CHAPTER 1

INTRODUCTION

1.1 Problem Background

Cancer is currently the second leading cause of death in the United States behind cardiovascular disease (American Cancer Society, 2012). As we can see from **Figure 1.1**, it is estimated that more than 1.6 million new cases of cancer were diagnosed in 2012, and the number of new cancer cases is expected to increase from about 1.5 million per year in 2010 to 1.9 million per year in 2020 (American Cancer Society, 2012). In Indonesia, according to KEMENKES (2013), the prevalence of cancer according to diagnosis by medical doctors is 1.4-1.5% by average (**Figure 1.2**), with cervical and breast cancer as the two highest prevalence (Kemenkes-RI, 2013). It is also reported that in 2012, there are about 8.2 million mortality cases caused by cancer (Kemenkes-RI, 2015). Because of the impact of cancer on the society, efforts to prevent or to treat cancer are an ongoing research interest (Bennett, Rojas, & Seefeldt, 2012).

Nicotinamide adenine dinucleotide (NAD) is a critical cofactor and substrate in cellular redox reactions such as mitochondrial oxidative phosphorylation, β oxidation, glycolysis, and citric acid cycle (Tan et al., 2013). The salvage of NAD is predominantly achieved by nicotinamide phosphoribosyltransferase (NAMPT), which catalyzes the rate-limiting formation of nicotinamide mononucleotide (NMN) from D-5-phosphoribosyl-1- pyrophosphate (PRPP) and nicotinamide (NAM) (S. Burgos, 2011).

The critical role of NAMPT in NAD^+ biosynthesis makes it an attractive target for the development of new anticancer agents, such as colorectal, ovarian, prostate, carcinomas, astrocytomas, melanoma, and lymphomas cancers. It is found that tumor cells have a high rate of NAD+ turnover owing to elevated adenosine diphosphate (ADP)-ribosylation activity, and NAMPT expression is upregulated in cancers (Kesherwani, Raghavan, Gunasekaran, & Velmurugan, 2018).

Inhibiting NAD biosynthesis by blocking the function of NAMPT is an attractive therapeutic strategy for targeting tumor metabolism (W. Wang et al., 2014).

Cellular metabolic adaptation is a fundamental requirement for malignant cell transformation, including NAD^+, which has been implicated as the substrate for important cancer-related enzymes such as ADP-ribose transferases, including poly-ADP-ribose polymerases (PARPs), sirtuins, and cyclic ADP (cADP) ribose synthases. NAMPT has several well-established links to cancer, including its differential expression in tumor tissue and its correlation with cancer therapy resistance and poor outcome in cancer patients. It is known that colorectal, ovarian, prostate, carcinomas, astrocytomas, and melanoma cancer tumors overexpress NAMPT relative to their benign counterparts, whereas higher levels of NAMPT expression correlate with more aggressive lymphomas and high-grade astrocytomas, with poor outcome in gastric and endometrial carcinomas (Roulston & Shore, 2016).

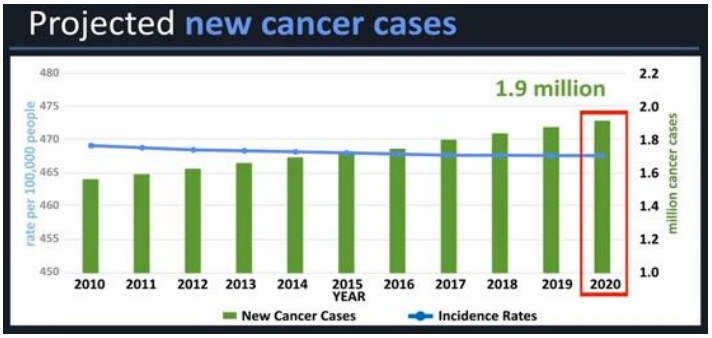

Figure 1. 1 Projected new cancer case according to Center for Disease Control and Prevention (2015)

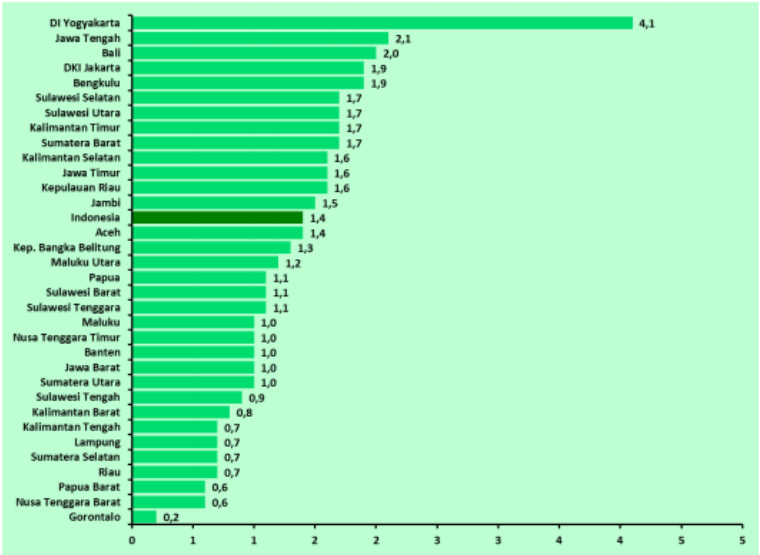

Figure 1. 2 Cancer Prevalence (%) based on medical doctor's diagnosis according to Indonesian province 2013. Source: RISKESDAS 2013

The cost of drug development and the low probability of technical success prove the critical need for improved efficiency of drug discovery and further investment in innovative technologies and processes that enhance the chances of bringing a compound to market as a drug (Claus & Underwood, 2002). *In silico* research was done with virtual screening and molecular docking to get the analysis of Quantitative Structure-Activity Relationship (QSAR). The output of the *in silico* study can be used as the bases for filtering or screening the available options and shrunk the possibility, so that it would be easier for selection in empirical research.

Research that utilizes computational approach has become an exciting method because of the rapidly growing availability of structural information across protein families, the accessibility to increased computational power at affordable cost compared to laboratory experiments, and there is a bit of improvement of the understanding on how to effectively apply virtual screening and molecular simulation technologies in research. The speed at which a virtual screen can be completed makes it effective at starting a project for which there are few or no compelling leads (Alvarez, 2004). One of the computational approach that is available to prove the presence of interactions between two structure molecules is Molecular Docking (Aldawsari et al., 2015). The ability to virtually screen compound libraries to improve enrichment of ligands progressed to experimental validation has provided countless lead compounds. The more availability of prior knowledge and published data, the higher the chance of success in the project, where proper critical observation or examination of available literature is essential. (Berry, et.al, 2015).

Nowadays, there are a lot of studies that resulted in finding the new potential inhibitor. A lot of NAMPT inhibitors have been found that are coming from different sources. However, there is still a lack of research that compares the binding of each one of them and also validates their structure. This obstacle will then limit the development of those molecules to be processed further. A field called **structural bioinformatics** provides a means for the downstream analysis of variation including homology modeling and molecular docking (Brown & Tastan Bishop, 2017).

1.2 Problem Formulation

The discovery of cancer drug has been a popular topic since a long time ago. Different methods have been applied to find the most effective drug. The increase of drug candidates makes the R&D department spends a lot more investment at the beginning. In other words, finding a method to narrow down the known NAMPT inhibitors with minimum cost is very important.

Molecular Simulation is a method that includes the use of computer to analyze molecules and predict certain conditions. Several types of simulation can be done, such as Molecular Docking and Structural Validation tools. Molecular Docking reveals the binding energy of ligand and the receptor. The best complex between ligand and receptor will have the lowest binding affinity, means that it requires less strength and more favorable to be bound. Structural Validation is used to check for the validity of the structure or the compound that is used in the experiment. Validation can also be done by comparing the result to the publicly available data such as from Protein Data Bank. As PDB has its own validation platform called World Wide Protein Data Bank Validation Service (wwPDB Validation), it is still found that close to 20% of the ligand structures from Protein Data Bank were reported to have some quality problems. Thus, it is essential to use other *in silico* methods to validate a structure before using it for a more complicated experiment such as Molecular Docking. Hence, a future study of the validated structure will be conducted in vitro for the highest possible complex and proven to be the drug candidate.

1.3 Research Objectives

- To identify the roles of *in silico* approach, which are molecular docking and structural validation method as a part of drug discovery development process.
- Utilizing *in silico* approach to compare among the known NAMPT inhibitor to find the best compound for anti-cancer drug.

1.4 Research Scope

- Literature Review and Data Gathering of the previous computational approaches involved in the finding of NAMPT inhibitors. The structure will be retrieved in the form of PDB file

downloaded from RSCB Protein Databank (http://www.pdb.org) and ligand database derived from PubChem (https://pubchem.ncbi.nlm.nih.gov/) or Protein Data Bank. This method will be resulted in both NAMPT molecule and a list of NAMPT inhibitors that have been known.

- Molecular Simulation: Two molecular simulation methods called Structural Validation Molecular Docking simulation will be done. Molecular Docking will use AutoDock Vina software and Structural Validation will use web tools, which are wwPDB Validation Service and WHAT_CHECK. Discovery Studio software will be used to support any additional method and to remove unnecessary compounds and visualization, and also Open Babel for file conversion.
- Analysis: The analysis of binding of each one of the inhibitors (ligand) and the NAMPT molecule will be done. The comparison between each of the interaction, then they will be analyzed, and structural validation will help to validate the control used for comparison with the new ligand and therefore generate the best inhibitor.