

ผลของการดัดแปลงโครงสร้างทางเคมีของกลาบริดิน
ต่อฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนสและฤทธิ์ต้านอนุมูลอิสระ

นางสาววารุณี จิรวัดนาพงศ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

สาขาวิชาเภสัชเคมีและผลิตภัณฑ์ธรรมชาติ

คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2549

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย



4 4 7 6 9 8 2 3 3 3

EFFECTS OF CHEMICAL STRUCTURE MODIFICATION OF GLABRIDIN
ON TYROSINASE INHIBITION AND FREE RADICAL SCAVENGING ACTIVITY

Miss Warunee Jirawattanapong

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Pharmaceutical Chemistry and Natural Products

Faculty of Pharmaceutical Sciences

Chulalongkorn University

Academic Year 2006

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Thesis Title EFFECTS OF CHEMICAL STRUCTURE MODIFICATION OF GLABRIDIN ON TYROSINASE INHIBITION AND FREE RADICAL SCAVENGING ACTIVITY


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Field of Study Pharmaceutical Chemistry and Natural Products

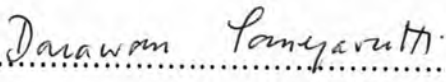
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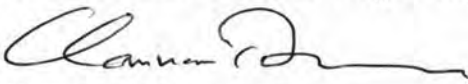
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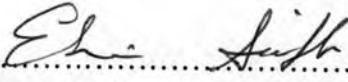
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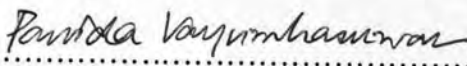
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การวิจัยนี้เป็นการศึกษาผลของการดัดแปลงโครงสร้างทางเคมีของกลาบรีดินซึ่งเป็นสารกลุ่ม isoflavan ที่แยกได้จากสารสกัดชะเอมเทศ ต่อการออกฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนสและฤทธิ์ต้านอนุมูลอิสระ ได้ทำการสังเคราะห์อนุพันธ์ของกลาบรีดินได้แก่ อนุพันธ์ diacyl 5 ชนิด และอนุพันธ์ dibenzoyl 7 ชนิด โดยเป็นการแทนที่หมู่ฟังก์ชัน phenolic hydroxyl ที่ตำแหน่ง C-2' และ C-4' ของกลาบรีดิน โดยใช้ acid anhydrides และ acid chlorides ตามลำดับ รวมทั้งสังเคราะห์อนุพันธ์ 3",4"-dihydroglabridin อีก 1 ชนิด

ได้นำอนุพันธ์ที่เตรียมได้ทั้งหมดมาทดสอบฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนสและฤทธิ์ต้านอนุมูลอิสระ พบว่า 3",4"-dihydroglabridin เป็นอนุพันธ์ชนิดเดียวที่มีฤทธิ์ดีกว่าสารตั้งต้น ซึ่ง 3",4"-dihydroglabridin มีฤทธิ์แรงในการยับยั้งเอนไซม์ไทโรซิเนสโดยมีค่า IC_{50} เท่ากับ $11.40 \mu M$ แต่มีฤทธิ์อ่อนในการต้านอนุมูลอิสระ จากการศึกษาถึงความสัมพันธ์ระหว่างโครงสร้างทางเคมีและการออกฤทธิ์พบว่า หมู่ฟังก์ชัน 4-substituted resorcinol มีความสำคัญต่อการออกฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนส และการที่ 3",4"-dihydroglabridin ไม่มี double bond ระหว่างคาร์บอนตำแหน่ง 3" และ 4" ทำให้มีความยืดหยุ่นของโครงสร้างในการเข้าทำปฏิกิริยากับเอนไซม์ได้ดีขึ้น ส่วนหมู่ฟังก์ชัน phenolic hydroxyl มีความสำคัญต่อการออกฤทธิ์ต้านอนุมูลอิสระ

การศึกษาความสามารถของการละลายในชั้นไขมันของอนุพันธ์ diacyl ester ของกลาบรีดิน ทำได้โดยการหาค่า partition coefficient (log P) ระหว่างชั้น *n*-octanol และชั้น phosphate buffer pH 5.5 พบว่าอนุพันธ์ชนิดที่ 18-20 มีค่า log P สูงกว่าสารตั้งต้น นอกจากนี้ยังได้ทำการศึกษาการเกิด hydrolysis ทั้งจากเอนไซม์และจากปฏิกิริยาเคมี พบว่าอนุพันธ์ diacetate เกิดปฏิกิริยา hydrolysis จากการเร่งของเอนไซม์เอสเทอร์สได้อย่างรวดเร็วโดยมีค่าครึ่งชีวิตเท่ากับ 2.34 นาที ขณะที่มีความคงตัวใน phosphate buffer ทั้งที่ pH 5.5 และ 7.4 ที่อุณหภูมิ $37^{\circ}C$ โดยมีค่าครึ่งชีวิตมากกว่า 15 วัน

สาขาวิชา เกษษเคมีและผลิตภัณฑ์ธรรมชาติ

ปีการศึกษา 2549

ลายมือชื่อนิสิต.....

ลายมือชื่ออาจารย์ที่ปรึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4476982333: MAJOR PHARMACEUTICAL CHEMISTRY AND NATURAL PRODUCTS

KEYWORD: GLABRIDIN/ CHEMICAL STRUCTURE MODIFATION/ TYROSINASE ACTIVITY/ FREE RADICAL SCAVENGING ACTIVITY

WARUNEE JIRAWATTANAPONG: EFFECTS OF CHEMICAL STRUCTURE MODIFATION OF GLABRIDIN ON TYROSINASE INHIBITION AND FREE RADICAL SCAVENGING ACTIVITY: THESIS ADVISOR: ASST. PROF. CHAMNAN PATARAPANICH, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. EKARIN SAIFAH, Ph.D., 137 pp.

This research was aimed to study the effects of structural modification of glabridin, a pyranoisoflavan isolated from licorice extract, on tyrosinase inhibitory and free radical scavenging activities. Five diacyl esters and seven dibenzoyl esters were successfully prepared on the phenolic hydroxyls at C-2' and C-4' of glabridin with the corresponding acid anhydrides and acid chlorides. The 3'',4''-dihydroglabridin was also synthesized.

All of the synthesized compounds were evaluated for tyrosinase inhibitory and free radical scavenging activities. Among the tested compounds, only the 3'',4''-dihydroglabridin exhibited higher activity than the parent compound. The 3'',4''-dihydroglabridin showed potent tyrosinase inhibitory activity with the IC₅₀ value of 11.40 μM, but it was a weak free radical scavenger. The study of structure-activity relationships of these synthesized derivatives indicate that the 4-substituted resorcinol skeleton is essential for tyrosinase inhibitory activity and the lacking of double bond between carbon atom 3'' and 4'' on the structure of the 3'',4''-dihydroglabridin, give more conformational flexibility to interact with the enzyme more effectively. While the phenolic hydroxyl moiety plays a critical role in free radical scavenging activity.

The lipophilicity of the diacyl esters were obtained as *n*-octanol/phosphate buffer pH 5.5 partition coefficient (log P). The esters 18-20 showed increased log P as comparison with the parent compound. In addition, the chemical and enzymatic hydrolysis of the glabridin diacetate ester has been investigated. The results indicated that the diacetate ester was rapidly hydrolyzed by porcine liver esterase with the half-life of 2.34 min, while it was sufficiently stable in phosphate buffer, both pH 5.5 and 7.4, at 37 °C with more than 15 days half-life.

Field of Study: Pharmaceutical Chemistry
and Natural Products

Academic Year: 2006

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ACKNOWLEDGEMENTS

I am deeply indebted to my thesis advisor, Assistant Professor Chamnan Patarapanich, and my thesis co-advisor, Associate Professor Ekarin Saifah, for their valuable advice, continual guidance, helpful discussion and suggestion throughout my period of study.

It is great pleasure to acknowledge Doctor Pisarn Jantaritthirathsamee of Garguar lab Co Ltd., for providing licorice extract (PT-40) as a starting material of this research work.

I would like to thank the Graduate School of Chulalongkorn University, for granting partial financial supports to conduct this investigation.

I would like to thank the Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences for providing 300 MHz NMR spectrometer and other scientific equipments, the Scientific and Technological Research Equipment Center, Chulalongkorn University for elemental analysis.

I am very grateful to Associate Professor Pensri Thongnopnua of Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University and to all my colleagues at Medicinal Plants Research Institute, Department of Medical Sciences, Ministry of Public Health, for their kindness and facilities.

I would also like to express my appreciation and thanks to all my friends at Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for their contributions and unforgettable friendships.

I would like to thank the members of thesis committee for their valuable suggestions and discussions.

Finally, I would like to express my special and deepest appreciation to my family, for their love, understanding and encouragement.

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ABBREVIATIONS AND SYMBOLS

%	=	percentage
δ	=	Chemical shift
μg	=	microgram
μl	=	microliter
μM	=	micromolar
$^{\circ}\text{C}$	=	degree Celsius
$^1\text{H-NMR}$	=	proton nuclear magnetic resonance
$^{13}\text{C-NMR}$	=	carbon-13-nuclear magnetic resonance
2D NMR	=	two dimensional nuclear magnetic resonance
ax	=	axial
AUC	=	area under curve
br d	=	broad doublet
br s	=	broad singlet
calcd	=	calculated
cm^{-1}	=	Reciprocal centimeter (unit of wave number)
conc	=	concentration
CDCl_3	=	deuterated chloroform
CH_2Cl_2	=	dichloromethane
CHCl_3	=	chloroform
Cu	=	copper
Cys	=	cysteine
d	=	doublet (for NMR spectra)
dd	=	doublet of doublets (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
DMSO-d_6	=	deuterated dimethylsulfoxide
DPPH	=	1,1-diphenyl-2-picrylhydrazyl
eq.	=	equatorial
EtOAc	=	ethyl acetate
g	=	gram
hr	=	hour
$^1\text{H-}^1\text{H COSY}$	=	$^1\text{H-}^1\text{H}$ correlation spectroscopy

HMBC	=	Heteronuclear Multiple Bond Correlation
HMQC	=	Heteronuclear Multiple Quantum Coherence
HRMS	=	high resolution mass spectrometry
Hz	=	hertz
IC ₅₀	=	Median inhibition concentration
IR	=	infrared spectrometry
IS	=	internal standard
<i>J</i>	=	coupling constant (for NMR spectra)
kDa	=	kilodalton
log P	=	partition coefficient
<i>m</i>	=	<i>meta</i>
m	=	multiplet (for NMR spectra)
mg	=	milligram
min	=	minute
ml	=	milliliter
<i>m/z</i>	=	mass to charge
M ⁺	=	molecular ion
MeOH	=	methanol
MHz	=	megahertz
mM	=	millimolar
mult	=	multiplicity (for NMR spectra)
nm	=	nanometer
NMR	=	Nuclear Magnetic Resonance
<i>o</i>	=	<i>ortho</i>
<i>p</i>	=	<i>para</i>
ppm	=	part(s) per million
Pd/C	=	Palladium on activated carbon
rpm	=	revolution per minute
RT	=	room temperature
s	=	singlet (for NMR spectra)
sec	=	second
spp	=	species
t	=	triplet (for NMR spectra)
td	=	triplet of doublet

TLC	=	Thin layer chromatography
TOF-MS	=	Time-of-Flight technique mass spectroscopy
Tyr	=	Tyrosine
UV	=	ultraviolet spectrophotometry