

การแยกและตัดแปลง โครงสร้างของออกซีเรสเวอราทรอลจากมะหาดเพื่อฤทธิ์ยับยั้งไทโรซิเนส

นายอาคม สอนสุทธิ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต

สาขาวิชาเภสัชเวท ภาควิชาเภสัชเวท

คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2549

ISBN 974-14-3820-6

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

ISOLATION AND STRUCTURE MODIFICATION OF OXYRESVERATROL FROM  
*ARTOCARPUS LAKOOCHA* FOR TYROSINASE INHIBITORY ACTIVITY

Mr. Acom Sornsute

A Thesis Submitted in Partial Fulfillment of the Requirements  
for the Degree of Master of Science in Pharmacy Program in Pharmacognosy

Department of Pharmacognosy  
Faculty of Pharmaceutical Sciences

Chulalongkorn University

Academic Year 2006

ISBN 974-14-3820-6

**491972**

Thesis Title ISOLATION AND STRUCTURE MODIFICATION OF  
OXYRESVERATROL FROM *ARTOCARPUS LAKOOCHA* FOR  
TYROSINASE INHIBITORY ACTIVITY

By Mr. Acom Sornsute

Field of study Pharmacognosy

Thesis Advisor Associate Professor Kittisak Likhitwitayawuid, Ph.D.

Thesis Co-advisor Associate Professor Sumphan Wongseripipatana, Ph.D.

---

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial  
Fulfillment of the Requirements for the Master's Degree.

..... Dean of the Faculty of Pharmaceutical Sciences

(Associate Professor Pompen Pramyothin, Ph.D.)


#### THESIS COMMITTEE

..... Chairman  
(Associate Professor Ekarin Saifah, Ph.D.)

..... Thesis Advisor  
(Associate Professor Kittisak Likhitwitayawuid, Ph.D.)

..... Thesis Co-advisor  
(Associate Professor Sumphan Wongseripipatana, Ph.D.)

..... Member  
(Poonsakdi Ploypradith, Ph.D.)

..... Member  
(Boonchoo Sritularak, Ph.D.)

อาคม สอนสุทธิ : การแยกและดัดแปลงโครงสร้างของออกซีเรสเวอราทรอลจากมะหาดเพื่อฤทธิ์ยับยั้งไทโรซิเนส. (ISOLATION AND STRUCTURE MODIFICATION OF OXYRESVERATROL FROM *ARTOCARPUS LAKOOCHA* FOR TYROSINASE INHIBITORY ACTIVITY) อ. ที่ปรึกษา : รศ.ดร. กิตติศักดิ์ ลิขิตวิทย์วสุฒิ, อ.ที่ปรึกษาร่วม : รศ.ดร. ลัมพันธ์ วงศ์เสรีพัฒนา, จำนวนหน้า 194 หน้า. ISBN 974-14-3820-6.

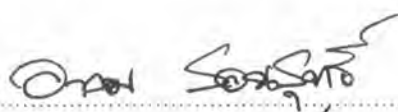
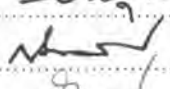

สารออกซีเรสเวอราทรอล (2,4,3',5'-tetrahydroxystilbene) เป็นสารหลักที่พบได้จากต้นมะหาด ซึ่งเป็นที่ทราบดีว่ามีฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนสที่แรง ในการศึกษาครั้งนี้ได้มุ่งไปยังการแยกและการดัดแปลงโครงสร้างของสารออกซีเรสเวอราทรอลเพื่อศึกษาฤทธิ์ในการยับยั้งเอนไซม์ไทโรซิเนส ในการแยกสารพบว่าการแยกด้วยอุปกรณ์ซอกเลต(soxhlet apparatus)และเอธานอลให้สารสกัดมากที่สุด และอลูมิเนียมออกไซด์เป็นสารดูดซับที่มีประสิทธิภาพดีในการแยกสารสกัด

ในครั้งนี้ได้พัฒนาการวิเคราะห์ที่ใช้รีเวอร์สเฟส เอชพีแอลซี จากการประเมินค่าความถูกต้อง ความแม่นยำ ความตรง ขอบจำกัดการตรวจวัด และขอบจำกัดการหาปริมาณ วิธีนี้ได้ใช้ในการหาปริมาณสารออกซีเรสเวอราทรอลจากสารสกัดพืชและปวกหาคัด ซึ่งผลเป็นที่น่าพอใจและน่าเชื่อถือ

ได้มีการเตรียมสารอนุพันธ์ขึ้นสิบสามอนุพันธ์ และได้ทดสอบฤทธิ์พบว่าสารที่เตรียมได้เกือบทั้งหมดสูญเสียฤทธิ์ในการยับยั้งเอนไซม์ แต่ไดไฮโดรออกซีเรสเวอราทรอลแสดงฤทธิ์ที่แรงกว่าออกซีเรสเวอราทรอลและกรดโคจิกที่มีการใช้ในผลิตภัณฑ์เครื่องสำอาง การศึกษาจลนศาสตร์ของเอนไซม์พบว่าสารไดไฮโดรออกซีเรสเวอราทรอลยับยั้งเอนไซม์แบบไม่แข่งขันซึ่งเหมือนกับออกซีเรสเวอราทรอล ในขณะที่กรดโคจิกยับยั้งเอนไซม์แบบผสม

นอกจากนี้ได้ศึกษาฤทธิ์ในการเป็นพิษต่อเซลล์ของสารอนุพันธ์ที่ได้จากการเติมหมู่เมทอกซิลแทนที่หมู่ไฮดรอกซีต่อเซลล์มะเร็งชนิด KB BC และ NCI พบว่าโครงสร้างแบบซัสของออกซีเรสเวอราทรอลที่เติมหมู่เมทอกซิลทั้งสี่หมู่แสดงฤทธิ์ยับยั้งที่แรงเมื่อเทียบกับอัลลิปีตินและดอกซีรูบิซิน

สิ่งที่พบจากการศึกษาครั้งนี้แสดงให้เห็นว่าออกซีเรสเวอราทรอลสามารถเป็นสารตั้งต้นที่ดีในการพัฒนาสารช่วยทำให้ผิวขาวและยาต้านมะเร็งต่อไป

ภาควิชา .....	เภสัชเวช .....	ลายมือชื่อนิสิต .....	
สาขาวิชา .....	เภสัชเวช .....	ลายมือชื่ออาจารย์ที่ปรึกษา .....	
ปีการศึกษา .....	2549 .....	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....	

# # 4676608933: MAJOR PHARMACOGNOSY

KEY WORDS: OXYRESVERATROL/ TYROSINASE/ *ARTOCARPUS LAKOOCHA*/ WHITENING/  
STILBENE/ CYTOTOXICITY

ACOM SORNROUTE : ISOLATION AND STRUCTURE MODIFICATION OF OXYRESVERATROL  
FROM *ARTOCARPUS LAKOOCHA* FOR TYROSINASE INHIBITORY ACTIVITY. THESIS ADVISOR  
: ASSOC. PROF. KITTISAK LIKHITWITAYAWUID, Ph.D., THESIS CO-ADVISOR : ASSOC. PROF.  
SUMPAN WONGSERIPIPATANA, Ph.D., 194 pp. ISBN 974-14-3820-6.

Oxyresveratrol (2,4,3',5'-tetrahydroxystilbene), a major compound found in the heartwood of *Artocarpus lakoocha* Roxb. (Moraceae), has been known for its potent tyrosinase inhibitory activity. This study focused primarily on the isolation and structure modification of oxyresveratrol for tyrosinase inhibitory activity. For the isolation experiments, it was found that the EtOH-soxhlet extraction method gave the highest yield of crude oxyresveratrol, and  $Al_2O_3$  was a good adsorbent for separation of the obtained extract.

An analytical method using reverse-phase high performance liquid chromatography was developed. The method was validated on the parameters for accuracy, precision, linearity, limit of detection and limit of quantitation. The method was used for quantitative determination of oxyresveratrol in plant extracts and Puag-Haad. The results were satisfactory and reliable.

Thirteen derivatives of oxyresveratrol have been prepared, and their anti-tyrosinase activity was determined. Almost all of the analogues prepared in this study were devoid of activity, but dihydroxyresveratrol showed more potent activity than did the parent compound and kojic acid, a strong tyrosinase inhibitor widely used in cosmetic products. Kinetic studies on the enzyme indicated that dihydroxyresveratrol had higher affinity to tyrosinase than the two compounds. Dihydroxyresveratrol, similar to oxyresveratrol, showed non-competitive inhibition property while kojic acid was a mixed-type inhibitor.

Additionally, cytotoxicity studies were performed on the O-methylated compounds using KB, BC and NCI-H187 cancer cells. *Cis*-tetra-O-methylated oxyresveratrol showed strong cytotoxicity, with potency comparable to those of ellipticine and doxyrubicin.

The findings in this study suggest that oxyresveratrol could be a good source of starting material for the development of whitening agents and anticancer drugs.

Department of ..... Pharmacognosy ..... Student's signature ..... *Acorn Sornroute*  
Field of study ..... Pharmacognosy ..... Advisor's signature ..... *K. Likhit*  
Academic year ..... 2006 ..... Co-advisor's signature ..... *S. Wongseripipatana* .....

## Acknowledgements

I wish to express my sincere gratitude and thanks to Associate Professor Dr. Kittisak Likhitwitayawuid, my thesis advisor and Associate Professor Dr. Sumphan Wongseripipatana, my thesis co-advisor, the present and the former Head of the Department of Pharmacognosy, respectively, for their close supervision, guidance, suggestions, supports and encouragements throughout the course of this study, as well as editing of this dissertation.

I am deeply indebted to Dr. Poonsakdi Ploypradith, Chulabhorn Research Institute and Professor Hiromitsu Takayama, Faculty of Pharmaceutical Sciences, Chiba University for their guidance, suggestion and encouragement which has enabled me to carry out this work. Their precious instructions are not only confined to the scientific study but also expanded to the general concepts and arts of life. The kindness and devotion that I received will be long remembered.

I would like to thank Associate Professor Dr. Wanchai De-Eknamkul, and Dr. Boonchoo Sritularak, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University for providing facilities and advice regarding the anti-tyrosinase activity evaluation.

I would like to express my sincere thanks to the Departments of Pharmaceutical Chemistry and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University for providing research facilities during my study.

I would also like to acknowledge the Graduate School of Chulalongkorn University for partial financial support.

Bioassay Research Facility of National Center for Genetic Engineering and Biotechnology (BIOTEC) is also gratefully acknowledged for cytotoxicity tests.

I wish to express my infinite gratitude to my friends for their love, understanding, assistance, and encouragement throughout this graduate study.

My final thank goes to my family. For all their love, understanding and support, there is no adequate way to express my gratitude.

# CONTENTS

	Page
ABSTRACT (Thai) .....	iv
ABSTRACT (English) .....	v
ACKNOWLEDGEMENT .....	vi
CONTENTS .....	vii
LIST OF TABLES.....	xiii
LIST OF FIGURES.....	xv
ABBREVIATIONS.....	xix
CHAPTER	
I INTRODUCTION.....	1
II HISTORICAL.....	5
1. Chemical constituents of <i>Artocarpus lakoocha</i> Roxb.....	5
2. Oxyresveratrol.....	10
3. Tyrosinase.....	10
3.1. Domain structure of mushroom tyrosinase .....	11
3.2. Study of mushroom tyrosinase.....	14
III EXPERIMENTAL.....	22
1. Sources of plant material.....	22
2. General techniques .....	22

2.1. Thin-layer chromatography (TLC) .....	22
2.2. Preparative thin-layer chromatography.....	23
2.3. Vacuum liquid column chromatography .....	23
2.4. Medium pressure liquid chromatography.....	24
2.5. Spectroscopy .....	24
2.5.1. Ultraviolet (UV) absorption spectra .....	24
2.5.2. Infrared (IR) absorption spectra .....	25
2.5.3. Mass spectra.....	25
2.5.4. Proton and Carbon-13 nuclear magnetic resonance ( <sup>1</sup> H- and <sup>13</sup> C-NMR) spectra.....	25
2.6. Solvents.....	25
3. Studies of methods for the isolation of oxyresveratrol.....	26
3.1. Extraction.....	26
3.1.1. The reflux method.....	26
3.1.2. The soxhlet method .....	26
3.1.3. The sonication method.....	26
3.1.4. The maceration method.....	26
3.2. Separation .....	29
3.3. Isolation of oxyresveratrol from Puag-Haad by MPLC.....	32
4. Method for quantitative analysis of oxyresveratrol.....	33
4.1. High performance liquid chromatography system .....	33



4.2. Internal standard .....	33
4.3. Standard curve .....	40
4.4. Method validation .....	42
4.4.1. Accuracy .....	42
4.3.2. Precision .....	43
4.3.3. Linearity .....	45
4.3.4. LOD and LOQ .....	46
5. Quantitative analysis of oxyresveratrol in extracts and Puag-Haad .....	47
6. Structure modification of oxyresveratrol .....	51
6.1. Preparation of oxyresveratrol tetra-ethylcarbonate (AS-1) .....	50
6.2. Preparation of oxyresveratrol tetra-diethylcarbamate (AS-2) .....	51
6.3. Preparation of oxyresveratrol tetra-acetate (AS-3) .....	51
6.4. Preparation of oxyresveratrol tetra-benzoate (AS-4) .....	52
6.5. Preparation of tetra- <i>O</i> -phenylmethyl oxyresveratrol (AS-5) .....	52
6.6. Preparation of tetra- <i>O</i> -methyl oxyresveratrol (AS-6) .....	53
6.7. Preparation of dihydroxyresveratrol (AS-7) .....	53
6.8. Preparation of 2,3'-di- <i>O</i> -methyl oxyresveratrol (AS-8), 4,3'-di- <i>O</i> -methyl oxyresveratrol (AS-9) and 2- <i>O</i> -methyl oxyresveratrol (AS-10) .....	54
6.9. Preparation of 2'-chloro-oxyresveratrol (AS-11) .....	55
6.10. Preparation of <i>cis</i> -tetra- <i>O</i> -methyl oxyresveratrol (AS-12) .....	55

6.11. Preparation of tetra- <i>O</i> -methyl dihydroxyresveratrol (AS-13) .....	56
7. Tyrosinase activity .....	61
7.1. Materials and instruments .....	61
7.2. Determination of tyrosinase inhibitory activity.....	61
7.3. Preparation of the reaction mixture .....	62
7.3.1 Preparation of 20 mM phosphate buffer (pH 6.8) .....	62
7.3.2 Preparation of 0.85 mM L-DOPA .....	62
7.3.3 Preparation of tyrosinase solution .....	62
7.3.4 Preparation of the test sample.....	62
7.4. Measurement of activity .....	62
7.4.1 Substrate-inhibitor pre-incubation method.....	62
7.4.2 Enzyme-inhibitor pre-incubation method .....	64
7.5. Preliminary evaluation of tyrosinase activity .....	64
7.6. Determination of IC <sub>50</sub> .....	64
7.7. Enzyme kinetics.....	65
8. Cytotoxicity assays .....	66
IV RESULTS AND DISCUSSION .....	67
1. Study of the isolation methods of oxyresveratrol from the heartwood of <i>Artocarpus lakoocha</i> .....	67
1.1. Extraction.....	67
1.2. Separation of the EtOH extract.....	67

1.3. Isolation of oxyresveratrol from Puag-Haad by MPLC.....	68
1.4. Purification.....	69
2. Analytical method for determination of oxyresveratrol in extracts and Puag-Haad.....	70
3. Structural characterization of the modified compounds.....	70
3.1. Structural characterization of oxyresveratrol tetra-ethylcarbonate (AS-1).....	70
3.2. Structural characterization of oxyresveratrol tetra-diethylcarbamate (AS-2).....	71
3.3. Structural characterization of oxyresveratrol tetra-acetate (AS-3).....	72
3.4. Structural characterization of oxyresveratrol tetra-benzoate (AS-4).....	73
3.5. Structural characterization of tetra- <i>O</i> -phenylmethyl oxyresveratrol (AS-5).....	74
3.6. Structural characterization of tetra- <i>O</i> -methyl oxyresveratrol (AS-6).....	74
3.7. Structural characterization of dihydrooxyresveratrol (AS-7).....	75
3.8. Structural characterization of 2,3'-di- <i>O</i> -methyl oxyresveratrol (AS-8).....	76
3.9. Structural characterization of 4,3'-di- <i>O</i> -methyl oxyresveratrol (AS-9).....	77
3.10. Structural characterization of 2- <i>O</i> -methyl oxyresveratrol (AS-10).....	77
3.11. Structural characterization of 6-chloro-oxyresveratrol (AS-11).....	78
3.12. Structural characterization of <i>cis</i> -tetra- <i>O</i> -methyl oxyresveratrol (AS-12).....	79

3.13. Structural characterization of tetra- <i>O</i> -methyl dihydroxyresveratrol (AS-13) .....	80
4. Tyrosinase activity .....	80
4.1. Screening for the tyrosinase inhibitory activity .....	80
4.2. Enzyme kinetics .....	84
5. Cytotoxic assay .....	89
V CONCLUSION .....	91
REFERENCES .....	93
APPENDIX .....	100
VITA .....	173

## LIST OF TABLES

Table		Page
1	Chemical investigations of <i>A. lakoocha</i> .....	5
2	Compounds inhibiting mushroom tyrosinase enzyme .....	14
3	Weight of extracts .....	27
4	Mixture of EtOH/CH <sub>2</sub> Cl <sub>2</sub> for gradient elution .....	30
5	Fractions obtained with silica gel as adsorbent .....	31
6	Fractions obtained with aluminium oxide as adsorbent.....	31
7	Fractions obtained with kieselguhr as adsorbent.....	31
8	Data of (AUC S), (AUC IS) and (AUC S/AUC IS) values of standard solutions .....	41
9	Calculation of WS <sub>i</sub> using the equation --- $y = 5 \times 10^7 x + 623683$ .....	43
10	Determination of accuracy.....	43
11	Precision experiment.....	44
12	Linearity experiment.....	45
13	Data of LOD and LOQ experiment .....	46
14	The AUC data of extracted sample .....	47
15	The calculated data from the injection .....	49
16	<sup>1</sup> H-NMR data .....	57
17	<sup>13</sup> C-NMR data .....	59
18	Effects on each tyrosinase activity are represented as percent inhibition, mean $\pm$ S.E. of three independent tests at 100 $\mu$ M .....	81
19	The 1/V value of oxyresveratrol with various substrate concentrations .....	84
20	Kinetic parameters of mushroom tyrosinase in the presence of oxyresveratrol .....	85
21	The 1/V value of AS7 dihydroxyresveratrol with various substrate concentrations.....	86
22	Kinetic parameters of mushroom tyrosinase in the presence of dihydroxyresveratrol.....	87

- 23 The  $1/V$  value of kojic acid with various substrate concentrations..... 87
- 24 Kinetic parameters of mushroom tyrosinase in the presence of kojic acid .. 88
- 25 Cytotoxicity activity of compounds against KB, BC, NCI-H187 cell lines ..... 89

## LIST OF FIGURES

Figure	Page
1 <i>Artocarpus lakoocha</i> Roxb. ....	4
2 Domain structure of tyrosinase from different groups of species .....	11
3 Schematic representation of the binuclear copper center.....	12
4 The Raper-Mason scheme of melanogenesis .....	13
5 The TLC chromatogram of oxyresveratrol .....	30
6 TLC chromatogram of each collected fraction .....	32
7 The HPLC chromatograms .....	34
8a Calibration curve of oxyresveratrol obtained from the AUC S / AUC IS ratio, equation: $Y = 22.509X + 0.3809$ .....	41
8b Calibration curve of oxyresveratrol obtained from the AUC S; equation -- $Y = 5 \times 10^7 X + 623683$ .....	41
9 Linearity experiment .....	45
10 LOD and LOQ experiment .....	46
11 Structure of compounds AS-1.....	71
12 Structure of compound AS-2 .....	72
13 Structure of compound AS-3 .....	73
14 Structure of compound AS-4 .....	73
15 Structure of compound AS-5 .....	74
16 Structure of compound AS-6 .....	75
17 Structure of compound AS-7 .....	76
18 NOE interactions observed for compound AS-8 .....	76
19 NOE interactions observed for compound AS-9 .....	77
20 NOE interactions observed for compound AS-10 .....	78
21 The COSY correlation observed for compound AS-11 .....	79
22 The COSY correlation observed for compound AS-12 .....	79
23 Structure of compound AS-13 .....	80

24	Dose-dependent inhibitory effects on mushroom tyrosinase by oxyresveratrol, dihydroxyresveratrol and kojic acid. ....	82
25	Dose-dependent inhibitory effects on mushroom tyrosinase by oxyresveratrol, dihydroxyresveratrol and kojic acid. ....	83
26	Lineweaver-Burk plot of mushroom tyrosinase in the presence of oxyresveratrol. ....	85
27	Lineweaver-Burk plot of mushroom tyrosinase in the presence of AS-7 .....	86
28	Lineweaver-Burk plot of mushroom tyrosinase in the presence of kojic acid. ....	88
29	The UV spectrum of compound AS-1 (in MeOH) .....	101
30	The IR spectrum of compound AS-1 (UATR) .....	102
31	The ESI mass spectrum of compound AS-1 .....	103
32	The 300 MHz $^1\text{H}$ -NMR spectrum of compound AS-1 (in $\text{CDCl}_3$ ) .....	104
33	The 75 MHz $^{13}\text{C}$ -NMR spectrum of compound AS-1 (in $\text{CDCl}_3$ ) .....	105
34	The UV spectrum of compound AS-2 (in MeOH) .....	106
35	The IR spectrum of compound AS-2 (UATR) .....	107
36	The ESI mass spectrum of compound AS-2 .....	108
37	The 300 MHz $^1\text{H}$ -NMR spectrum of compound AS-2 (in $\text{CDCl}_3$ ) .....	109
38	The 75 MHz $^{13}\text{C}$ -NMR spectrum of compound AS-2 (in $\text{CDCl}_3$ ) .....	110
39	The UV spectrum of compound AS-3 (in MeOH) .....	111
40	The IR spectrum of compound AS-3 (UATR) .....	112
41	The ESI mass spectrum of compound AS-3 .....	113
42	The 300 MHz $^1\text{H}$ -NMR spectrum of compound AS-3 (in $\text{CDCl}_3$ ) .....	114
43	The 75 MHz $^{13}\text{C}$ -NMR spectrum of compound AS-3 (in $\text{CDCl}_3$ ) .....	115
44	The UV spectrum of compound AS-4 (in $\text{CHCl}_3$ ) .....	116
45	The IR spectrum of compound AS-4 (UATR) .....	117
46	The ESI mass spectrum of compound AS-4 .....	118
47	The 300 MHz $^1\text{H}$ -NMR spectrum of compound AS-4 (in $\text{CDCl}_3$ ) .....	119
48	The 75 MHz $^{13}\text{C}$ -NMR spectrum of compound AS-4 (in $\text{CDCl}_3$ ) .....	120
49	The UV spectrum of compound AS-5 (in $\text{CHCl}_3$ ) .....	121
50	The IR spectrum of compound AS-5 (UATR) .....	122



51	The 300 MHz $^1\text{H}$ -NMR spectrum of compound AS-5 (in $\text{CDCl}_3$ ).....	123
52	The 75 MHz $^{13}\text{C}$ -NMR spectrum of compound AS-5 (in $\text{CDCl}_3$ ).....	124
53	The UV spectrum of compound AS-6 (in MeOH) .....	125
54	The IR spectrum of compound AS-6 (UATR) .....	126
55	The TOF MS spectrum of compound AS-6.....	127
56	The 300 MHz $^1\text{H}$ -NMR spectrum of compound AS-6 (in $\text{CDCl}_3$ ).....	128
57	The 75 MHz $^{13}\text{C}$ -NMR spectrum of compound AS-6 (in $\text{CDCl}_3$ ).....	129
58	The UV spectrum of compound AS-7 (in MeOH) .....	130
59	The IR spectrum of compound AS-7 (UATR) .....	131
60	The ESI mass spectrum of compound AS-7 .....	132
61	The 300 MHz $^1\text{H}$ -NMR spectrum of compound AS-7 (in DMSO) .....	133
62	The 75 MHz $^{13}\text{C}$ -NMR spectrum of compound AS-7 (in DMSO).....	134
63	The UV spectrum of compound AS-8 (in MeOH).....	135
64	The IR spectrum of compound AS-8 (UATR) .....	136
65	The ESI mass spectrum of compound AS-8.....	137
66	The 300 MHz $^1\text{H}$ -NMR spectrum of compound AS-8 (in $\text{CDCl}_3$ ) .....	138
67	The 75 MHz $^{13}\text{C}$ -NMR spectrum of compound AS-8 (in $\text{CDCl}_3$ ).....	139
68	The 300 MHz NOESY spectrum of compound AS-8 (in $\text{CDCl}_3$ ).....	140
69	The UV spectrum of compound AS-9 (in MeOH) .....	141
70	The IR spectrum of compound AS-9 (UATR) .....	142
71	The ESI mass spectrum of compound AS-9.....	143
72	The 300 MHz $^1\text{H}$ -NMR spectrum of compound AS-9 (in $\text{CDCl}_3$ ).....	144
73	The 75 MHz $^{13}\text{C}$ -NMR spectrum of compound AS-9 (in $\text{CDCl}_3$ ).....	145
74	The 300 MHz NOESY spectrum of compound AS-9 (in $\text{CDCl}_3$ ).....	146
75	The UV spectrum of compound AS-10 (in MeOH) .....	147
76	The IR spectrum of compound AS-10 (UATR) .....	148
77	The ESI mass spectrum of compound AS-10 .....	149
78	The 300 MHz $^1\text{H}$ -NMR spectrum of compound AS-10 (in Acetone-d).....	150
79	The 75 MHz $^{13}\text{C}$ -NMR spectrum of compound AS-10 (in Acetone-d).....	151
80	The NOESY spectrum of compound AS-10 (in $\text{CDCl}_3$ ) .....	152
81	The UV spectrum of compound AS-11 (in MeOH) .....	153

82	The IR spectrum of compound AS-11 (UATR) .....	154
83	The ESI mass spectrum of compound AS-11.....	155
84	The 500 MHz $^1\text{H}$ -NMR spectrum of compound AS-11 (in $\text{CDCl}_3 + \text{CD}_3\text{OD}$ ).....	156
85	The 75 MHz $^{13}\text{C}$ -NMR spectrum of compound AS-11 (in $\text{CDCl}_3 + \text{CD}_3\text{OD}$ ) .....	157
86	The 500 MHz $^1\text{H}$ - $^1\text{H}$ COSY spectrum of compound AS-11 (in $\text{CDCl}_3 + \text{CD}_3\text{OD}$ ).....	158
87	The UV spectrum of compound AS-12 (in MeOH).....	159
88	The IR spectrum of compound AS-12 (UATR) .....	160
89	The TOF MS spectrum of compound AS-12.....	161
90	The 300 MHz $^1\text{H}$ -NMR spectrum of compound AS-12 (in $\text{CDCl}_3$ ).....	162
91	The 75 MHz $^{13}\text{C}$ -NMR spectrum of compound AS-13 (in $\text{CDCl}_3$ ).....	163
92	The 300 MHz $^1\text{H}$ - $^1\text{H}$ COSY spectrum of compound AS-12 (in $\text{CDCl}_3$ ) .....	164
93	The 300 MHz NOESY spectrum of compound AS-12 (in $\text{CDCl}_3$ ).....	165
94	The UV spectrum of compound AS-13 (in MeOH) .....	166
95	The IR spectrum of compound AS-11 (in $\text{CDCl}_3$ ) .....	167
96	The ESI mass spectrum of compound AS-13.....	168
97	The 300 MHz $^1\text{H}$ -NMR spectrum of compound AS-13 (in $\text{CDCl}_3$ ) .....	169
98	The 75 MHz $^{13}\text{C}$ -NMR spectrum of compound AS-13 (in $\text{CDCl}_3$ ).....	170
99	The 300 MHz $^1\text{H}$ -NMR spectrum of oxyresveratrol (in DMSO).....	171
100	The 75 MHz $^{13}\text{C}$ -NMR spectrum of oxyresveratrol (in DMSO).....	172

## ABBREVIATIONS

BC	=	breast cancer cells
brs	=	broad singlet
$^{\circ}\text{C}$	=	degree celsius
$^{13}\text{C-NMR}$	=	carbon-13 nuclear magnetic resonance
$\text{CDCl}_3$	=	deuterated chloroform
$\text{CHCl}_3$	=	chloroform
cm	=	centimeter
COSY	=	correlation spectroscopy
$\delta$	=	chemical shift
d	=	doublet
$\text{DMSO-}d_6$	=	deuterated dimethylsulphoxide
ESIMS	=	electrospray ionization mass spectrometry
EtOAc	=	ethyl acetate
$\epsilon$	=	molar absorptivity
g	=	gram
$^1\text{H-NMR}$	=	proton nuclear magnetic resonance
HPLC	=	high performance liquid chromatography
Hz	=	hertz
IR	=	infrared

$J$	=	coupling constant
KB	=	human epidermoid carcinoma cells of the nasopharynx
L	=	liter
m	=	multiplet
$[M+H]^+$	=	protonated molecular ion
MHz	=	megahertz
$\mu\text{g}$	=	microgram
mg	=	milligram
$\mu\text{l}$	=	microliter
ml	=	milliliter
$\mu\text{m}$	=	micrometer
mm	=	millimeter
MeOH	=	methanol
nm	=	nanometer
NMR	=	nuclear magnetic resonance
NOESY	=	nuclear overhauser effect correlation spectroscopy
ppm	=	part per million
ppt	=	part per thousand
q	=	quartet
s	=	singlet
sp.	=	species

t = triplet

TLC = thin layer chromatography

UV = ultraviolet