UNIVERSITI PUTRA MALAYSIA

MECHANISMS OF ANTITUMOUR ACTIVITY OF 3, 19-(2-BROMOBENZYLIDENE) ANDROGRAPHOLIDE (SRJ09)

LIM SIANG HUI

FPSK(M) 2007 14
IMPORTANT

The following manuscript "MECHANISMS OF ANTITUMOUR ACTIVITY OF 3,19-(2-BROMOBENZYLIDENE)ANDROGRAPHOLIDE (SRJ09)" is submitted to the School of Graduate Studies, Universiti Puta Malaysia, in fulfilment of the requirements for the Degree of Master of Science by Lim Siang Hui. This manuscript can only be used for personal viewing and no part of this manuscript may be reprinted, linked to, or otherwise redistributed, in any form or by any means, without first obtaining the prior written consent of the author.

If you wish to reprint or reproduce this work, or if you have any enquiries, please email Lim Siang Hui (limsianghui@hotmail.com)
MECHANISMS OF ANTITUMOUR ACTIVITY OF 3,19-(2-BROMOBENZYLIDENE)ANDROGRAPHOLIDE (SRJ09)

By

LIM SIANG HUI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science
September 2007
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

MECHANISMS OF ANTITUMOUR ACTIVITY OF 3,19-(2-BROMOBENZYLIDENE)ANDROGRAPHOLIDE (SRJ09)

By

LIM SIANG HUI

September 2007

Chairman: Associate Professor Johnson Stanslas, PhD

Faculty: Medicine and Health Sciences

To date, most of the clinical cytotoxic anticancer drugs target all rapidly dividing cells and are non-selective in their mechanism of action by disrupting essential components that are crucial to both malignant and normal cells. Hence, the search for more effective and selective anticancer drugs is currently being researched actively involving the various entities of the drug discovery programme. We at UPM have shown that andrographolide (AGP), a compound isolated from a local herb, Andrographis paniculata, to have anticancer activity in vitro and in vivo. In order to improve the antitumour properties of AGP, semi-synthetic derivatives of this compound were synthesised in our laboratory, with the aim of identifying the most promising anticancer compound among the AGP derivatives and to elucidate the mechanism(s) of action of the compound. The in vitro antitumour study showed that 3,19-(2-bromobenzylidene)andrographolide (SR09) displayed better antitumour activity when compared with AGP and other derivatives namely 3,19-(2-chlorobenzylidene)andrographolide (SRJ11) and 3,19-(3-chloro-4-fluorobenzylidene) andrographolide (SRJ23). The antitumour activity of AGP, SRJ09, SRJ11 and SRJ23 was shown to be not compromised by P-glycoprotein activities in MES-SA Dx5 multidrug resistant cell line. The time-course study
revealed SRJ09 had a rapid acting interval compared with AGP. SRJ09 was previously shown to induce G1-phase cell cycle arrest and in this study the effect was shown attributed to increased of p21 (CDK inhibitor) expression without affecting the expression of cyclin D1. Apoptosis was the main mode of cell death induced by SRJ09 and was p53 and bcl-2 independent, which might suggest that SRJ09 act through the extrinsic apoptotic pathway. A simple pharmacokinetic study was performed in Balb/c for the purpose of dose selection for in vivo study revealed that SRJ09 had a relatively short half-life but was able to reach in vitro cytotoxic concentration range. In a subsequent in vivo antitumour study, SRJ09 delayed quadruple tumour growth by 4 day in HCT-116 colon cancer xenografted mice treated with 400 mg/kg SRJ09 (q4d×3) when compared with control. In conclusion, SRJ09 have been proven as a lead anticancer agent given to its ability to induce in vitro cell cycle arrest and apoptosis and to have in vivo antitumour activity. Therefore, further studies in improving the anticancer properties of SRJ09 by chemical modification will be advantageous.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

MEKANISME AKTIVITI ANTIKANSER 3,19-(2-BROMOBENZILEDINE)ANDROGRAPHOLIDE (SRJ09)

Oleh

LIM SIANG HUI

September 2007

Pengerusi: Profesor Madya Johnson Stanslas, PhD

Fakulti: Perubatan dan Sains Kesihatan

Sehingga kini, kebanyakan agen antikanser klinikal bertindak ke atas sel yang membahagi dengan cepat dan mempunyai mekanisme yang tidak selektif dengan mengganggu komponen asas yang penting untuk kedua-dua sel kanser dan sel normal. Oleh yang demikian, penemuan agen antikanser yang lebih efektif dan selektif kini dikaji dengan aktif dengan melibatkan pelbagai entiti program penemuan ubatan. Kajian kami di UPM menunjukkan bahawa andrographolide (AGP), sebatian yang dipencil dari Andrographis paniculata, mempunyai aktiviti antikanser secara in vitro and in vivo. Untuk meningkatkan potensi antitumor AGP, terbitan semisintetik sebatian ini disintesis di makmal kami, bertujuan untuk mengenalpasti sebatian antikanser yang poten di antara terbitan AGP dan seterusnya memahami mekanisme tindakannya. Secara in vitro, 3,19-(2-bromobenzylidene)andrographolide (SRJ09) menunjukkan kelebihan aktiviti antikanser berbanding dengan AGP dan terbitan yang lain seperti 3,19-(2-chlorobenzylidene)andrographolide (SRJ11) dan 3,19-(3-chloro-4-fluorobenzylidene)andrographolide (SRJ23). Aktiviti antikanser AGP, SRJ09, SRJ11 dan SRJ23 didapati tidak dipengaruhi oleh aktiviti P-glikoprotein sel MES-SA Dx5 yang resistan terhadap pelbagai agen antikanser. Kajian berlandaskan masa
menunjukkan SRJ09 mempunyai sela masa tindakan yang pantas berbanding dengan AGP. Kajian terdahulu menunjukkan bahawa SRJ09 merencat kitaran sel pada fasa G1 dan dalam kajian ini, kesan ini didapati berpunca dari peningkatan ekspresi p21 (perencat kinase bergantung siklin) dan tidak menpengaruhi ekspresi siklin D1. Kajian yang selanjutnya menunjukkan bahawa apoptosis merupakan punca utama kematian sel yang diaruh oleh SRJ09 dan adalah bebas daripada regulasi p53 dan Bcl-2. Untuk tujuan penentuan dos kajian in vivo, kajian farmakokinetik ringkas ke atas mencit Balb/c menunjukkan bahawa SRJ09 mempunyai separuh hayat yang pendek tetapi mampu mencapai julat konsentrasi sitotoksik in vitro. Dalam kajian in vivo, SRJ09 melewatkan pertumbuhan tumor kepada empat kali ganda selama empat hari dalam mencit yang dixenograf dengan kolon kanser HCT-116 yang dirawat dengan 400 mg/kg SRJ09 (q4d x 3) berbanding dengan kawalan. Kesimpulannya, SRJ09 dikenalpasti sebagai agen antikanser pilihan utama kerana kebolehannya untuk merencat kitaran sel dan mengaruh apoptosis secara in vitro serta menunjukkan aktiviti antikanser secara in vivo. Justeru itu, kajian lanjutan untuk memperbaiki aktiviti antikansernya melalui modifikasi kimia adalah bermanfaat.
ACKNOWLEDGEMENTS

During the course of my graduate study, many individuals have generously contributed their time and support in making the completion of this project possible. I would like to take this opportunity to extend my sincere gratitude to those who had provided guidance in every step of the way. Without them, I will not have achieved my success today.

First and foremost, I would like to acknowledge my excellent supervisor, Associate Professor Dr Johnson Stanslas, for his persistent direction and constructive guidance throughout my study. I would also like to heartily thank him for spending his precious time in reading and correcting this thesis. It has been a real pleasure and a positive learning experience working in your laboratory as a graduate student. As demanding as these years has been, they were equally enjoyable and unforgettable.

My appreciation is also extended to Prof Dr Zauyah Yusuf and Dr Nazil Salleh for their valuable advice and guidance throughout the course of this project.

A very special appreciation is extended to all the members of CRDD, especially Audrey, Sagi, Tang, Guru, Velan, Viknes, Jebril and Wijayahadi for being there as a friend, a labmate and for providing many wonderful time and memories which I will treasured forever. In addition, my appreciation is also sincerely extended to Hamzani, Rijalana, and staffs of IMR (especially Mala and Zul) for their continuous help during my research work.
I would also like to thank my family especially my parents for their constant and loving support over the years. I am forever grateful.

Lastly, I would like to extend my deepest gratitude to Hoon Koon for always being there and understanding and believing in everything that I do. Her relentless support and devoted companionship during the course of my study are also greatly appreciated.
I certify than an Examination Committee met on 19\textsuperscript{th} September 2007 to conduct the final examination of Lim Siang Hui on his Master of Science thesis entitled “Mechanisms of Antitumour Activity of 3,19-(2-bromobenzylidene)andrographolide (SRJ09), a Semisynthetic Derivative of Andrographolide” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Muhammad Nazrul Hakim Abdullah, PhD**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Asmah Rahmat, PhD**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Mohamad Aziz Dollah, PhD**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Mohd Zaini Asmawi, PhD**  
Professor  
School of Pharmaceutical Sciences  
Universiti Sains Malaysia  
(External Examiner)

__________________________  
Hasanah Mohd Ghazali, PhD  
Professor/ Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia  

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

**Johnson Stanslas, PhD**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Mohammad Nazil Salleh, PhD**  
Lecturer  
School of Life Sciences  
Technology Park Malaysia Academy  
(Member)

**Zauyah Yusuf, PhD**  
Professor  
Department of Medical Microbiology and Immunology  
Universiti Kebangsaan Malaysia  
(Member)

_______________________  
AINI IDERIS, PhD  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 22 January 2008
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

___________________
LIM SIANG HUI

Date:
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vi</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>viii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xviii</td>
</tr>
</tbody>
</table>

## CHAPTER

1. **INTRODUCTION**
   1.1 Overview 1
   1.2 Objectives 3

2. **LITERATURE REVIEWS**
   2.1 Cancer Biology And Development 4
      2.1.1 Self-sufficiency in Growth Signals 6
      2.1.2 Insensitivity to Antigrowth Signals 7
      2.1.3 Evading Apoptosis 8
      2.1.4 Limitless Replicative Potential 9
      2.1.5 Sustained Angiogenesis 10
      2.1.6 Tissue Invasion and Metastasis 11
   2.2 Modalities of Anticancer Treatment 12
      2.2.1 Chemotherapy 13
         2.2.1.1 Alkylating Agents 14
         2.2.1.2 Antimetabolites 15
         2.2.1.3 Anthracyclines 17
         2.2.1.4 Antimicrotubule Agents 19
         2.2.1.5 Topoisomerase Inhibitors 21
         2.2.1.6 Hormonal Agents 23
   2.3 Cell Death Pathways As Chemotherapeutic Targets 24
      2.3.1 Apoptosis 25
         2.3.1.1 The Intrinsic Pathway 27
         2.3.1.2 The Extrinsic Pathway 28
      2.3.2 Necrosis 29
   2.4 Andrographolide 32

3. **MATERIALS AND METHODS**
   3.1 Materials 37
      3.1.1 Cell Lines 37
      3.1.2 Reagents and Chemicals 37
      3.1.3 Antibodies and Protein Ladder 38
      3.1.4 Laboratories Wares 39
      3.1.5 Instrumentations 39
   3.2 Methods 40
3.2.1  Preparation of Drugs  40
3.2.2  Cell Culture  40
3.2.3  Cryogenic Preservation and Recovery  41
3.2.4  Microculture MTT (Tetrazolium) Assay  42
3.2.5  Stability Study  43
  3.2.5.1  Sample Collection and Extraction  43
  3.2.5.2  High Performance Liquid Chromatography (HPLC)  44
3.2.6  Cell Morphological Study Using DNA Fluorochromes  45
3.2.7  DNA Fragmentation Analysis  47
3.2.8  Western Blotting  48
  3.2.8.1  Isolation of Total Proteins from Cancer Cells  48
  3.2.8.2  SDS-PAGE Electrophoresis  49
  3.2.8.3  Semi-Dry Western Blotting and Immunodetection  50
  3.2.8.4  Stripping and Reprobing Membranes  51
3.2.9  Pharmacokinetic Study  52
  3.2.9.1  Dosing of Animal with Vehicle and Compound  52
  3.2.9.2  Sample Collection and Handling  52
  3.2.9.3  Extraction Procedure  53
  3.2.9.4  HPLC Analysis  53
3.2.10  In Vivo Chemotherapeutic Study  54
  3.2.10.1  Animals and Environmental Control  54
  3.2.10.2  Development of Human Tumour Xenografts in Nude Mice  55
    3.2.10.3  In Vivo Antitumoural Assay  56
3.2.11  Statistical Analysis  57

4  IN VITRO ANTITUMOUR PROPERTIES OF NEW DERIVATIVES AGP  58
4.1  Introduction  58
4.2  Results and Discussion  60
  4.2.1  Growth Inhibitory Effects of AGP and Its Derivatives  60
  4.2.2  AGP and SRJ09 Exposure Interval Effects on Cell Growth Inhibition  65
  4.2.3  Effect of AGP, SRJ09 and SRJ11 against Multidrug Resistant Cell Line  69
  4.2.4  Stability of AGP and SRJ09 in Complete Culture Medium  73
4.3  Summary  77
5 REGULATION OF CELL CYCLE ARREST AND 
CELL DEATH BY SRJ09  
5.1 Introduction 78
5.2 Results and Discussion 79
  5.2.1 Identification of The Mode(s) of Cell Death Induced by SRJ09 79
  5.2.2 DNA Fragmentation Induced by SRJ09 on HCT-116 85
  5.2.3 Effects of SRJ09 on p53, Bcl-2, Cyclin D1 and p21 Protein Expression in MCF-7 and HCT-116 cells 86
5.3 Summary 95

6 IN VIVO ANTITUMOUR PROPERTIES OF SRJ09 97
6.1 Introduction 97
6.2 Results and Discussion 98
  6.2.1 Pharmacokinetics of SRJ09 in Balb/C Mice 98
  6.2.2 Effects of SRJ09 on HCT-116 Xenografted Nude Mice 102
6.3 Summary 107

7 GENERAL DISCUSSION AND CONCLUSION 108
7.1 General Discussion 108
7.2 Conclusion 110
7.3 Recommendation of Future Research 111

REFERENCES 113
APPENDICES 123
BIODATA OF THE AUTHOR 129
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Morphological changes and biochemical features between apoptosis and necrosis</td>
<td>25</td>
</tr>
<tr>
<td>3.1</td>
<td>Dilutions of antibodies</td>
<td>51</td>
</tr>
<tr>
<td>3.2</td>
<td>Pharmacokinetic parameters calculation</td>
<td>54</td>
</tr>
<tr>
<td>4.1</td>
<td>GI(_{50}) values (in (\mu)M) of AGP, SRJ09, SRJ11, SRJ23 and DOX against a panel of cancer cell lines</td>
<td>61</td>
</tr>
<tr>
<td>4.2</td>
<td>TGI values (in (\mu)M) of AGP, SRJ09, SRJ11, SRJ23 and DOX against a panel of cancer cell lines</td>
<td>62</td>
</tr>
<tr>
<td>4.3</td>
<td>LC(_{50}) values (in (\mu)M) of AGP, SRJ09, SRJ11, SRJ23 and DOX against a panel of cancer cell lines</td>
<td>63</td>
</tr>
<tr>
<td>4.4</td>
<td>GI(<em>{50}), TGI and LC(</em>{50}) values (in (\mu)M) of AGP, SRJ09, SRJ11, SRJ23 and DOX in HUVEC cell line</td>
<td>64</td>
</tr>
<tr>
<td>4.5</td>
<td>Growth inhibitory parameters of AGP and SRJ09 at different exposure intervals against MCF-7 cell line</td>
<td>67</td>
</tr>
<tr>
<td>4.6</td>
<td>Growth inhibitory parameters of AGP and SRJ09 at different exposure intervals against HCT-116 cell line</td>
<td>69</td>
</tr>
<tr>
<td>4.7</td>
<td>Growth inhibitory parameters of AGP, SRJ09 and SRJ11 against human sarcoma cell line MES-SA and its multidrug resistant sublines MES-SA/Dx5</td>
<td>72</td>
</tr>
<tr>
<td>6.1</td>
<td>Pharmacokinetic parameters obtained after single i.p bolus administration of 100 mg/kg SRJ09</td>
<td>99</td>
</tr>
<tr>
<td>6.2</td>
<td>The % T/C values of SRJ09 treatment at various doses on each measurement day</td>
<td>104</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Acquired capabilities that sustained cancer development</td>
<td>5</td>
</tr>
<tr>
<td>2.2</td>
<td>Chemical structure of cyclophosphamide</td>
<td>15</td>
</tr>
<tr>
<td>2.3</td>
<td>Deoxythymidine synthesis pathway as antimetabolites target</td>
<td>16</td>
</tr>
<tr>
<td>2.4</td>
<td>Chemical structures of methotrexate and 5-fluorourasil</td>
<td>17</td>
</tr>
<tr>
<td>2.5</td>
<td>Chemical structures of doxorubicin and daunorubicin</td>
<td>18</td>
</tr>
<tr>
<td>2.6</td>
<td>Chemical structures of vinca alkaloids and taxane</td>
<td>20</td>
</tr>
<tr>
<td>2.7</td>
<td>Chemical structures of topoisomerase I inhibitor and topoisomerase II inhibitor</td>
<td>23</td>
</tr>
<tr>
<td>2.8</td>
<td>Apoptosis through the extrinsic and intrinsic pathway</td>
<td>26</td>
</tr>
<tr>
<td>2.9</td>
<td>The induction of necrosis caused by the inhibition of cellular energy production, imbalance of intracellular calcium flux, generation of ROS, and activation of non-apoptotic proteases</td>
<td>32</td>
</tr>
<tr>
<td>2.10</td>
<td>Chemical structure of AGP</td>
<td>33</td>
</tr>
<tr>
<td>2.11</td>
<td>Chemical structures of semisynthetic derivatives of AGP</td>
<td>35</td>
</tr>
<tr>
<td>3.1</td>
<td>Three endpoints parameter of GI&lt;sub&gt;50&lt;/sub&gt;, TGI and LC&lt;sub&gt;50&lt;/sub&gt; as determined from a dose-response growth curves</td>
<td>43</td>
</tr>
<tr>
<td>4.1</td>
<td>NCI Self-Organize Maps (SOM) of AGP and its derivatives into similar pattern of growth inhibition to standard agents with known molecular target</td>
<td>59</td>
</tr>
<tr>
<td>4.2</td>
<td>Representative dose-response curves of time interval exposure effect of AGP and SRJ09 against MCF-7 cell growth</td>
<td>66</td>
</tr>
<tr>
<td>4.3</td>
<td>Representative dose-response curves of time intervals exposure effect of AGP and SRJ09 on HCT-116 cell growth</td>
<td>68</td>
</tr>
<tr>
<td>4.4</td>
<td>Representative dose-response curves of growth inhibitory effects of AGP, SRJ09, SRJ11 and DOX against MES-SA and MES-SA Dx5 cells</td>
<td>71</td>
</tr>
<tr>
<td>4.5</td>
<td>Stability of AGP and SRJ09 in RPMI 1640 culture medium</td>
<td>74</td>
</tr>
<tr>
<td>4.6</td>
<td>Structures of camptothecin and lovastatin and their respective hydroxyl acid form</td>
<td>75</td>
</tr>
</tbody>
</table>
4.7 Proposed hydrolysis of andrographolide lactone form to hydroxyl acid form

4.8 Dose-response curves of growth inhibitory effects of freshly prepared (0 hr) and 24 hr pre-incubated SRJ09 against MCF-7 cells

5.1 Fluorescence micrographs (400X) of HCT-116 cells after AO/PI staining

5.2 Induction of apoptosis and necrosis by SRJ09 in MCF-7 cells at various concentration and time points

5.3 Induction of apoptosis and necrosis by SRJ09 in HCT-116 cells at various concentration and time points

5.4 Fluorescence micrographs of untreated control and SRJ09-treated HCT-116

5.5 DNA fragmentation in HCT-116 cells treated with SRJ09 for 24 hr

5.6 Western blot analysis of p53 apoptotic regulatory protein in SRJ09-treated MCF-7 cells

5.7 Western blot analysis of bcl-2 apoptotic regulatory protein in SRJ09-treated MCF-7 cells

5.8 Western blot analysis of cyclin D1 cell cycle regulatory protein in SRJ09-treated MCF-7 cells

5.9 Western blot analysis of p21 cell cycle regulatory protein in SRJ09-treated MCF-7 cells

5.10 Western blot analysis of p53 apoptotic regulatory protein in SRJ09-treated MCF-7 cells

5.11 Western blot analysis of bcl-2 apoptotic regulatory protein in SRJ09-treated MCF-7 cells

5.12 Western blot analysis of cyclin D1 cell cycle regulatory protein in SRJ09-treated MCF-7 cells

5.13 Western blot analysis of p21 cell cycle regulatory protein in SRJ09-treated MCF-7 cells

6.1 SRJ09 plasma concentration-time profile in mice following single i.p bolus administration of 100 mg/kg SRJ09
6.2 Logarithm of SRJ09 plasma concentration-time profile in mice following single i.p bolus administration of 100 mg/kg SRJ09

6.3 Growth of HCT-116 tumour xenografts in nude mice

6.4 A comparative effect of SRJ09 treatment on tumour volume in HCT-116 xenografted nude mice with the vehicle control

6.5 Percentage of relative body weight of mice receiving various SRJ09 doses
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>AGP</td>
<td>andrographolide</td>
</tr>
<tr>
<td>AIF</td>
<td>apoptosis-inducing factor</td>
</tr>
<tr>
<td>AO</td>
<td>acridine orange</td>
</tr>
<tr>
<td>APAF</td>
<td>apoptotic protease activating factor-1</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>area under curve</td>
</tr>
<tr>
<td>bp</td>
<td>base pair</td>
</tr>
<tr>
<td>$C_0$</td>
<td>initial concentration</td>
</tr>
<tr>
<td>$Ca^{2+}$</td>
<td>calcium ion</td>
</tr>
<tr>
<td>CDK</td>
<td>cyclin dependent kinase</td>
</tr>
<tr>
<td>Cl</td>
<td>clearance</td>
</tr>
<tr>
<td>$C_{max}$</td>
<td>maximum concentration</td>
</tr>
<tr>
<td>$CO_2$</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>DD</td>
<td>death domain</td>
</tr>
<tr>
<td>DISC</td>
<td>death-inducing signalling complex</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNase</td>
<td>deoxyribonuclease</td>
</tr>
<tr>
<td>DOX</td>
<td>doxorubicin</td>
</tr>
<tr>
<td>DR</td>
<td>death receptors</td>
</tr>
<tr>
<td>dTMP</td>
<td>2'-deoxythymidine-5'-phosphate</td>
</tr>
<tr>
<td>dUMP</td>
<td>2’-deoxyuridine-5’-phosphate</td>
</tr>
<tr>
<td>ECGS</td>
<td>endothelial cell growth supplement</td>
</tr>
</tbody>
</table>
EDTA ethylenediaminetetraacetic acid
EGF epidermal growth factor
FADD Fas-associated death domain
FasL Fas ligand
FBS foetal bovine serum
FGF fibroblast growth factors
Fig figure
GI_{50} 50% growth inhibition concentration
HCl hydrochloride acid
HPLC high performance liquid chromatography
HRP horse radish peroxidase
HUVEC human umbilical vein endothelial cell
i.p. intraperitoneum
IGF insulin-like growth factor
IgG immunoglobulin type-G
K_{el} elimination rate constant
LC_{50} 50% lethal concentration
MAPK mitogen-activated protein kinase
MDR multidrug resistance
MgCl_2 magnesium chloride
MTT 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
NaCl natrium chloride
NAD nicotinamide adenine dinucleotide
NCI National Cancer Institute
PAGE polyacrylamide gel electrophoresis
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP</td>
<td>poly(ADP-ribose) polymerase</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PBS-T</td>
<td>PBS containing 0.1% Tween 20</td>
</tr>
<tr>
<td>PDGF</td>
<td>platelet-derived growth factor</td>
</tr>
<tr>
<td>PI</td>
<td>propidium iodide</td>
</tr>
<tr>
<td>RIP</td>
<td>receptor-interacting protein</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RNase</td>
<td>ribonuclease</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxidative species</td>
</tr>
<tr>
<td>RR</td>
<td>resistant ratio</td>
</tr>
<tr>
<td>RTV</td>
<td>relative tumour volume</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulphate</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error mean</td>
</tr>
<tr>
<td>Smac</td>
<td>second mitochondria-derived activator of caspases</td>
</tr>
<tr>
<td>SOM</td>
<td>self-organise maps</td>
</tr>
<tr>
<td>T½</td>
<td>half-life</td>
</tr>
<tr>
<td>TEMED</td>
<td>N,N,N‘,N’-tetramethylethylenediamine</td>
</tr>
<tr>
<td>TGF</td>
<td>tumour growth factor</td>
</tr>
<tr>
<td>TGI</td>
<td>total growth inhibition</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>time to achieved C&lt;sub&gt;max&lt;/sub&gt;</td>
</tr>
<tr>
<td>TNF</td>
<td>tumour necrosis factor</td>
</tr>
<tr>
<td>TNFR</td>
<td>tumour necrosis factor receptor</td>
</tr>
<tr>
<td>TRAIL</td>
<td>tumour necrosis factor-related apoptosis inducing ligand</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
</tbody>
</table>
V  
volt

$V_d$  
volume of distribution

VEGF  
vascular endothelial growth factor

w/v  
weight over volume
CHAPTER 1

INTRODUCTION

1.3 Overview

Cancer is a new growth of tissue in which cell multiplication is uncontrolled and progressive. Unlike benign tumour cells, cancer cells exhibit the properties of invasion and metastasis and are highly anaplastic. In 2002, it is reported that cancer is the second major cause of death, which account 12.5% of total deaths worldwide (The World Health Report 2003). Surgical excision, radiation therapy and chemotherapy are the main approaches to treat cancer. Unlike surgery and radiation therapy, chemotherapy is considered a systemic treatment in which the drugs circulate in the blood circulations to eradicate cancer micrometastases at distant sites from the original cancer. Therefore, chemotherapy is often administered in conjunction with local therapy such as surgery and radiation to obtained optimal effects. The use of anticancer drugs as part of the treatment strategy for cancer has greatly improved the overall prognosis of cancer patients.

To date, most of the anticancer drugs target all rapidly dividing cells and are non-selective in their mechanism of action by disrupting essential components or metabolic pathways that are crucial to both malignant and normal cells (Ewesuedo and Ratain 2003). Therefore, scientists are still looking for more effective and type-selective antitumour drugs with minimum side effects. Several strategies were employed which include chemical modification or combinatorial chemistry involving
existing natural and synthetic products, structure-based drug design and new natural products discovery. The search for new natural products from the vast biodiversity is an importance source of structural diversity that yield unusual and unexpected lead structures which served as starting points for chemical modification to derive an optimal drug (Young 1999).

In the early 2000, andrographolide (AGP) a compound isolated from *Andrographis paniculata* received much attention as a candidate to be developed into anticancer agents. AGP was shown to inhibit tumour growth both *in vitro* and *in vivo* (Stanslas et al. 2001; Rajagopal et al. 2003). AGP was also reported to cause $G_1$ phase cell cycle arrest (Stanslas et al. 2001; Rajagopal et al. 2003) and apoptosis (Cheung et al. 2005; Kim et al. 2005; Zhou et al. 2006). Shortly, AGP was derivatised through various chemical modifications in an effort to improve its antitumour potential (Nanduri et al. 2004; Jada et al. 2006). The derivatives synthesised by coupling of two hydroxyl groups in AGP (C-3 and C-19) by reacting AGP with benzaldehydes having different functional groups are generally shown to have an improvement in terms of antitumour activity (Jada et al. 2006). Hence, in this study several lead derivatives were selected and evaluated, in order to identify the most potent anticancer compound among AGP derivatives and their mechanism(s) of action.