PREPARATION OF BETULINIC ACID DERIVATIVES IN ORGANIC SOLVENTS

By

ANISHAH BINTI ISSAK

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

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Faculty : Science and Environmental Studies

Betulinic acid has been identified as a potential natural product in medical field. The objectives of this research were to discover a new method for the modification of betulinic acid's functional groups (C-3 and C-28 position) and to evaluate its activity against cancer cell. Modification at C-3 position was firstly carried out using acetate anhydride/pyridine with chemical reaction of 79% yield and was successfully modified using enzymatic reaction using Novozyme 435 in 85% yield. The later reaction is a new method for the preparation of 3β-acetoxy-lup20(29)-ene-28-oic acid using enzymatic reaction.

Further modification at C- 28 position was carried out through an enzymatic reaction to produce betulinic acetate ester (3β-acetoxy-lup20(29)-ene-28-decanoate). Immobilized lipase from *Candida antartica* and *Mucor miehei* (Novozyme 435 and Lipozyme) were used. Novozyme 435 gave better esterification product of 3β-acetoxy-lup20(29)-ene-28-

decanoate. To our knowledge, the esterification of 3β-acetoxy-lup20(29)-ene-28-decanoate using enzymatic reaction has not been reported previously.

The effect of various parameters such as reaction time (1-24 h), initial water activity a_w (0.11 - 0.90), substrate molar ratio of 1-decanol to 3β-axetoxy-lup20(29)-ene-28-oic acid (1:1 to 1:30) and various organic solvents on the esterification reaction was studied. Novozyme 435 was observed performed well in this study and gave maximum percent conversions of esterification under the following condition: reaction time (24 h), initial water activity a_w (0.33), substrate molar ratio (1:15) in chloroform.

These betulinic acid derivatives were then evaluated for their inhibitory activity against human leukemia cell line (HL60). Our results suggested that betulinic acid derivatives reduce their cytotoxicity compared with betulinic acid itself.

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

PENYEDIAAN TERBITAN ASID BETULINIK DI DALAM PELARUT ORGANIK

Oleh

ANISHAH BINTI ISSAK

Mei 2004

Pengerusi : Profesor Madya Faujan Ahmad, Ph. D

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Betulinik asid telah dikenalpasti sebagai satu hasil semulajadi yang berpotensi di dalam bidang perubatan. Objektif penyelidikan ini adalah untuk mencari satu kaedah baru untuk pengubahsuaian kumpulan berfungsi (kedudukan C-3 dan C-28) pada betulinik asid dan menilai aktivitinya terhadap sel kanser. Pengubahsuaian pada kedudukan C-3 adalah pertama kali dilakukan menggunakan asetat anhidrida/piridin dengan tindakbalas kimia pada 79 % dan berjaya mengubahsuaian dengan tindakbalas enzim mennggunakan Novozim 435 pada 85%. Tindakbalas terkini ini merupakan kaedah baru untuk menyediakan 3β-asetoksi-lup20(29)-en-28-oik asid menggunakan tindakbalas enzim.

Pengubahsuaian seterusnya pada kedudukan C-28 telah dilakukan melalui tindakbalas enzim untuk menghasilkan betulinik asetat ester (3β-asetoksi-lup20(29)-en-28-dekanoat).

Geraksekat enzim daripada *Candida antartica* dan *Mucor meihei* (Novozim 435 dan Lipozim) telah digunakan. Novozim 435 telah memberi hasil pengesteran 3β-asetoksi-lup20(29)-en-28-dekanoat yang lebih baik. Untuk pengetahuan semua, pengesteran 3β-asetoksi-lup20(29)-en-28-dekanoat menggunakan tindakbalas enzim belum pernah dilaporkan sebelum ini.

Kesan pelbagai parameter seperti masa tindakbalas (1-24 jam), aktiviti air awal a_w (0.11-0.99), pecahan mol reaktan 1-dekanol kepada 3β-asetoksi–20(29)-lup-en-28-oik asid (1:1 hingga 1:30) dan pelbagai pelarut organik pada tindakbalas pengesteran telah dikaji. Novozim 435 telah dikenalpasti sebagai enzim terbaik di dalam kajian ini dan peratus perubahan maksimum pengesteran ini pada keadaan: masa tindakbalas (24 j), aktiviti air awal a_w (0.33), pecahan mol reaktan (1:15) di dalam klorofom

Terbitan betulinik asid ini kemudiannya telah dinilai aktivitinya terhadap sel leukimia manusia (HL60). Keputusannya mencadangkan yang terbitan betulinik asid mengurangkan ketoksikan berbanding betulinik asid itu sendiri.

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I certify that an Examination Committee met on 11th May 2004 to conduct the final examination of Anishah Issak on her Master Degree dissertation entitled "Preparation of Betulinic Acid Derivatives in Organic Solvents" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommended that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions

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