Stapled Peptides Inhibitors: A new Window For Target Drug Discovery

Ameena M. Ali

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PhD thesis

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By

Ameena Mohamed Ali
Born on 7\textsuperscript{th} September 1985
In Manama, Kingdom of Bahrain
Supervisors:
Prof. Alexander Dömling
Dr. Sayed Goda

Co-Supervisors:
Dr. Matthew Groves

Assessment Committee:
Prof. Frank Dekker,
Prof. Gerrit Poelarends
Prof. Stefan Knapp
To my Mum, who inspired me by her dreams
  My Dad, who trust my competencies
  My gifted sisters & brothers
  My life joinery Friends
  Thanks for your patience, support and love

  And absolutely, Jack
  For his enormous contribution to getting me where I am today
  You are not only a best friend & family to me,
  but also an unofficial and additional supervisor

  I am gratefully dedicate this dissertation
  To you My Hidden Soldiers…
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General introduction and scope of the thesis
Protein-protein interactions (PPIs) have been linked and approved to play a main role in human disease progress making them very interesting targets for bioactive molecules such as, inhibitors to block these interactions and improve our prospects of developing therapeutic agents. As a result, tremendous progress has been expanded in the development of these therapeutic agents and their use as PPI inhibitors. Most of these inhibitors are belonged to two main drug classes: small molecules and biologics. Small molecule compounds have the ability to penetrate the cell membrane to reach their intracellular targets or “hydrophobic pockets” and proved their efficacy in binding and interrupting PPI. However, shallow and large protein surfaces are not accessible with small molecules, which fostered the emergence of another therapeutic class - so-called biologics. This class comprises bioactive proteins that are typically >5000 Da in size and validated as potent and selective binders to their target protein surface, but their use is generally limited to extracellular hits and they have poor oral availability. As a consequence, a number of challenging intracellular targets are not approachable by small molecules or biologics and have been therefore termed “undruggable”. Recently, a new class of therapeutic agents engaged in targeting and inhibiting intracellular PPI that are beyond the reach of small molecules and biologics has emerged, ie. peptides. Peptides retain excellent surface recognition to the target surface and have minimal toxicity. However, the major challenge about the peptides that they suffer from proteolytic instability and low cell permeability. All-hydrocarbon stapling of the helical peptides provides an opportunity to stabilize the bioactive confirmation of the peptides, protect from proteolysis and enhance their drug-like properties and target affinity.

In this thesis, we investigate the feasibility of stapling to reinforce the secondary structure of the folded peptides and enhance their binding to the target surface. This was accomplished by applying this technique to our oncogenic PPI target, p53-MDM2, as one of the strategies to treat cancer. We designed p53-based hydrocarbon stapled peptides and introduced the multicomponent reaction (MCR) technique as a potent method in staple synthesis that were linked to α-helix peptides at $i,i+4$ stapling position. Furthermore, we used the microscale thermophoresis (MST) technique to investigate the binding affinities of the later stapled peptides toward our target protein, being the first to apply this new technology on MDM2 and stapled peptides. Furthermore, we were able to solve three co-crystal structures of three stapled peptides in complex with a mutant MDM2 to reveal their binding mode to the hydrophobic cleft of MDM2, as well as, the role of the staple in these interactions. Our structures are considered to be novel.

It was a challenging approach to express our target insoluble proteins, the WT- and mutant MDM2, within the inclusion bodies (IBs) of the E.coli cells, moreover, preserving the topology of these surfaces. For that we developed a system for the refolding buffers screening in order to determine the correct refolding conditions in reliable and time saving manner using the differential scanning fluorimetry (DSF). By applying this systematic buffer screen on MDM2 proteins, we were successfully able to get stable and properly refolded proteins in correct buffer system, pH and essential additives, which allowed the production of MDM2 proteins for structural and biophysical studies.
As inhibiting PPIs has become an attractive goal for drug discovery, in this thesis we investigate and solve the structure of Pex4p:Pex22p\textsuperscript{S} complex as a novel PPI from the yeast *Hansenula polymorpha* to elucidate the complex role in peroxisomal recycling. Since peroxisomes are major cellular compartment of eukaryotic cells and are involved in a variety of metabolic functions and pathways. Thus, mutation in one of the main recycling peroxins proteins are linked to human peroxisome biogenesis disorder including Pex1, Pex6, Pex10, and Pex4:Pex22 complex.

Therefore, this thesis reveled the discovery of new drug targets PPI, which is the case of Pex4-Pex22\textsuperscript{S} and the development of new strategies to design new PPI inhibitors toward p53-MDM2 interaction, called hydrocarbon stapled peptides. Theses peptides showed an enhancement in their binding affinities to MDM2 and the novel high-resolution structures expand our understanding of their binding mode within MDM2 hydrophobic cleft. Moreover, the contribution of the staple in this interactions and the topological conformational changes that occurred on the target protein interface while in a binding mode. All together approve the efficacy of stapling and stapled peptides in PPI disruption with high potency and selectivity, making this class as new window of discovery for therapeutic peptides targeting different human diseases. This could be possible if the permeability and the oral availability of the staple peptides is overcome. Efforts by the current research are underway to improve these limitations and enhance the pharmacokinetics of the therapeutics peptides to become more potent drugs.