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In Silico Study of Natural Pyridoacridine Against CDK-2 and CDK-6 Cancer

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ABSTRACT

Cancer is a major public health problem and leading cause of deaths worldwide. The number of cancer cases in the world per year is predicted to reach up to 23.6 million by 2030. Cancer is the over-proliferation of the cells that can be controlled by the cell cycle. The cyclin-dependent kinases (Cdks) are protein that is activated during cell cycle transitions. Cdk-2 is responsible as a target of a checkpoint in the S phase cell cycle. Cdk-6 as a transcriptional regulator to support homeostatic in the cell cycle. Cdk-2 and Cdk-6 are now used as the candidate for cancer treatment. Thus, this study is aimed to determine the potential of Pyridoacridine containing natural anticancer pigments (PCNPs) using the molecular docking approach. In this work, molecular docking using Glide is performed to know glide Gscore and Glide energy. Thus, this study explains the pathway Cdk-2 and Cdk-6 as a therapeutic target in apoptosis. The result of the study was meridine compound of PCNPs has the best score in Cdk-2 and Cdk-6. So, PCNPs may inhibit Cdk-2 and Cdk-6 in cancer. PCNPs is one of the anticancer potential candidates to develop. The amino acid residues like leucine and valine can be an important amino acid that can bind and interact with protein kinase cdk-2 and cdk-6. The mechanism action of PCNPs as an inhibitor protein kinase in cancer.

Key words *: CDK-2. Cdk-6, Docking, Pyridoacridine*

Introduction

Cancer is the deadliest disease and a leading cause of mortalities worldwide. Liver cancer is one of the fastest-growing types of cancer in the world. Uncontrolled cellular proliferation in the liver can be caused by damage cell cycle regulatory checkpoint namely cyclin-dependent protein kinase 2 (cdk-2) and cdk 6 (Brumby and Helena, 2005). Cdk-2 can control the G1/S phase while cdk-6 can control the G1 phase and the retinoblastoma protein (Rb) transcription. Both of these proteins can express and activated in cell lines and hepatocellular carcinoma (HCC). Thus, the important role of cdk-2 and cdk-6 is to regulate cell proliferation. Cdk-2 can be activated by cyclin A and cyclin E. At the late G1 phase, Cdk-2 and cyclin E form a complex that triggers a G1–S phase transition through phosphorylation of Rb (Asghar *et al.* 2015). The kinase activity is higher in HCC than non-tumor liver tissue in 84% of the cases for cdk-2 (Li *et al.*, 2002). Cdk-2 and cdk-6 inhibitors are being developed as new therapeutic strategies in cancer treatment.

Natural products play an important role as inhibitor cancer candidates and therapeutic agents to decrease malignancy level and tumor progression. More than 60% of anticancer agents are derived from a natural compound potential candidate. Natural compounds can be obtained from several plants, microorganisms, and marine organisms

(Natalia *et al.,* 2017). The discovery of natural compounds is crucial to control the tumor cell proliferation, and as anticancer therapy (Amin *et al.,* 2015). Thus, we must understand the molecular mechanism of the cell cycle regulation and interaction between protein target and drug candidate from natural products (Rao *et al.,* 2012; Johansson and Persson, 2008).

Pyroacridine was part of some pigments such as varamine, violacein, amphimedine, fascaplysin, monascin, chinikomycin that have the anticancer potential (Molinski, 1993; Srinivasan and Devi, 2011; Ali, 2012; Shi and Pan, 2011). In this study, we determine Pyridoacridine containing natural anticancer pigments (PCNPs). The selected PCNPs were amphimedine, deoxyamphimedine, neoamphimedine from Xestospongia and Amphimedon species, meridine (Corticium niger sponge or another species: *Amphicarpa meridiana*) and varamine A from *Lissoclinum vareau* (Marshall *et al.,* 2009; Schmitz, 1983; Molinski and C. M. Ireland, 1989). This natural product yet discovered the mechanism of action to inhibit cell progression through cdk-2 and cdk-6. This review can explain the importance of PCNPs as an inhibitor cancer progression.

To discover the potential of bioactive compound from a marine organism, pyroacridine have aromatic and alkaloid compounds reported can inhibit cancer cell (Longley *et al.,* 1993; Vikas Sharma *et al.,* 2016). Based on some publication before, pyridoacridine act as DNA intercalating agents, and nucleic acid intercalation. Most pyridoacridines have been reported to have significant cytotoxicity because a highly planar electron-deficient aromatic ring system can intercalate DNA and in cell growth (Marshall and Barrows, 2004). Pyridoacridines can act as antibacterial, antifungal, antiviral, and antiparasitic activities (De Guzman, 1999; Feng *et al.,* 2010, Fuente, 2001; Schmitz, 1991; McCarthy, 1992).

Meridine compounds from Amphicarpa meridiana have antifungal and antitumor activity Meridine can inhibit cdk protein kinase activity, prevent proliferation, induce apoptosis, and interfere activity of cell division and death (Lyskov, S., and Gray, 2008; Elgazar, 2018). In addition, neoamphimedine from Xestospongia can stimulate to poisomerase II to catenate DNA to a high molecular weight complex (Franco *et al.,* 1998). The research before reported that bright crimson pigments, varamine A were isolated from the Fijian ascidian *Lissoclinum vareau* can exhibited cytotoxicity of leukemia cells (Molinski and Ireland, 1998; Kobayashi, *et al.* 1991). From all these results, it appears that pyridoacridine derivatives are good candidates for design drugs as an antitumor and anticancer activity.

The bioactive compound from natural products have potential as anti-inflammation, antibacterial and anticancer. Drug candidates are expected to be a better treatment for people with liver cancer. The development of research in the field of bioinformatics has the potential to facilitate the process of drug discovery. One of the methods used through in silico (Lyskov *et al.,* 2008). The in silico study can explain the interaction between phytochemistry and molecule targets for developing novel candidate drugs. The reports of virtual screening 60.000 secondary metabolites natural products may have inhibitor activity (Elgazar *et al.,* 2018).

This study determines the interaction between secondary metabolites from natural products PCNPs. In silico methods can help explain the linking between ligand and protein with minimum energy towards cdk-2 and cdk-6 as the target protein. The aim of this study to find out the potential of PCNPs as anticancer especially liver cancer and analysis the amino acid interaction between PCNPs and cdk-2 and cdk-6 . This study is important as one of the methods to design a novel drug that fit with liver cancer patient and to increase some information of natural products compound *et al.,* 2015; Patz *et al.,* 2014).

Materials and Methodology

The in silico test was carried out using cdk-2 and cdk-6. The 3D structure is obtained from the protein data bank (www.pdb.org) using the PDB code. The 3D structure of the drug candidate components was drawn using a software maestro. The interaction between ligands and amino acid residues on the active site was carried out using the Grid software. The energy affinity of the PCNPs and the target protein was carried out with a Grid score. The lowest energy affinity is chosen to determine the most potent drug candidate. Furthermore, the study of the cdk-2 and cdk-6 pathways that bind to PCNPs in liver cancer was carried out to determine the mechanism of action of the drug candidate. Finally, the protein structure was minimized to Root Mean Square Deviation (RMSD) value with a score <2. Thus, analysis of in-

teraction ligand and protein target was done to determine amino acid residues that interact between ligand and protein.

Results and Discussion

The results of the virtual screening of PCNPs against cdk-2 and cdk-6 were determined. The bond energies have been tested by RMSD and compared with crystallographic ligands. PCNPs as drug candidates can be done quickly and easily with the help of molecular docking. The virtual screening of drug candidates from natural ingredients helps to discover new molecules and the activity of the targeted compounds.

In this review, it was reported that the cdk-2 and cdk-6 are protein kinases that provide domains essential for enzymatic activity. Cdks play important roles in cell cycle regulators, modulate transcription, and tumorigenesis (Malumbres, 2014). This study can explain that the drug candidates in the form of PCNPs can act as cdk-2 and cdk-6 inhibitors which deregulation in cancer.

The importance of cdk as cancer : protein kinases themselves are commonly regulated by phosphorylation: either directly via phosphorylation of the kinase domain or indirectly via prephosphorylation of the substrate. Activation of cyclin-dependent kinase 2 (cdk-2) and mitogen-activated protein (MAP) kinases (MAPKs), for example, occurs via phosphorylation of residues within a flexible activation loop (Johnson *et al.,* 1996). Protein kinases are often very specific to the substrates they phosphorylate (Pinna and Ruzzene, 1996). So, cdk-2 and cdk-6 can be specific targets of cancer therapy.

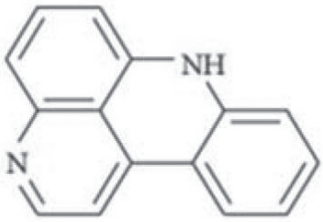

Fig. 1. Basic structure of pyrioacridines

Docking has been successfully performed between PCNPs with cdk-2 and cdk-6. The docking results show that meridine is the most potent compound among other pyroacridine analoges. This is evidenced by the lowest energy affinity value pro-

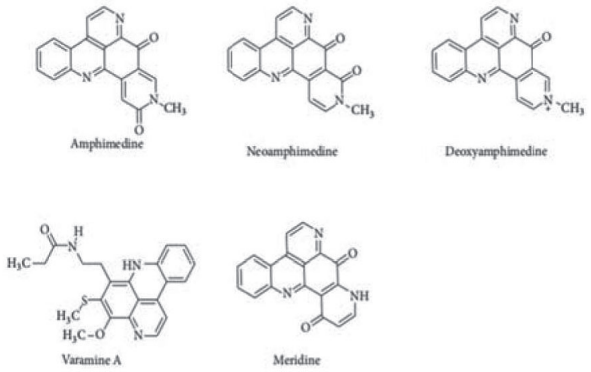

Fig. 2. The structure of pyroacridines analogue

duced on meridine with a score of -6.21 when it binds to cdk-2 and a score of -9.53 when it binds to cdk-6. At cdk-2 the meridine score was no better than the inhibitor, namely SU9516 with a score of -8.997, but at cdk-6, the meridine score was lower by -9.53 compared to the inhibitor, namely palbociclib. So, meridine was one of the pyroacridines analogues that potent as inhibitor cdk-2 and cdk-6 in cancer.

The interaction amino acid between meridine and cdk-2 was leucine. Leucine's roles in initiating mitochondrial metabolism through the stimulation of mitochondrial biogenesis, fatty acid oxidation, and p53 signaling. Previous studies have shown that leucine supplementation can be helpful in cancer therapy (Bruckbauer and Zemel, 2011; Bruckbauer *et al.* 2012). In an in vitro study in four melanoma cell lines, leucine deprivation was shown to increase caspase-3- mediated apoptosis (Sheen *et al.,* 2012).

Leucine and valine, are essential amino acids, and they have recently emerged as predictors for the future risk of cancers and diabetes (Hattori *et al.,* 2017; Stiles *et al.,* 2015). Overexpression of valine catabolism resulting in mitochondrial damage (Reuter *et al.,* 2014; Yunlong Shan, 2019). Meridine may inhibit the expression of valine metabolism in cancer. The

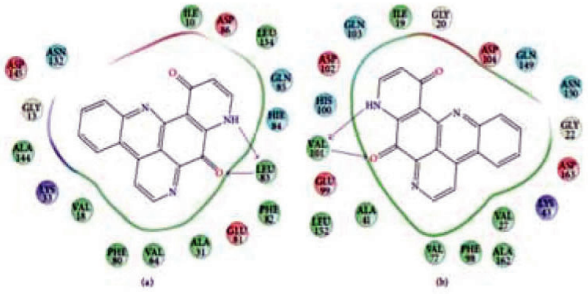

Fig. 3. Amino acid interaction between of PCNPs (meridine) against cdk-2 (a) and cdk-6 (b)

valine catabolic inhibitor along with other drugs can control the level progression of cancer (Taniguchi *et al.,* 1996).

Various pyridoacridines has the ability as anticancer activities via different mechanism: inhibition of DNA/RNA/protein synthesis, inhibition of topoisomerase, binding with DNA cleavage/catenation/damage of DNA and cell cycle arrest (Molinski TF 1993; Proksch *et al.* 1998; Concetta Imperatore, 2014). We can conclude that pyridoacridine includes of alkaloids that work as anticancer with mechanism protein kinase inhibitor (cdk-2 and cdk-6).

Pyridoacridines are a class marine-derived alkaloids that share an 11H-pyrido[4,3,2-mn]acridine skeleton. Pyridoacridine alkaloids are important as

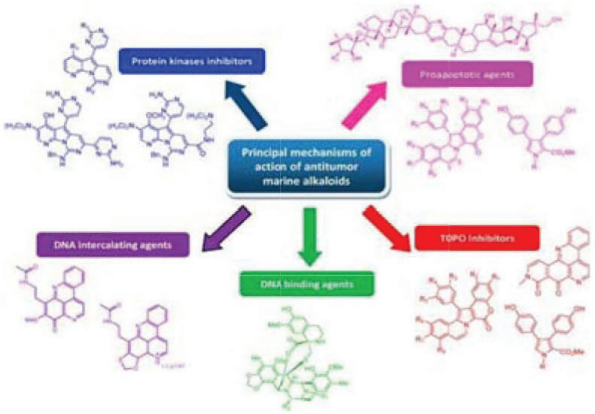

Fig. 4. Principal mechanism action of alkaloids

biological activities including cytotoxicity activity, production of reactive oxygen species (ROS), and topoisomerase inhibition. These activities depend on pyridoacridine skeleton (Marshall and Barrows, 2004).

Most pyridoacridines have been reported to have significant cytotoxicity due to a highly planar electron-deficient aromatic ring system that can intercalate DNA, resulting in the inhibition of cell growth (Marshall and Barrows, 2004). Amphimedine compounds show the anticancer effect to inhibit TOPO II, produce reactive oxygen species, cause the release of calcium from the sarcoplasmic reticulum, induce neuronal differentiation, and bind nucleotide receptors (Marshall and Barrows, 2004; Carroll, 2005). Amphimedine, neoamphimedine, and deoxyamphimedine have the same skeleton but different structures, so their activity may can be different. Neoamphimedine inhibits topoisomerase II, while amphimedine is relatively nontoxic at the same dose level and deoxyamphimedine damages DNA independent of topoisomerase enzymes through the generation of reactive oxygen species (Marshall *et al.,* 2009). Based on this study, meridine can interact with cdk-2 and cdk-6 as protein kinase and has low energy affinity. It can be assumed that the role of meridine as a protein kinase inhibitor.

Conclusion

In summary, the PCNPs especially meridine has the potential of anticancer drugs to develop with another therapy. The amino acid residues like leucine and valine can be an important amino acid that can bind and interact with protein kinase cdk-2 and cdk-6. The mechanism action of PCNPs as an inhibitor protein kinase in cancer and enhance apoptosis level. The benefit of this study is the docking result and analysis can be used to obtain the potential of compound alkaloids like PCNPs as new candidate drug potential as inhibitor cancer. This result can continue to study the effectivity of PCNPs compound with in vivo and in vitro research. The in silico technique can be used to study the potential of novel compounds as anticancer, antitumor, and anti inflammation. In addition, a multidisciplinary approach (organic chemistry, biochemistry, molecular biology, and molecular genetics) is needed to develop the potential of natural products as anticancer drug discovery.

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