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ENZYMATIC SYNTHESIS OF 3-*O*-ACYLBETULINIC ACID DERIVATIVES AND PREDICTION OF ACYLATION USING RESPONSE SURFACE METHODOLOGY AND ARTIFICIAL NEURAL NETWORK ANALYSES

MANSOUR GHAFFARI MOGHADDAM

FS 2010 26

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By

MANSOUR GHAFFARI MOGHADDAM

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

February 2010

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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February 2010

Chairman: Professor Faujan H. Ahmad, PhD

Faculty: Science

In this study, 3-*O*-acyl-betulinic acid derivatives were synthesized by the reaction of betulinic acid with various anhydrides using lipase as a biocatalyst in organic solvents. The reaction between betulinic acid and phthalic anhydride was chosen as the model reaction for optimization studies. The immobilized lipase from *Candida antarctica* (Novozym 435) was selected as a biocatalyst. The effects of different reaction parameters were investigated and optimized in the model reaction using *one-variable-at-a-time* technique for the first time. Optimum conditions to produce 3-*O*-phthalyl-betulinic acid up to 61.8% were observed at a reaction time of 24 hours; amount of enzyme, 176 mg; betulinic acid to phthalic anhydride molar ratio of 1:1; amount of celite, 170 mg and 6 mg of K_2CO_3 in a mixture of *n*-hexane-chloroform (1:1, v/v) as organic solvent at 55°C.

The response surface methodology (RSM), based on a five-level, four-variable central composite rotatable design (CCRD), was employed to evaluate the effects of synthesis

parameters of the model reaction. Using the RSM analysis, it was observed that the maximum yield of 3-*O*-phthalyl-betulinic acid (65.8%) could be obtained using 145.6 mg of enzyme, reaction temperature of 53.9°C, reaction time of 20.3 hours and betulinic acid to phthalic anhydride molar ratio of 1:1.11. The actual experimental value obtained was at 64.7%.

Artificial neural network (ANN) was successfully developed to model and predict the enzymatic synthesis of 3-*O*-phthalyl-betulinic acid. The network consists of an input layer, a hidden layer and an output layer. Inputs for the network were reaction time, reaction temperature, enzyme amount and substrate molar ratio, while the output was percentage isolated yield of ester. Four different training algorithms, belonging to two classes, namely gradient descent and Levenberg-Marquardt, were used to train ANN. The best results were obtained from the quick propagation algorithm (QP) with 4-9-1 topology. Based on the ANN analysis, the optimal conditions to obtain the highest yield were 148.3 mg enzyme, reaction temperature of 53.1°C, reaction time of 20.3 hours and betulinic acid to phthalic anhydride molar ratio of 1:1.24. The predicted and actual yields were 64.9 and 64.3%, respectively. In this work, the ANN and RSM analysis were investigated on the enzymatic synthesis of 3-*O*-phthalyl-betulinic acid for the first time.

Finally, several betulinic acid esters (compounds 57-66) were synthesized using the optimal operation conditions which were obtained by the RSM technique. Esterification of betulinic acid with various anhydrides was performed at 54ºC in a mixture of *n*-hexane-chloroform (1:1, v/v) for 20.3 hours, catalyzed by Novozym 435, gave 24.7 to 79.3% yield. Five new compounds (58, 62, 64, 65 and 66) were synthesized for the first time in the present study.

In brief, the anti-cancer activity of betulinic acid (1) and its 3-*O*-acylated derivatives (compounds 57-66) were evaluated against human lung carcinoma (A549) and human ovarian (CAOV3) cancer cell lines. In particular, compounds (59), (61) and (63) were found to show IC_{50} < 10 µg/ml against A549 cancer cell line tested and showed better cytotoxicity than betulinic acid. In the ovarian cancer cell line, all betulinic acid derivatives prepared revealed weaker cytotoxicity than betulinic acid.

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

SINTESIS ENZIMATIK TERBITAN ASID 3-*O*-ASILBETULINIK DAN ASILASI PREDIKSI MENGGUNAKAN KAEDAH TINDAK BALAS PERMUKAAN DAN RANGKAIAN NEURAL BUATAN

Oleh

MANSOUR GHAFFARI MOGHADDAM

Februari 2010

Pengerusi: Profesor Faujan H. Ahmad, PhD

Fakulti: Sains

Di dalam kajian ini, terbitan asid 3-*O*-asil-betulinik telah disintesiskan daripada tindak balas asid betulinik dan pelbagai anhidrida dengan menggunakan lipase sebagai biopemangkin dalam kehadiran pelarut organik. Tindak balas antara asid betulinik dan ftalik anhidrida telah dipilih sebagai model untuk kajian pengoptimuman. Lipase immobilized dari *Candida antarctica* (Novozim 435) telah dipilih sebagai biopemangkin. Sementara itu, kesan untuk pelbagai parameter juga telah dikaji dan dioptimumkan sebagai model tindak balas dengan menggunakan teknik *satu-variasi-pada-satu-masa* untuk pertama kali. Keadaan optimum untuk penghasilan asid 3- *O*-fthalil- betulinik sehingga 61.8% telah diperolehi dalam masa tindak balas 24 jam, kuantiti enzim 176 mg, asid betulinik kepada ftalik anhidrida nisbah molar 1:1, kuantiti celit 170 mg dan 6 mg K_2CO_3 dalam campuran klorofom-*n*-heksana (1:1, v/v) sebagai pelarut organic pada suhu 55°C.

Kaedah tindak balas permukaan (RSM) berdasarkan lima peringkat, empat pemalar bolehubah rekabentuk komposit putaran tengah (CCRD) telah digunakan untuk menilai kesan parameter sintesis. Menggunakan analisis RSM, hasilan maksimum asid 3-*O*-fthalil-betulinik (65.8%) telah didapati dengan menggunakan 145.6 mg enzim, suhu reaksi pada 53.9°C, masa reaksi pada 20.3 jam dan asid betulinik kepada ftalik anhidrida pada nisbah molar 1:1.11. Nilai untuk experimen sebenar yang terdapat adalah sebanyak 64.7%.

Rangkaian neural buatan (ANN) telah berjaya membangunkan pemodelan dan ramalan untuk sintesis enzimatik asid 3-*O*-fthalil-betulinik. Rangkaian ini mengandungi lapisan masukan iaitu lapisan terlindung dan lapisan keluaran. Masukan untuk rangkaian adalah masa reaksi, suhu reaksi, kuantiti enzim dan nisbah molar substrak, sementara keluaran adalah peratus hasilan ester yang terpisah. Empat latihan algoritma yang berbeza tertakluk kepada dua kelas, iaitu Gradient Descent dan Levenberg–Marquardt telah digunakan untuk percubaan ANN. Keputusan terbaik telah didapati dari algoritma Propagasi Maju (QP) dengan topologi 4-9-1. Berdasarkan analisis ANN, dan keadaan optimum untuk mendapatkan hasil tertinggi adalah 148.3 mg enzim, suhu reaksi pada 53.1°C, masa reaksi pada 20.3 jam dan asid betulinik kepada ftalik anhidrida pada nisbah molar 1:1.24. Hasil ramalan dan hasil sebenar masing-masingnya adalah 64.9 dan 64.3%. Dalam kajian ini, analisis ANN dan RSM telah dikaji ke atas sintesis enzimatik untuk asid 3-*O*-ftalil-betulinik pada pertama kali.

Akhirnya, beberapa ester asid betulinik (57-66 sebatian) telah disintesis dengan menggunakan keadaan operasi optimum yang terdapat dalam teknik RSM. Pengesteran untuk asid betulinik dengan pelbagai anhidrida telah dijalankan pada suhu 54ºC dalam

campuran *n*- heksana-klorofom (1:1, v/v) bagi 20.3 jam dimangkinkan dengan Novozim 435, memberi 24.7% sehingga 79.3% hasilan. Lima sebatian baru (58, 62, 64, 65 and 66) telah disintesiskan untuk pertama kali dalam kajian semasa.

Secara ringkas, aktiviti anti-kanser untuk asid betulinik asid (1) dan derivatif 3-*O*-asilan (sebatian 57-66) telah dinilaikan ke atas karsinoma peparu manusia (A549) dan kanser sel stem ovari manusia (CAOV3). Sebatian (59), (61) and (63) menunjukkan IC_{50} < 10 µg/ml ke atas cubaan A549 kanser sel stem dan memperolehi sitotoksik yang lebih baik daripada asid betulinik. Dalam kanser sel stem ovari, semua derivative asid betulinik menunjukkan sitotoksik yang lemah daripada asid betulinik.

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I certify that a Thesis Examination Committee has met on 25 February 2010 to conduct the final examination of Mansour Ghaffari Moghaddam on his thesis entitled "Enzymatic Synthesis of 3-*O*-Acylbetulinic Acid Derivatives and Prediction of Acylation using Response Surface Methodology and Artificial Neural Network Analyses" 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 the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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Date: 15 April 2010

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

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Date: 13 May 2010

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 Universiti Putra Malaysia or at any other institution.

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MANSOUR GHAFFARI MOGHADDAM

Date: 22 March 2010

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