I. Scholarly Merit

The candidate's thesis entitled "***Application of computer-aided drug discovery methods for targeting the oncogenic activity of MYC in prostate cancer***" is reviewed below. The doctoral work is dedicated to the development of small molecule inhibitors in the context of identifying new drug therapies for combatting prostate cancer (PCa). As therapeutic options for advanced stages of PCa are limited and mostly focused towards drugs targeting the androgen receptor, new innovative PCa therapeutics with novel mechanisms of action are highly needed. That is where the work of the candidate takes place.

As Myc oncoproteins were identified a key driver for severe PCa, the candidate is interested in identifying clinically viable small-molecules targeting Myc, despite the numerous challenges associated to that target. **Overall, this excellent thesis describes the application of state-of-the-art computer-assisted drug design (CADD) technologies towards the discovery of novel inhibitors targeting the transforming activity of Myc in PCa.** Several potent compounds of high interest were identified virtually and later confirmed experimentally. This research showcases what CADD methods and software tools, when used according to the best protocols and guidelines, are capable of achieving, even for the most challenging biological targets.

Notably, the reviewer appreciated:

- the candidate underscoring the fact that PCa is caused by a spectrum of genetic and molecular abnormalities, the different scoring systems for PCa, and the very detailed background/context of the current molecular discovery regarding PCa (especially with AR and obviously MYC – the focus of this thesis);

- the well-written description of the MYC gene/proteins and signaling pathway in regard to PCa;

- the excellent review of all reported MYC binders/disruptors/stabilizers, including some candidates entering clinical trials;

- the use of state-of-the-art CADD methods and associated software, all integrated in a logical modeling and screening protocol;

- the successful discovery of molecular hits such as VPC-70551, which represents a potent and stable c-Myc-Max inhibitor, with the potential to lead to the first Myc-Max drug candidate for treatment of CRPC.

- the overall success of the thesis demonstrating the paradigm change of the fact that Myc was considered "undruggable". The candidate clearly demonstrates they could be one (or several) molecular way(s) to succeed.

A successful dissertation:

i. presents a contribution to knowledge, **[Yes this is the case here]**

ii. is likely to have an impact on the discipline and/or in an applied domain, **[Yes this is the case here]**

iii. describes a coherent body of work whose depth and scope justify the granting of a doctoral degree. **[Yes this is the case here – the candidate used the best CADD methods and software available today]**

While meeting these general criteria, a dissertation's content and presentation should comply with the more detailed expectations below:

iv. The research undertaken is contextualized clearly, and accurately references the larger field of knowledge on the topic; **[Yes this is the case here]**

v. The methods used are described in detail, relevant to the research question(s), and employed appropriately **[Yes this is the case here]**

vi. The research results are reported fully and clearly. **[Yes this is the case here – all tables and figures are publication ready]**

vii. The analyses and conclusions drawn from the research are well-justified and integrated into the larger field of knowledge. **[Yes this is the case here]**

viii. The implications and limitations of the research are fully discussed. **[Yes this is the case here]**

ix. The writing of the document is of a professional standard. **[Yes this is the case here – the document is very well written]**

Overall this is an excellent and impressive work.

II. Recommended revisions.

Here are specific recommendations for minor revisions:

- Update Figure 1.9 with annotations from the text (e.g., H1, H2, LZ)

- What is the PDB ID for the structures of Figure 1.10? Resolution? Curation needed?

- Add a table to recapitulate all PDB structures relevant to Myc drug discovery

- Add a table to recapitulate all compounds mentioned in Chapter 1 with info about their target, binding site, and experimental potency (in vitro and in vivo if available)

- Since the candidate is using the Schrodinger package, can she use SiteMap to characterize the three binding sites (pages 129-130) with a D score?

- Page 133 – add the 25 μM in the text next to the inhibition values

- Redo Figure 3.24 as it is hard to read

III. Overall Recommendation.

Please select one of the following statements:

1. I recommend that the candidate proceed to oral defense.

a) Only minor revisions are needed (see previous section).

b) Substantive revisions are needed, but they can be resolved in the context of an oral defense and subsequent final revisions.

2. I recommend that the candidate not proceed to oral defense.

a) Major revisions are needed before the examination can continue. [Normally, the examination is postponed to allow the candidate to revise the dissertation, which is then submitted to the original external examiner and to one new external examiner for review.]

b) The dissertation is unacceptable; it is fundamentally flawed and therefore beyond revision.

IV. Questions for Oral Defense

Here is a short list of questions to be answered by the candidate:

Q1- Page 112 – How good is the sequence alignment for C-Myc? [partially answered on page 175] Once the homology model was obtained, what type of post-processing was done to it? MD runs?

Q2- Pages 129-130 – Were these three sites previously known? Since the candidate is using a PDB-deposited crystal structure (1NKP), what else was known about those three sites in the literature? Among all the small molecule binders/inhibitors listed in Chapter1, is there one of them that is targeting one of those three sites? (this is [partially] answered on page 168)

Q3- Page 133 – Can the candidate comment on the choice of features to define the pharmacophore used for molecular filtering?

Q4- Page 136 – What is the docking score of VPC-70063? What is among the best scores obtained by the 69 experimentally-tested compounds? Did the candidate run MM/GBSA calculations for other confirmed actives? Confirmed inactives?

Q5- Page 159 – Could the candidate comment on potential other drugs containing the hydrazide linker?