UNIVERSITI PUTRA MALAYSIA

THE SYNTHESIS AND BIOACTIVITY OF 2,6-BISBENZYLIDENECYCLOHEXANONE, PYRAZOLINE, CHALCONE AND OXADIAZOLE DERIVATIVES AND COMPUTATIONAL STUDIES ON SOME OF THESE COMPOUNDS

LAM KOK WAI

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By

LAM KOK WAI

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2010

DEDICATION

To my parents, sister and little brother,

When I was deemed to fail, you stand beside me whispering supportive words and cherish me along the way. Another great chapter of life in me was born because of you. Thank you!

Abstract of thesis presented to the Senate of University Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

THE SYNTHESIS AND BIOACTIVITY OF 2,6-*BIS*BENZYLIDENECYCLOHEXANONE, PYRAZOLINE, CHALCONE AND OXADIAZOLE DERIVATIVES AND COMPUTATIONAL STUDIES ON SOME OF THESE COMPOUNDS

By

LAM KOK WAI

October 2010

Chairman : Professor Nordin Hj. Lajis, PhD

Institute : Bioscience

In the first part of this thesis fulfillment, a series of forty four 2,6-*bis*benzylidenecyclohexanone, pyrazoline, pyrazole and isoxazole derivatives were synthesized and evaluated for inhibitory activities on IFN-γ/LPS-activated RAW 264.7 cells and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Three compounds **4-8**, **4-9** and **4-11a** possessed significant nitric oxide (NO) inhibitory activities as compared to N-Nitro-L-Arginine Methyl Ester (L-NAME) and curcumin with an IC_{50} value of 6.68 ± 0.16 μM, 6.09 ± 0.46 μM and 6.84 ± 0.12 μM, respectively. Apparently, the suppression effect upon NO secretion was not due to cell death since the active compounds did not suppress the cell viability in close proximity to the IC_{50} of NO inhibition. Meanwhile compound **4-11** (IC_{50} = 13.27 ± 1.78 μM)

bearing adjacent hydroxyl groups recorded the highest radical scavenging activity as compared to quercetin **(**IC_{50} = 21.46 ± 0.85 µM). The binding mode of compound **4-8** (2,6-*bis*(4-hydroxy-3-methoxybenzylidene)cyclohexanone, **BHMC**) at the active site of p38*α* MAP kinase (PDB code 1a9u) was investigated using **AUTODOCK** 4.2 program. Both the hydroxyl groups of **BHMC** were involved in hydrogen bonding with residues, including Methionine 109 (2.086Å) and Phenylalanine 169 (2.137Å) with the calculated free binding energy of -6.96 kcal/mol. One of the phenyl groups was clearly seen occupying the hinge region, while the other ring filled the cavity at the back of the ATP-site.

In the second part of this thesis, a further forty six chalcone derivatives were synthesized and evaluated for anti-inflammatory activity on RAW 264.7 cells. Among these compounds, chalcones bearing the furanyl group showed remarkable results as anti-inflammatory agents. Both compounds **5-2d** and **5-2j** were identified as the most potent NO inhibitor on IFN-γ/LPS-activated RAW 264.7 cells with IC_{50} values of 2.51 ± 0.42 µM and 2.26 ± 0.47 µM, respectively. In order to examine the structure-activity relationship, a 3D QSAR analysis was carried out by comparative molecular field analysis (CoMFA) method on the selected chalcones. Partial least square analysis produced a statistically coherent model with good predictive value, r^2 = 0.989 and a good cross validated value, q^2 = 0.583. The binding mode of compound **5-2a** (3-(2-hydroxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one, **HMP**) at the active site of p38*α* MAP kinase (PDB code 1a9u) was investigated using **AUTODOCK** 4.2 program. The hydroxyl group was involved in hydrogen bonding with the backbone amide of Methionine 109 nitrogen atom with the calculated free binding energy of

-6.15 kcal/mol. The methylfuranyl moiety was clearly seen occupying the hydrophobic back pocket where the p38α gatekeeper residue, Threonine 106 resided.

In the final part of the thesis, a series of twenty four oxadiazole and triazolothiadiazole derivatives were synthesized and evaluated for their mushroom tyrosinase inhibitory activity. Five derivatives were found to display high inhibition activity ranging from 0.87 to 1.49 μM. Compound **6-5** exhibited the highest activity with IC_{50} value of 0.87 ± 0.16 μM. The *in silico* protein-ligand docking using **AUTODOCK** 4.1 was successfully performed on compound **6-5** with significant binding energy value of -5.58 kcal/mol. The docking results also showed that the tyrosinase inhibition might be due to the metal chelating effect of thione functionality in compounds **6-1** until **6-5**. Further studies revealed that the presence of hydrophobic groups such as cycloamine derivatives played an important role in the inhibition. The piperazine moiety in compound **5** appeared to be involved in an extensive hydrophobic contact and a 2.9 Å hydrogen bond with residue Glutamic acid 182 in the active site.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah Doktor Falsafah

SINTESIS DAN KEAKTIFAN BIOLOGI DIARYLPENTANOID TERBITAN 2,6-*BIS*BENZILDIENASIKLOHEKSANONE, PIRAZOLINA, CHALCONE DAN OKSADIAZOL DAN KAJIAN SIMULASI PENGKOMPUTERAN BAGI SEBATIAN TERPILIH

Oleh

LAM KOK WAI

Oktober 2010

Chairman : Professor Nordin Hj. Lajis, PhD

Institute : Biosains

Pada bahagian pertama tesis ini, empat puluh empat siri terbitan 2,6-*bis*benzildienasikloheksanone dan pyrazolina telah disintesis dan dikaji untuk aktiviti keradangan pada sel RAW 264.7 yang diaktifkan oleh IFN-γ/LPS dan aktiviti perencatan 1,1-diphenyl-2-picrylhydrazyl (DPPH) radikal. Tiga sebatian **4-8**, **4-9** dan **4-11a** menunjukkan aktiviti keradangan yang signifikan masing-masing dengan nilai IC_{50} iaitu 6.68 ± 0.16 μM, 6.09 ± 0.46 μM and 6.84 ± 0.12 μM berbanding dengan N-Nitro-L-Arginine Methyl Ester (L-NAME) dan curcumin. Umumnya, perencatan pembebasan radikal nitrik oxida (NO) melalui aktiviti keradangan bukan disebabkan oleh faktor kematian sel kerana sebatian itu tidak membunuh sel pada IC_{50} perencatan itu. Selain itu, sebatian **4-11** (IC_{50} = 13.27 ± 1.78 μM) yang mempunyai kumpulan hidroksi bersebelahan merekodkan aktiviti perencatan radikal bebas tertinggi

berbanding dengan quercetin (IC_{50} = 21.46 ± 0.85 μM). Sebatian **4-8** (2,6-*bis*(4-hidroksi-3-metoxibenzildiena)sikloheksanone, **BHMC**) mengikat dirinya di tapak aktif MAP kinase (PDB code 1a9u) dapat diselidiki melalui program **AUTODOCK** 4.2. Kedua-dua kumpulan hidroksi **BHMC** terlibat dalam perikatan hidrogen dengan amino asid Methionine 109 (2.086Å) and Phenylalanine 169 (2.137Å) masing-masing mencatatkan tenaga perikatan bebas, 6.96 kcal/mol. Salah satu kumpulan fenil memenuhi ruang pertukaran manakala satu lagi terletak pada bahagian belakang tapak ATP.

Pada bahagian kedua tesis ini, empat puluh enam terbitan chalcone telah disintesis dan dikaji untuk aktiviti keradangan pada RAW 264.7. Di antara semua sebatian yang disintesis, sebatian yang mengandungi kumpulan furan menunjukkan aktiviti anti-keradangan yang signifikan. Sebatian **5-2d** dan **5-2j** dikenalpasti sebagai agen anti nitrik oxida yang paling aktif ke atas sel yang diaktifkan oleh IFN-γ/LPS dengan nilai IC_{50} iaitu 2.51 ± 0.42 μM and 2.26 ± 0.47 μM. Bagi mengaji hubungan di antara struktur dan aktiviti sebatian, analisis 3D-QSAR telah dijalankan melalui langkah kerja 'comparative molecular field analysis' (CoMFA) ke atas chalcones yang dikenalpasti. Analisis 'Partial Least Square' menunjukkan kajian model statistic yang signifikan dengan r^2 = 0.989 dan q^2 = 0.583. Lakaran peta elektrostatik dan sterik yang dijanakan pada model CoMFA akan membantu kami dalam merekabentuk ubat anti-keradangan berkesan pada masa hadapan. Sebatian **5-2a** (3-(2-hydroxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one, **HMP**) mengikat dirinya di tapak aktif MAP kinase (PDB code 1a9u) dapat diselidiki melalui program **AUTODOCK** 4.2. Kumpulan hidroksi terlibat dalam pengikatan hydrogen dengan kumpulan atom nitrogen amida

Methionine 109 mencatatkan nilai tenaga perikatan bebas pada -6.15 kcal/mol. Kumpulan metilfuranil memenuhi ruang hidrofobik yang terletak pada p38α gatekeeper, Threonine 106.

Pada bahagian terakhir tesis, dua puluh empat terbitan oxadiazole telah dirangka, disintesis dan dikaji untuk aktiviti anti-tyrosinase pada cendawan. Lima deriviti didapati merekodkan aktiviti perencatan yang tinggi dalam julat 0.87 hingga 1.49 μM. Sebatian **6-5** menunjukkan aktiviti perencatan aktiviti tyrosinase tertinggi pada nilai 0.87 ± 0.16 μM. 'Docking' protein-ligand *in silico* berjaya dijalankan dengan menggunakan **AUTODOCK** 4.1 pada sebatian **6-5** dengan nilai tenaga perikatan bebas yang signifikan pada -5.58 kcal/mol. Daripada keputusan 'docking', perencatan enzim tyrosinase mungkin disebabkan oleh kesan logam chelasi yang disebabkan oleh kehadiran fungsi 'thione' pada sebatian **6-1** sehingga **6-5**. Pengajian seterusnya mendapati kehadiran kumpulan hidrofobik seperti cycloamina deriviti memainkan peranan penting dalam fungsi perencatan. Kumpulan piperazine pada sebatian **6-5** terlibat dalam sentuhan hidrofobik berterusan dan 2.9 Å perikatan hydrogen dengan amino asid Glutamic acid 182 pada tapak aktif.

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I would like to express my utmost thanks to both of my parents and sister for giving me the best and wonderful life for the past 27 years. Their rapport and never say-die spirit have encouraged me to go for one step further in what I would think I could not achieve in the first place. My special thanks to my little young brother for completing my personal life apart from research.

To my friends and colleagues especially from H.E.J Karachi and Laboratory of Natural Products, I would like to offer you my hearty gratitude for your constant support and inspiration. One of the joys of working in these laboratories was to be with a group of young and motivated researchers like you guys.

Furthermore, I would also like show my appreciation and gratitude to those who have contributed to my research. Finally, I was grateful to my god for blessing me a healthy body and an enlighten mind.

I certify that a Thesis Examination Committee has met on 20th October 2010 to conduct the final examination of Lam Kok Wai on his thesis entitled 'The Synthesis and Bioactivity of 2,6-*Bis*benzylidenecyclohexanone, Pyrazoline, Chalcone and Oxadiazole Derivatives and Computational Studies on Some of These Compounds' in accordance with the Universities and University Colleges Act 1971 and the constitution of the University Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the PhD. Members of the Thesis Examination Committee were as follows:
Members of the Thesis Examination Committee were as follows:

Gwendoline Ee Cheng Lian, Phd
Professor
Faculty of Science
University Putra Malaysia
(Chairman)

Karen A. Crouse, Phd
Professor
Faculty of Science
University Putra Malaysia
(Internal Examiner)

Bimo A. Tejo, Phd
Lecturer
Faculty of Science
University Putra Malaysia
(Internal Examiner)

Khalid M. Khan, Phd
Professor
H.E.J Research Institute of Chemistry
University of Karachi
(External Examiner)

Bujang Kim Huat, Phd
Professor and Deputy Dean
School of Graduate Studies
University Putra Malaysia
Date:

This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor Philosophy. The members of the Supervisory Committee were as follows:

Md Nordin B Hj. Lajis, Phd
Professor
Institute of Bioscience
University Putra Malaysia
(Chairman)

Syahida Binti Ahmad, Phd
Doctor
Faculty of Biotechnology and Biomolecular Sciences
University Putra Malaysia
(Member)

Mohd Basyaruddin Bin Abdul Rahman, Phd
Professor
Faculty of Science
University Putra Malaysia
(Member)

Hasanah Mohd Ghazali, Phd
Professor and Dean
School of Graduate Studies
University Putra Malaysia
Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University Putra Malaysia or at any other institutions.

Lam Kok Wai
Date: 20 October 2010

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