UNIVERSITI TEKNOLOGI MARA

INTERACTION OF BILE PIGMENTS AND 4 (METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE (NNK) WITH CYP2A6 AND CYP2A13

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Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science

Faculty of Pharmacy

September 2023

ABSTRACT

Bilirubin and biliverdin are bile pigments produced from the breakdown of senescent red blood cells. Other than mainly known to aid in the digestion of fats, the free radical scavenging property and the inverse relationship between serum bilirubin levels and cancer risk has been widely reported. The 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is a procarcinogen found in tobacco smoke. Metabolism of NNK mediated by CYP2A6 and CYP2A13 resulted in formation of free radical with DNA damaging property. Inhibitor of NNK metabolism has been of interest to decrease the production of free radicals that contribute to the development of tobacco products-induced lung cancer. The interactions of bile pigments with CYP2A6 and CYP2A13 enzymes still largely unknown. Fundamental knowledge on the interaction of bile pigments and NNK with CYP2A6 and CYP2A13 enzymes, is important as part of understanding the protective role of bile pigments in human during NNK-induced carcinogenesis. Therefore, in this study, the interaction of bile pigments with the active site and other possible binding sites in the enzyme structures of CYP2A6 and CYP2A13 was explored. The inhibitory effect of bilirubin and NNK on the coumarin 7-hydroxylation mediated by CYP2A6 was also investigated. Molecular docking using AutoDock (ADV) software was performed to computationally dock the bile pigments to the active sites of CYP2A6 and CYP2A13 enzymes. Next, the interactions between the top docked poses of ligands produced by ADV was visualized by PyMOL and PoseView software. Based on the binding energy, NNK exhibited lower binding energy to active sites of CYP2A6 compared to bile pigments, but bile pigments exhibited lower binding energy to CYP2A13 active sites than NNK. Nevertheless, the ligands were found interacted with key amino acid residues at the active sites of CYP2A6 and CYP2A13 crystal structures. DoGSiteScorer was used to predict allosteric sites of both enzymes. Three potential binding pockets have been identified for both enzymes. Results from enzyme inhibition assay showed the interaction of CYP2A6 with coumarin, bilirubin and NNK. Bilirubin inhibits the hydroxylation of coumarin to 7-hydroxycoumarin (umbelliferone) in a dose dependent manner, and the percentage of inhibition by bilirubin was directly proportional to the concentration of umbelliferone produced. However, the same pattern was not observed when NNK was interacted with CYP2A6. Regardless, the interaction of bile pigments with CYP2A6 and CYP2A13 observed in the *in silico* and enzyme inhibition have elucidated the interaction of bilirubin with CYP2A6.

ACKNOWLEDGEMENT

In the name of Allah, the Most Merciful, the Most Gracious. I am thankful to Allah, who gave me the courage, strength, guidance and love to complete this challenging journey.

Foremost, I would like to express my sincere gratitude to my supervisor Dr Salfarina Ramli for the continued support of my master study and research, for her patience, motivation, and immense knowledge.

I owe a deep sense of gratitude to Dr A'edah Abu Bakar for her helpful advice and the opportunity she gave me to further my study in this field.

To my supervisory committee, thank you to Dr Siti Azma Binti Jusoh @ Yusof, who always gave me her best to guide me in molecular docking and always encourage me to accomplish this task. My thanks also go to Dr Hasseri Halim for his inspiration and kindness.

I would like to show my greatest appreciation to my colleagues in the lab, Akmal Arif, Nurliana, Evana, Fatin Amelina, Jemain, Hisham and Nor Atiqah, who colour up my lab and keep me strive towards the end. My deepest thought goes to my besties, Nuraainaa Zawani, Nur Mahira Husna, Nur Fatihah and Fatin Fashihah, who have always been there for me in shine or rain.

Last but not least, special thanks go to my family. Words cannot express how grateful I am to my beloved mother, , father, , my siblings; Nurul Ain Syazana, Faris Asyraf, Nur Humaira, Ahmad Mifdhal Aiman, Irfan Darwisy, Nur Hana Kamila and my nephews; Aisy Fayyadh, Ayden Adham Firas, Muhammad Seth Anaqi and Arasy Fahim, for all of the sacrifices that you have made on my behalf. Your prayers have sustained me this far. Thank you for unconditional love and this master's degree is dedicated to all of you.

This acknowledgement also goes to the Ministry of Higher Education for the research grant of FRGS/1/2017/STG04/UITM/02/3 and Universiti Teknologi MARA for the facilities and supports, especially to the Head of Department of Postgraduate and Research, Faculty of Pharmacy UiTM; Assoc. Prof. Dr Meor Mohd Redzuan and the staffs. Finally, I would like to thank everybody who has taken part in the realization of this journey directly and indirectly. Thank you so much.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Bile pigments; bilirubin and biliverdin, are endogenous substances produced during heme degradation (Ryter, 2012). The normal range for total bilirubin is 0.1–1.2 mg/dL, while conjugated bilirubin levels is within the range of 0.1–0.3 mg/dL. Total bilirubin levels exceeding 1.2 mg/dL are considered high, while levels surpassing 2.5 mg/dL are classified as hyperbilirubinemia (Gazzin et al., 2016).

Within the normal range, biological property of bile pigments includes anti-mutagenicity, antioxidant properties, and ability to bind to certain important enzymes. It was also proposed that unconjugated bilirubin acts as an antioxidant by donating a hydrogen radical from a C10-bridge or an amide group to free radicals, forming stable carbon-centered radicals that then react with other free radicals to form new covalent bonds or free oxygen (Trieu Hong, 2016.).

Smoking, obesity, diabetes, ionizing and ultraviolet radiation, and air pollution have been associated with an increase in the production of reactive oxygen species (ROS) within the body. In response to this oxidative stress, the body depends on endogenous antioxidants and the intake of antioxidant-rich vegetables and fruits to effectively scavenge free radicals and maintain a balanced redox state. It is widely recognized that the dysregulation of ROS production can lead to detrimental effects on DNA, proteins, and lipids, ultimately initiating or promoting the progression of cancer (Inoguchi et al., 2021). Numerous studies have measured serum bilirubin levels following chemical exposure. For instance, elevated serum bilirubin levels observed in subjects after the consumption of herbal extracts can indicate the toxicity of those extracts (Hemalatha et al., 2019). Additionally, bilirubin levels have been monitored as an indicator of heme oxygenase activity or the status of endogenous antioxidants in cancer patients (Suh et al., 2018).

Cigarette smoke contains approximately 4000 compounds, among which at least 200 are toxicants, while 80 are classified as suspected carcinogens. The abundance of free radicals and oxidants in cigarette smoke engenders oxidative stress, a significant contributing factor (Masood et al., 2015). While smoking tobacco products provides