UNIVERSITI PUTRA MALAYSIA

MECHANISM OF THE ANTI-INFLAMMATORY ACTION OF 3-(2-HYDROXY-PHENYL)-1-(5-METHYL-FURAN-2-Y-L) PROPENONE (HMP)

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By

LIEW CHOI YI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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July 2010

Chairman: Professor Daud Ahmad bin Israf Ali, PhD

Faculty: Medical and Health Sciences

Chalcones, a subgroup of flavonoids, are found in many plants and many synthetic analogues have been artificially synthesized. Many natural and synthetic chalcones exhibit varying degress of anti-inflammatory activity. In an attempt to discover more potent anti-inflammatory compounds, 3-(2-hydroxyphenyl)-1-(5-methyl-furan-2-y-l) propenone (HMP) was evaluated for its ability to inhibit the synthesis of major proinflammatory mediators and cytokines in interferon-γ (IFN-γ)- and lipopolysaccharide (LPS)-induced RAW 264.7 cells and phorbol myristate acetate (PMA)-differentiated/LPS-induced U937 cells in study I, II, III and IV respectively. The 96-well plate assays included cell viability test, griess, chemical scavenging assay and enzyme-linked immunosorbent assays were conducted meanwhile western blotting, reverse transcription-polymerase chain reactions, immunoprecipitation, kinase assay, electrophoretic mobility shift assay and docking experiment were applied for molecular

detection throughout the studies. In study I, II and III, HMP suppressed the production of nitric oxide (NO) at doses as low as 0.78 μM, prostaglandin E2 (PGE2) and interleukin (IL)-1β secretion at doses of 12.5 μM and above meanwhile tumor necrosis factor (TNF)-α and IL-6 secretion at 25 μM with significant inhibitory effects ($p < 0.05$). HMP did not affect the secretion of chemokines IL-8 and monocyte chemotactic protein-1 (MCP-1) and the anti-inflammatory cytokine IL-10. HMP showed a dose-dependent inhibition of NO synthesis as demonstrated from NO secretion and inducible nitic oxide synthase (iNOS) expression. For study III and IV in which western blotting and kinase assay were conducted, the inhibition of NO synthesis was related to the inhibition of p38 phosphorylation and potent inhibition of p38 kinase activity that led to significant inhibition of phosphorylation of activating transcription factor (ATF)-2. This effect in turn caused significant inhibition of activating protein (AP)-1-DNA binding which partially explains the inhibitory effect upon the synthesis of iNOS. Interestingly, HMP failed to alter phosphorylation of extracellular-signal-related-kinase (ERK) 1/2 and Jun N-terminal kinase (JNK) and did not affect their kinase activity. Furthermore, HMP also failed to inhibit phosphorylation of Inhibitory protein κB (I-κB), nuclear translocation of p65 nuclear factor-κB (NF-κB) and DNA binding of p65 NF-κB. Phosphorylation of signal transducers and activators of transcription (STAT)-1 was also unaffected by HMP. Molecular docking experiments confirmed that HMP fits well in the highly conserved hydrophobic pocket of p38 MAP kinase. In conclusion, in contrast to many anti-inflammatory chalcones, HMP shows a higher selectivity toward NO inhibition therefore providing an interesting drug lead that has potentially less side effects.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

MEKANISME ANTI-RADANG 3-(2-HYDROXY-PHENYL)-1-(5-METHYL-FURAN-2-Y-L) PROPENONE (HMP)

Oleh

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Calkon, sejenis subkelompok flavonoid, diterima dalam banyak tumbuhan telah dihasilkan dalam bentuk analog sintetik. Kebanyakan calkon semula jadi ataupun analog sintetik menunjukkan pelbagai darjah aktiviti anti-radang. Untuk menemui lebih banyak anti-radang *compound*, keupayaan 3-(2-hidroksi-fenil)-1-(5-metil-furan-2-il) propenone (HMP) telah dikaji untuk menghalang mediator pro-inflamasi dan sitokin yang dihasilkan daripada induksi oleh interferon (IFN)-γ dan lipopolisakarida (LPS) dalam sel- sel RAW264.7 dan juga induksi oleh LPS dalam sel-sel U937 yang telah ditransformasi oleh forbol miristat asetat (PMA) dalam kajian I, II, III dan IV masing-masing. Ujian-ujian *96-well plate* dimana termasuknya kajian tentang kebolehhidupan sel, *griess*, *chemical scavenging* dan asai imunoserapan terangkai enzim telah digunakan. *Western blotting*, tindak balas rantaian *reverse transcription-polymerase*, pengimunomendakan, ujian kinase, ujian syif kegerakan elektroforesis dan eksperimen

docking juga digunakan dalam keseluruhan projek ini. HMP dapat menghalang pengeluaran oksida nitrik (NO) pada dos serendah 0.78 μM, prostaglandin E2 (PGE2) and IL-1 pada dos 12.5 μM dan ke atas serta tumor nekrosi faktor (TNF)- dan IL-6 pada dos 25 μM dengan $p < 0.05$ dalam ujian-ujian I, II and III masing-masing. HMP tidak dapat mempengaruhi penghasilan IL-8 dan *monocyte chemotactic protein* (MCP)-1 serta sitokin anti-inflamasi (IL-10). Pengurangan NO oleh HMP adalah disebabkan oleh sekatan terhadap rembesan NO dan ekspres iNOS. Melalui *western blotting* dan ujian kinase dalam ujian III and IV, penyekatan terhadap fosforilasi p38 protein dan juga aktiviti enzim-enzim ini yang akan menghasilkan *phospho-ATF2* juga dikurangkan. Kesan-kesan tersebut menyebabkan pula berlakunya halangan terhadap *activating protein* (AP)-1 aktiviti pengikatan di mana halangan inilah yang menyebabkan pengeluaran NO oleh HMP. HMP tidak berkesan terhadap fosforilasi oleh *extracellular-signal-related-kinase* (ERK) 1/2 dan *Jun N-terminal kinase* (JNK) serta activiti-activiti oleh enzim-enzim ini. Selain itu, HMP juga gagal menyekat fosforilasi oleh *inhibitory protein κB* (I-κB) ataupun *signal transducers and activators of transcription* (STAT)-1 dan nuklear translokasi serta *DNA binding* oleh nuklear faktor-κB (NF-κB) Pengajian molekul docking telah membuktikan bahawa penyekatan p38 oleh HMP adalah secara selektif. Tambahan pula, HMP menunjukkan persamaan dalam pola pengikatan terhadap halaman aktif p38 *kinase* apabila dibandingkan dengan SB 203580 (p38 inhibitor selektif) tersebut dalam pengajian tersebut. Secara kesimpulannya, keputusan-keputusan ini menunjukkan bahawa tindakan anti-radang oleh HMP adalah lebih selektif, dijangka dapat mengurangkan akibat sampingan jika dibandingkan dengan calkon-calkon yang lain.

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I certify that a Thesis Examination Committee has met on date of viva voce to conduct the final examination of Liew Choi Yi on her Master of Science thesis entitled 'Mechanism of anti-inflammatory action of 3-(2-hydroxy-phenyl)-1-(5-methyl-furan-2-y-l) propenone (HMP)' in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998.The Committee recommends that the student be awarded degree of Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for the synthesis of tested compounds, docking experiment, 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 Universiti Putra Malaysia or at any other institution.

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LIEW CHOI YI

Date: 19 July 2010

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