



UNIVERSITI PUTRA MALAYSIA

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

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By

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**OPTIMIZED SYNTHESIS OF LIPASE-CATALYZED 3-O-(3',3'-
DIMETHYLSUCCINYL)-BETULINIC ACID BY IMMOBILISED NOVOZYME
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June 2010

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

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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 antartica* (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-*O*-(3',3'-dimethylsuccinyl)-betulinic acid (**5**). Based on the analysis of ridge max, the optimum conditions for the synthesis of 3-*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-*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-*O*-(3',3'-dimethylsuccinyl)-betulinic acid showed nontoxic activity against human T-promyelocytic leukemia (HL-60) and human breast cancer (MCF-7) with $IC_{50} > 30$ μ g/ml. However, it has better activity against human cervical carcinoma cancer (HeLa) (IC_{50} 1.9 μ g/ml) compared to betulinic acid (IC_{50} 4.8 μ g/ml). Interestingly, both compound were highly inactive against mouse embryonic fibroblast normal cell line (3T3) with $IC_{50} > 30$ μ g/ml.



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

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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'-dimethilsuksinil)-asid betulitik (**5**). Analisis menunjukkan bahawa keadaan optimum untuk sintesis 3-*O*-(3',3'-dimethilsuksinil)-asid betulitik (**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 betulitik (**1**) dan 1.0 mmol 2,2-dimethilsuksinik 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-kanser asid betulitik dan terbitannya, 3-*O*-(3',3'-dimethilsuksinil)-asid betulitik (**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'-dimethilsuksinil)-asid betulitik (**5**) tidak toksik terhadap penyakit leukemia (HL-60), kanser payudara (MCF-7) dan kanser serviks (HeLa) dengan nilai $IC_{50} > 30 \mu\text{g/ml}$. walaubagaimanapun, sebatian ini mempunyai aktiviti yang lebih baik untuk melawan kanser serviks (HeLa) ($IC_{50} 1.9 \mu\text{g/ml}$) berbanding asid betulitik (**1**) ($IC_{50} 4.8 \mu\text{g/ml}$). Menariknya, kedua-dua sebatian ini (asid betulitik (**1**) dan 3-*O*-(3',3'-dimethilsuksinil)-asid betulitik (**5**)) adalah sangat tidak aktif terhadap sel normal tikus (3T3) dengan nilai $IC_{50} > 30 \mu\text{g/ml}$.



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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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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.

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Date: 18 October 2010



TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vi
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xi
LIST OF FIGURES	xiii
LIST OF SCHEMES	xv
LIST OF ABBREVIATIONS	11
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	6
Betulinic acid	6
Bioactivity of betulinic acid and its derivatives	7
Anti Human Immunodeficiency Virus (HIV)	7
Anti-cancer activity	12
Anti-inflammatory activity	15
Antiviral activity	16
Preparation of Betulinic Acid	18
Synthesis of Betulinic Acid Derivatives by Chemical Reactions	19
3- <i>O</i> -(3',3'-Dimethylsuccinyl)-betulinic acid	24
Enzymatic synthesis of betulinic acid derivatives	28
Enzyme	30
Enzymes in organic solvent	30
Novozyme 435	31
Factors affecting activity and selectivity of lipase	32
Temperature	32
Organic solvent	33
Enzyme concentration	33
Substrate concentration	34
Response Surface Methodology	35
Application of RSM to the enzymatic Reactions	38
3 MATERIALS AND METHODS	
Materials and Chemicals	42
Thin Layer Chromatography (TLC)	42
Fourier Transform-Infrared Spectroscopy (FT-IR)	44
Direct Induction Probe-Mass Spectrometry (DIP-MS)	44



Nuclear Magnetic Resonance (NMR)	44
Synthesis of 3- <i>O</i> -(3',3'-dimethylsuccinyl)-betulinic acid	45
Esterification reaction	45
Isolation and purification for betulinic acid ester	45
Optimization Studies	46
Study on Individual Parameter Effects on Enzymatic	47
Reaction between Betulinic Acid and 2,2-	
dimethylsuccinic anhydride	
Effect of Different Reaction Time on the	47
Esterification Reaction	
Effect of Different Reaction Temperature on the	47
Esterification Reaction	
Effect of Different Amount of Enzyme on the	47
Esterification Reaction	
Effect of Different Amount of Anhydride on the	48
Esterification Reaction	
Effect of Reused Enzyme on the Esterification	48
Reaction	
Study on Interactive effects of Enzymatic reaction	48
Parameters and their Optimization Using Response	
Surface Methodology (RSM)	
RSM Design	49
Statistical and Graphical Analyses	49
Reaction Optimisation and Model Validation	50
Reaction for Optimization	51
Scaling –Up of 3- <i>O</i> -(3',3'-dimethylsuccinyl)-betulinic	51
acid Production	
Bioassay Screening Method	52
Cytotoxic Activity	53
PRELIMINARY STUDY	
Synthesis of bi-functional betulinic acid derivatives	54

4

RESULTS AND DISCUSSION

Analysis of Betulinic Acid	55
Spectral characterization of betulinic acid	55
Esterification Reaction (Reaction of Betulinic Acid With	55
2,2-Dimethylsuccinic Anhydride)	55
Identification of the product	
Optimization of Esterification by Individual Parameters	58
Effect of Reaction Time	60
Effect of Different Reaction Temperature	71
Effect of Different Amount of Enzyme	71
Effect of Different Amount of Anhydride (molar	72
ratio)	73



	Effect of Reused Enzyme on the Esterification Reaction	75
	Optimization of Esterification Reaction by RSM	76
	Analysis of Variance (ANOVA)	76
	Regression Analysis	80
	Response Surface Analysis	81
	Interactive Effect of Reaction Time and Reaction Temperature (X_1X_2)	81
	Interactive Effect of Reaction Time and Amount of Enzyme (X_1X_3)	82
	Interactive Effect of Reaction Temperature and Amount of Enzyme (X_2X_3)	83
	Optimization and Model Validation	84
	Scaling –Up production of 3- <i>O</i> -(3',3'-dimethylsuccinyl)-betulinic acid	85
	Biological Activity Studies	86
	Preliminary Result	90
	Biological activity of preliminary product	93
5	CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH	
	Conclusions	95
	Recommendations for further studies	97
	REFERENCES	98
	APPENDICES	105
	BIODATA OF STUDENT	126
	LIST OF PUBLICATIONS	127

