UNIVERSITI TEKNOLOGI MARA

IN VITRO EVALUATION OF SMALL MOLECULES INHIBITORY ACTIVITY AGAINST APOBEC3B CYTOSINE DEAMINASE ENZYME

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Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science
(Molecular Biology)

Faculty of Applied Sciences

August 2022

ABSTRACT

Human APOBEC3B (A3B) is a member of the APOBEC3 (A3) family of cytidine deaminases, which function as DNA mutators and restrict viral pathogens and endogenous retrotransposons. APOBEC3B has both C-terminal catalytic (CTD) and N-terminal pseudo-catalytic (NTD) of zinc domains, only the CTD in APOBEC3B has been identified to have enzymatic activity. Even though APOBEC3B has important role in innate immunity, it was also identified as a major source of genetic heterogeneity in several human cancers. Therefore, therapeutic interventions using small molecules in modulating APOBEC3B may represent a strategy for cancer prevention and treatment. In this study, a pharmacoinformatics approach was adopted to narrow down potential chemical compounds against the APOBEC3B enzyme. Virtual screening of 2000 drug-like molecules from the National Cancer Institute (NCI) Development Therapeutics Program (DTP) drug library was performed using docking programs; GOLD and AutoDock/Vina against the X-ray crystallography structure of APOBEC3B (PDB ID: 5TD5). Among the docked compounds, nordracorubin, NSC641233, and raloxifene (RAL) showed the best binding energies towards APOBEC3B on both AutoDock/Vina and GOLD. Several significant interactions were observed between APOBEC3B and the three hits, including hydrogen bonding and pi-pi stacking. The three compounds and aurintricarboxylic acid (ATA); a positive control molecule, were further studied to validate their APOBEC3B inhibitory activities *in vitro* using a Fluorescence Resonance Energy Transfer (FRET) based assay. The three compounds with different concentrations (0.781 - 25 μM) were tested against purified recombinant APOBEC3B protein (80ng/ μL) incubated with ssDNA substrate containing the target cytosine. Among the test compounds, only nordracorubin and raloxifene showed inhibition of APOBEC3B in the FRET assay, while NSC641233 showed no effect against the APOBEC3B enzyme. Nordracorubin exhibited more APOBEC3B inhibitory potency (IC_{50}: 7.92 ± 0.68 μM) than raloxifene (IC_{50}: 14.95 ± 0.52 μM). Interestingly, there was no statistically significant difference between nordracorubin and ATA, indicating the efficacy of nordracorubin compared with the positive control. In addition, the cytotoxic activity of nordracorubin, raloxifene, ATA, and doxorubicin against MDA-MB-231 breast cancer cells was evaluated using the MTT assay. Nordracorubin showed a higher cytotoxic effect on MDA-MB-231 cell line (IC_{50}: 6.85 ± 0.58 μM) than ATA and raloxifene (IC_{50}: 7.92 ± 1.10 and 43.65 ± 2.25 μM), respectively. There were no significant differences between the IC_{50} values of nordracorubin and the standard cytotoxic drug. These results suggest that nordracorubin has an important inhibitory activity on APOBEC3B and could be further developed to enhance its efficacy against APOBEC3B in targeting early mutations in the development of precancerous lesions and ultimately prevent chemotherapy resistance.

ACKNOWLEDGEMENT

Firstly, I wish to thank Allah for giving me the opportunity to embark on my Master's and for completing this long and challenging journey successfully. My gratitude and thanks go to my supervisor Prof. Ts. Dr. Mohd Ilham Adenan, who accepted me as his student. His dedication, support, and constant supervision have been great motivation for me to complete my research. I am also very grateful for the financial help I received during my study and his patience in seeing me through this project. My greatest appreciation goes to my co-supervisor Dr Ng Kwok Wen, for his scientific discussion and his guidance throughout this process.

I would also like to thank all the staff and friends at the Institute of Science (IOS) and tissue culture Puncak Alam, especially Mr Yazid and Madam Mastura Ma'ad, for giving me a place and chance to use the equipments there. To my seniors, Nor Atiqah Binti Jusril, Zuriati Zahari, Nelofar, and all my friends; thank you for your guidance and support throughout this project. The kind and friendly atmosphere which they provided enable me to enjoy doing this research.

Finally, I am dedicating this to my parents; Mohamud Mohamed Egal and Aisha Abdul-kadir Hussein, who have been the supportive backbone for the duration of my study. Alhamdulillah.

This study was funded by Research Grant (100-IRMI/GOV 16/6/2) (013/2019)] from Ministry of Agriculture, Malaysia.

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