UNIVERSITI PUTRA MALAYSIA

OPTIMIZED SYNTHESIS OF LIPASE-CATALYZED SYNTHESIS OF 3-O-(3',3'-DIMETHYSUCCINYL)-BETULINIC ACID BY IMMOBILISED NOVOZYME 435

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By

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirement for Degree of Master

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June 2010

Chairman: Prof. Dr. Faujan Bin H. Ahmad, PhD

Faculty: Science

The derivative of betulinic acid, 3-O-(3',3'-dimethylsuccinyl)-betulinic acid (5) was successfully synthesized by the reaction of betulinic acid and 2,2-dimethylsuccinic anhydride, catalyzed by immobilized lipase from Candida antarctica (Novozyme 435) in chloroform. The structure of the product was determined by spectroscopic methods. Effects of different reaction parameters were investigated and optimized in the model reaction. Optimum conditions to produce 3-O-(3',3'-dimethylsuccinyl)-betulinic acid (5) up to 78.1 % were observed at reaction time; 24 h, amount of enzyme; 100 mg, betulinic acid (1) (0.055 mmole) to 2,2-dimethylsuccinic anhydride (0.055 mmole) substrate molar ratio; 1:1 at 50 °C.
Response surface methodology (RSM) based on a five-level, three variables and central composite rotatable design (CCRD) was employed to evaluate the interactive effects of the parameters used in the synthesis methodology such as reaction time, temperature and enzyme amount. It was observed that, simultaneous increase in reaction time, temperature and amount of enzyme will increase the yields of 3-\textit{O}-(3',3'-dimethylsuccinyl)-betulinic acid (5). Based on the analysis of ridge max, the optimum conditions for the synthesis of 3-\textit{O}-(3',3'-dimethylsuccinyl)-betulinic acid (5) were as follows: 53.6 °C of reaction temperature, 28.15 hours of reaction time and 122 mg of enzyme for 1.0 mmol of betulinic acid (1) and 1.0 mmol of 2,2-dimethylsuccinic anhydride. The optimum predicted for percentage yield was at 83.93 % in which agree well with the actual value of 84.38 %.

In brief, the anticancer activity of betulinic acid (1) and 3-\textit{O}-(3',3'-dimethylsuccinyl)-betulinic acid (5) were evaluated against cultured human T-promyelocytic leukemia (HL-60), human breast cancer (MCF-7), human cervical carcinoma cancer (HeLa) and mouse embryonic fibroblast normal cell line (3T3) cells lines. In particular, 3-\textit{O}-(3',3'-dimethylsuccinyl)-betulinic acid showed nontoxic activity against human T-promyelocytic leukemia (HL-60) and human breast cancer (MCF-7) with IC\textsubscript{50} > 30 μg/ml. However, it has better activity against human cervical carcinoma cancer (HeLa) (IC\textsubscript{50} 1.9 μg/ml) compared to betulinic acid (IC\textsubscript{50} 4.8 μg/ml). Interestingly, both compound were highly inactive against mouse embryonic fibroblast normal cell line (3T3) with IC\textsubscript{50} > 30 μg/ml.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana

OPTIMUM SINTESIS TINDAKBALAS-PEMANGKINAN BAGI 3-O-(3',3'-DIMETHILSUKSINIL)-ASID BETULINIK MENGGUNAKAN NOVOZYME 435

Oleh

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Terbitan asid betulinik, 3-O-(3',3'-dimethilsuksinil)-asid betulinik (5) telah berjaya dihasilkan melalui tindakbalas antara asid betulinik dan 2,2-dimethilsuksinik anhidrida menggunakan enzim daripada Candida antartica (Novozyme 435) sebagai pemangkin tindak balas dalam kloroform. Struktur sebatian hasil tindak balas ditentukan melalui analisis spektroskopi. Kesan untuk pelbagai parameter juga telah dikaji dan dioptimumkan sebagai model tindak balas. Keadaan optimum untuk menghasilkan 3-O-(3',3'-dimethilsuksinil)-asid betulinik (5) sehingga 78.1% telah diperolehi dalam masa tindak balas 24 jam, kuatiti enzim 100 mg, asid betulinik (1) (0.055 mmol) kepada 2,2-dimethilsuksinik anhidrida (0.055 mmol) nisbah molar substrak; 1:1 pada suhu 50 °C.
Analisis kaedah permukaan respon (RSM) telah digunakan untuk menilai kesan interaktif bagi tindak balas sintesis pada pelbagai parameter yang digunakan seperti masa tindak balas, suhu tindak balas dan jumlah pemangkin terhadap hasil tindak balas sebatian 3-O-(3',3'-dimethylsulksinil)-asid betulinik (5). Analisis menunjukkan bahawa keadaan optimum untuk sintesis 3-O-(3',3'-dimethylsulksinil)-asid betulinik (5) adalah seperti berikut: 53.6 °C suhu tindak balas, 28.15 jam masa tindak balas dan 122 mg jumlah pemangkin bagi 1.0 mmol asid betulinik (1) dan 1.0 mmol 2,2-dimethylsulksinik anhidrida yang digunakan. Peratusan hasil tindak balas yang dijangkakan adalah sebanyak 83.93% dimana hasil ini bertepatan dengan hasil sebenar tindak balas iaitu sebanyak 84.38%.

Secara ringkasnya, aktiviti anti-kanse asid betulinik dan terbitannya, 3-O-(3',3'-dimethylsulksinil)-asid betulinik (5) telah diuji untuk melawan penyakit leukemia (HL-60), kanser payudara (MCF-7), kanser serviks (HeLa) dan sel normal tikus (3T3). Hasil kajian menunjukkan bahawa 3-O-(3',3'-dimethylsulksinil)-asid betulinik (5) tidak toksik terhadap penyakit leukemia (HL-60), kanser payudara (MCF-7) dan kanser serviks (HeLa) dengan nilai IC₅₀ > 30 μg/ml. walaubagaimanapun, sebatian ini mempunyai aktiviti yang lebih baik untuk melawan kancer serviks (HeLa) (IC₅₀ 1.9 μg/ml) berbanding asid betulinik (1) (IC₅₀ 4.8 μg/ml). Menariknya, kedua-dua sebatian ini (asid betulinik (1) dan 3-O-(3',3'-dimethylsulksinil)-asid betulinik (5)) adalah sangat tidak aktif terhadap sel normal tikus (3T3) dengan nilai IC₅₀ > 30 μg/ml.
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I certify that a Thesis Examination Committee has met on 18 October 2010 to conduct the final examination of Siti Aminah Binti Gunong @ Mohd Shah on her thesis entitled “Optimized synthesis of lipase-catalyzed 3-O-(3',3'-dimethylsuccinyl)-betulinic acid by immobilised Novozyme 435” in accordance with the Universities and Universities Colleges Act 1971 and the Construction of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Degree of Master.

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Date: 23 December 2010
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement of the degree of master. The members of supervisory committee were as follows:-

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Date:
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 of Universiti Putra Malaysia or at any other institution.

SITI AMINAH BINTI GUNONG @ MOHD SHAH

Date: 18 October 2010
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