UNIVERSITI TEKNOLOGI MARA

SYNTHESIS, BIOACTIVITY EVALUATION AND COMPUTATIONAL STUDIES OF BISINDOLYLMETHANE AND FLAVONE DERIVATIVES

SYAHRUL IMRAN BIN ABU BAKAR

Thesis submitted in fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Applied Sciences

May 2016

AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

Name of Student:	:	Syahrul Imran bin Abu Bakar
Student I.D. No.	:	2012348831
Programme	:	Doctor of Philosophy (Science)
Faculty	:	Applied Sciences
Thesis Title	:	Synthesis, bioactivity evaluation and computational studies of bisindolylmethane and flavone derivatives

Signature of Student	:	
Date	:	May 2016

ABSTRACT

Bisindolylmethane and flavone are well-known natural product scaffolds having important pharmacophores and they have gained tremendous interest owing to their remarkable potency and activity profile towards various target diseases. In this study novel bisindolylmethanes and flavones were synthesized to identify potential inhibitors for bacterial infection, cancer, and diabetes. One hundred twenty-nine (129) bisindolylmethane derivatives (Schiff base, thiourea, sulfonamide, and hydrazone) and 43 flavone derivatives (hydrazone and ether) were synthesized, evaluated for various *in vitro* bioactivities, and analyzed through computational studies to identify possible inhibition mechanisms. Antibacterial activity of bisindolylmethane Schiff bases showed that most compounds moderately inhibit *Salmonella typhi*, *S. paratyphi* A and *S. paratyphi* B bacterial strains. The results also reveals that compounds having halides and nitro substituents showed best antibacterial activity. Bisindolylmethane thioureas and sulfonamides were tested for carbonic anhydrase II inhibition activity. Molecular docking results suggest that nitro substituent at *para* position interacts well with Zn^{2+} ion and interferes with the Zn-OH-Thr199-Glu106 hydrogen bond network. Bisindole hydrazone in this study were synthesized through a three-step reaction. β-Glucuronidase inhibitory property of some derivatives were found to be very potent (0.1-83.5 μM). Docking studies showed that active compounds should have two or more hydroxyl groups substituted on carbon adjacent to each other for good interactions to take place. Hydroxyl group at *meta* position of **269** was found to interact with important amino acids Glu450 and Glu540. With regards to flavone hydrazones and α-glucosidase inhibitory activity, thirty derivatives (**288-317**) were found to be active (0.7-30.7 μM). Compound **288** (0.7 ± 0.2 μM) was the most active compound in the series. QSAR model developed using Discovery Studio (DS) 2.5 had successfully predicted the pIC_{50}. Molecular docking on α-glucosidase was able to identify possible binding modes responsible for the inhibitory activity. Benzohydrazone linkage enhances rotatability and allows *N*-benzylidene moiety to interact with residues like Glu276, His348, and Asp349. In the final part of this thesis, 155 synthesized derivatives consisting of bisindolylmethanes (anilines, Schiff bases, thioureas and sulfonamides) and flavones (hydrazones and ethers) were evaluated for their antiproliferative activity against lung, breast, colon, nasopharyngeal, and endometrial cancer cell lines followed by molecular docking studies. Docking results suggest that bisindolylmethane thiourea and sulfonamide adopt different inhibition mechanisms. Thiourea derivative **191** was able to fit in the S1' hydrophobic pocket of MMP-2 protein, while sulfonamide **224**, which was too bulky for MMP-2 protein, was able to fit into DDX3 protein. Molecular docking for Schiff base and thiourea derivatives of bisindolylmethane suggest that they inhibit through the same mechanism by targeting HER2 protein. Schiff base and thiourea interact with residues in the phosphate binding pocket and also hinge region of HER2 protein. In general, the synthesized compounds represent potential leads for future drug discovery. In this study, efficient methodologies for synthesis of novel bisindolylmethanes and flavone were developed. Biological properties of the derivatives were evaluated experimentally and rationalized through computational analysis, providing important information on vital structural features. Integrating computational analysis in drug design is a suitable approach towards obtaining drug candidates of better potency.

ACKNOWLEDGEMENT

First of all, thanks to Allah S.W.T, the compassionate, and the merciful, for giving me the ability, health and knowledge to complete this Ph.D research.

This Ph.D research would not have been possible without the help of many people in so many ways. I would like to express my deepest appreciation to my supervisor, Professor Dr Nor Hadiani Ismail, whose constant guidance helped me in completing this study, from the thesis proposal up to the thesis manuscript. Without her guidance, endless advice and persistent help, this study would not have been possible. Massive thanks for the opportunities and exposure in preparing me to gain self-confidence in everything I do. My appreciation also goes to my co-supervisor, Dr Muhammad Taha, for his ideas, guidance and encouragement to complete my research.

To my parents, Abu Bakar bin Mahmud and Rehana Begum binti Mohd Iqbal, for their continuous prayers and support throughout the journey. Thank you for allowing me to go after my dreams and passion, and letting me be the person I am today. Everything I am today, I owe it to both of you. My sincerest gratitude also goes to my beloved siblings; Shahrul Nizam, Syahrul Maliq, and Nur Najieha who have been cheering me up to keep me going. Not forgetting my wife, Nadiah Syariani Mohd Shariff that had been giving constant support towards the end of my study. Thank you for your patience throughout these years and know that I love you all to the moon and back.

A very special thanks to my lab mates; Faiezah Abdullah, Mohamad Azlan Shah, Herizal Ali Nuzar, Muhammad Afifi Mohd Faridz, and Mastura Arbin for being loyal company throughout my study. Thank you for staying through thick and thin, and the moral support along the way. I am also grateful to fellow postgraduate colleagues and staff at Atta-ur Rahman Institute of Natural Product Discovery (AuRIns). This journey would not have been fun and memorable without their company. Thank you for the knowledge and perspective sharing, which I found very helpful for future use.

My gratitude goes out to all those who facilitated along this study process especially Professor Dr Khalid Mohammed Khan, who has provided me with financial support and space in his laboratory at International Centre of Chemical and Biological Sciences (ICCBS) in University of Karachi for me to complete the derivatization for bisindolylmethane. I would like to express my appreciation to Professor Dr Habibah Wahab, Dr Maywan Hariono, and Dr Muhammad Yusuf for allowing me to perform some computational studies at their Pharmaceutical Design and Simulation (PhDS) laboratory in School of Pharmaceutical Chemistry, Universiti Sains Malaysia (USM) Pulau Pinang.

I would like to express my gratitude to Ministry of Higher Education for awarding me the MyBrain15 scholarship. Last but not least, massive thanks to everyone involved in this study directly or indirectly, people without whom this thesis might not have been written, and to whom I am greatly indebted.

Thank you.

TABLE OF CONTENTS