease Society UK) and during subsequent research collaborations while holding predominantly teaching-focused academic positions (2014-2015, University of Nottingham, and 2015-2022, University of Warwick). The outcome includes eight publications, with the candidate as the first or corresponding author in five of them. These publications, published in high-impact journals in the fields of chemistry and biomedical sciences, underscore the significance of the studies and highlight the candidate's prowess in conducting high-quality research.

Dr. Kitson's primary expertise lies in organic and synthetic chemistry, evident through his authorship in journals such as The Journal of Organic Chemistry, Nature Chemistry, Chemical Communications, Organic and Biomolecular Chemistry, and Tetrahedron. His publications, where he serves as the first and/or corresponding author, share a common theme of devising elegant synthetic routes for assembling complex molecules with potential biological activity.

To establish the scientific foundation for his work, Dr. Kitson has substituted a traditional Introduction chapter with a co-authored review article published in The Journal of Organic Chemistry in 2013. This review article offers an overview of the molecular chaperone Heat Shock Protein 90 (HSP90) and its inhibitors, with a specific emphasis on two classes of inhibitors—those derived from two natural products, geldanamycin and radicicol. The article serves as a valuable resource to contextualize the research undertaken by Dr. Kitson.

The structure of the Habilitation thesis accentuates Dr. Kitson's contributions, with a primary focus on the synthetic chemistry and characterization of compound identity, purity, and specific physicochemical and structural attributes. Subsequently, it underscores the utilization and applications of these chemical tools to enhance our understanding of biology and pharmacology.

A significant focus of the research, and the most productive, revolves around C19-analogues of geldanamycin—a benzoquinone ansamycin known for its inhibitory activity against the molecular chaperone HSP90. Geldanamycin, a natural product, gained attention for its potent anti-cancer activity but also presented unwanted toxicities, some target-related and others stemming from its chemical structure. In particular, Dr. Kitson's research targets the C19 site on the benzoquinone ring, susceptible to nucleophilic attack by biomolecules, such as thiols, potentially causing some of the toxicities associated with geldanamycin. His

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innovative approach involves blocking this site, aiming to mitigate target-unrelated toxicities while preserving the biological activity against HSP90.

During this work, Dr. Kitson addressed significant challenges in the synthesis of geldanamycin derivatives by devising several coupling methods, including an optimized Stille coupling method (Nature Chemistry 2013), and a modified Suzuki–Miyaura protocol, and other optimized procedures (Chem. Commun. 2013 and Tetrahedron 2021). Unlike prior methods, these approaches were compatible with delivering a spectrum of derivatives needed for structure-activity relationship studies. The optimized coupling methods also enabled the synthesis of 19-substituted geldanamycin derivatives in the quantity and purity required for high-standard biological investigations.

To enable the characterization of these 19-substituted geldanamycin derivatives, Dr. Kitson has demonstrated effectiveness as a scientist through his adept ability to establish collaborations with researchers possessing complementary expertise. These collaborations were instrumental in conducting essential structural and biological studies with the synthesized C19-series of compounds, leading to publications in Molecular Pharmacology (2014, 2015) in collaboration with David Ross and his team at the University of Colorado Anschutz Medical School, and in Proceedings of the National Academy of Sciences USA in collaboration with Ariberto Fassati and his team at the Wohl Virion Centre and Medical Research Council Centre for Medical Molecular Virology, Division of Infection and Immunity, University College London.

A particularly rewarding outcome of these collaborations is the potential biological applications of the C19-series, as evidenced by their retained cellular activity in cancer cell models while exhibiting reduced toxicity on normal cells) (Nature Chemistry 2013; Molecular Pharmacology 2014). A study in collaboration with the Ross lab was published in Molecular Pharmacology, 2015 to support that one of the C19-series compounds, namely 19-phenyl-geldanamycin could ameliorate mutant a-Synuclein oligomer formation and toxicity in SH-SY5Y cells in culture, presumably through its ability to induce heat shock protein expression and activate autophagy. Intriguingly, the C19-series did not retain the ability of other HSP90 inhibitors to prevent HIV-1 reactivation in CD4+ T cells (PNAS 2014), echoing the recently acknowledged complexity of the HSP90 target and the poorly understood relationship between target modulation and biological activity.

Another notable outcome of the collaborations outlined above is the utilization of these agents to probe mechanistic questions concerning the hepatotoxicity observed with the parent geldanamycin-type compounds. Specifically, certain compounds from the C19-series demonstrated resistance to nucleophilic attack by glutathione, resulting in significantly reduced toxicity in freshly isolated hepatocytes. This finding supports the notion that arylation of glutathione by the benzoquinone moiety of the parent ansamycins is a major mechanism contributing to their observed hepatotoxicity (Molecular Pharmacology, 2014).

An intriguing aspect of Dr. Kitson’s studies lies in an observed change in the conformation of the ansa ring, a structural feature of the ansamycin molecules, upon the addition of substituents at the 19-position. This enforced conformational switch of the trans-amide group of geldanamycin into the cis-form, essential for HSP90 binding, may support an optimized conformational stabilization of the ensuing molecules, facilitating interaction with HSP90. This is in contrast to the parent geldanamycin, which adopts a trans conformation in solution, reverting to cis when geldanamycin becomes HSP90 bound. This is an observation that merits future exploration in the context of target binding and modulation.

The cumulative results strongly indicate the potential of blocking the C19 site to alleviate some of the off-target toxicities associated with the parent compounds, while also potentially serving as a pharmacophore for the design of therapeutically meaningful agents, as proposed by Dr. Kitson. Given the time lapse between these studies and the current proposal, gaining insights into the current status and any subsequent investigations—both chemical and biological—stemming from Dr. Kitson's pioneering work in this area would have been valuable. I concur with Dr. Kitson that the C19-series presents an intriguing pharmacophore with more to be discovered, both mechanistically and therapeutically. However, should research continue in this domain by Dr.

Kitson, it is advisable that the high-quality chemistry efforts he has demonstrated align with the evolving developments and complexities of the HSP90 target, as elucidated by recent studies. In this context, I am curious to know whether Dr. Kitson has envisioned or initiated follow-up studies to explore the potential conformational stabilization achieved in the C19-series. This could provide valuable insights, particularly regarding its impact on HSP90 isoform binding and interaction with pathologic forms of HSP90.

In conclusion, Dr. Kitson's contributions, as evidenced in this thesis, expand our understanding of HSP90 inhibitors and offer promise for influencing the development of novel therapeutic agents. His adept synthesis of complex molecules opens doors to intriguing possibilities in drug discovery and targeted therapies. Dr. Kitson's meticulous work offers valuable insights into HSP90 inhibitors and their potential therapeutic applications. As the scientific community continues to explore the intricacies of HSP90 modulation, Dr. Kitson's research provides a thoughtful guide, suggesting promising avenues for future investigations.

Should you have any questions or require further information, please feel free to contact me.

Sincerely,

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