UNIVERSITI PUTRA MALAYSIA

3,19-DIACETYL-14-DEOXY-11,12- DI DEHYDROANDROGRAPHO LIDE (SRS27) ANTAGONISES INFLAMMATORY RESPONSE AND OXIDATIVE STRESS IN IN VITRO AND IN VIVO ASTHMA MODELS

LIM CHEE WOEI

FPSK(p) 2014 10
3,19-Diacetyl-14-deoxy-11,12-didehydroandrographolide (SRS27) antagonises inflammatory response and oxidative stress in \textit{in vitro} and \textit{in vivo} asthma models

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DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

2014
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By

LIM CHEE WOEI

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

June 2014
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

3,19-DIACETYL-14-DEOXY-11,12-DIDEHYDROANDROGRAPHLIDE (SRS27) ANTAGONISES INFLAMMATORY RESPONSE AND OXIDATIVE STRESS IN IN VITRO AND IN VIVO ASTHMA MODELS

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June 2014

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Corticosteroids and non-steroidal anti-inflammatories are the most effective treatments for a variety of inflammatory conditions such as asthma. Steroids act by blocking transcription factors, such as nuclear factor (NF)-κB and activator protein (AP)-1 to down-regulate a vast array of pro-inflammatory genes, whereas NSAIDs specifically target cyclo-oxygenase (COX) activity to reduce prostaglandin production. However, the use of both types of drugs is associated with unwanted side effects, and a significant proportion of patients are steroid resistant. Thus, there is an urgent need to develop novel anti-inflammatory drugs to replace or complement present day therapy.

The bioactive compounds from the famous Asian herb Andrographis paniculata (known locally in Malaysia as Hempedu Bumi) have been studied for almost a century. The focus has been placed on the identification of antiinflammatory and anticancer agents. The herb contains two main diterpenoid constituents named andrographolide (AGP) and 14-deoxy-11,12-didehydroandrographolide (DDAG). AGP and DDAG were found to exhibit anti-asthma effects by inhibiting inflammatory responses in an allergic mouse asthma model. As such, both of them could act as novel replacement for current anti-inflammatory drugs. However, due to inadequacies of both compounds in terms of drug-like properties, DDAG analogues were semisynthesised to tackle these shortcomings.

Among the analogues, 3,19-diacetyl-14-deoxy-11,12-didehydroandrographolide (SRS27) was proven to inhibit cysteinyl leukotriene (CysLT) and nitric oxide (NO) synthesis in mouse macrophages, like AGP. However, DDAG on the other hand, failed to exhibit such activity. SRS27 was less toxic compared with AGP, which suggests that a simple chemical modification of DDAG produces a compound with CysLT and NO inhibitory activity similar to AGP but maintained the toxicity profile similar to DDAG. It is interesting to note that other analogues such as SRS28, SRS49, SRS76 and SRS83 with chemical modifications on the same carbon numbers
3 and 19 of DDAG were unable to show inhibition of CysLT and NO synthesis.

Consequently, the potential anti-inflammatory effect of SRS27 was investigated in ovalbumin (OVA)-induced mouse asthma model. The compound was administered in a prophylactic manner and showed a substantial decrease in asthma parameters. SRS27 at 3 mg/kg twice daily for three days consecutively significantly reduced OVA-induced total cell such as macrophages, eosinophils, lymphocytes and neutrophils, as well as inflammatory cytokines such as IL-4, IL-5, IL-13 and eotaxin in bronchoalveolar lavage (BAL) fluid. The compound also suppressed serum IgE production. In addition, SRS27 suppressed mucus hyper-secretion and expression of inflammatory mediators such as TNF-α, MCP-1, Muc5ac, RANTES, IL-33 and iNOS. Mechanistically, the compound inhibited lung NF-κB p65 nuclear translocation. In line with this observation, p65 NF-κB nuclear translocation was also found to be inhibited by the compound in A549 lung cancer cell line. Notably, this inhibition was not a result of cell toxicity as peripheral blood count in normal BALB/C mice treated with 3 mg/kg of SRS27 was not affected. The acute toxicity in mice further supported this idea, which indicated SRS27 is indeed a safe compound, just like DDAG. A pharmacokinetic study in Balb/C mice at 3 mg/kg single dose revealed SRS27 that had a relatively short half-life but was able to achieve a concentration range of 13- 19 µM concentration that could be related to in vitro anti-asthma activities. SRS27 is the first known DDAG derivative tested positive in a mouse asthma model and as such this compound could serve as a prototype and template for future improvement as a potential prophylactic agent to control asthma.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

3,19-DIACETYL-14-DEOXY-11,12-DIDEHIDROANDROGRAPHOLIDE (SRS27) MENGHALANG RESPON INFLAMASI DAN TEKANAN OKSIDASI DI DALAM MODEL ASMA IN VITRO DAN IN VIVO

Oleh

LIM CHEE WOEI

Jun 2014

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Kortikosteroid dan drug anti-inflamasi non steroid (DAINS) merupakan rawatan yang paling berkesan untuk pelbagai keadaan radang seperti asma. Steroid bertindak dengan menyekat faktor transkripsi, seperti factor nuklear (NF) κB dan pengaktif protein (AP) -1 untuk merencat pelbagai gen pro-keradangan, manakala DAINS bertindak ke atas aktiviti siklooksigenase (COX) dengan mengurangkan sintesis prostaglandin. Walaubagai manakala, penggunaan kedua-dua jenis ubat tersebut memberi kesan sampingan yang tidak diingini, dan sebahagian besar daripada pesakit mengalami kerintangan terhadap steroid. Oleh sebab itu, keperluan untuk membangunkan ubat anti-inflamasi yang baharu untuk menggantikan atau sebagai pelengkap bagi terapi yang sedia ada adalah amat kritikal.


Antara analog yang dikaji, 3,19-diacetyl-14-deoxy-11,12-didehidandroandrographolide (SRS27) telah dibuktikan dapat merencat sintesis cysteinyl leukotriene (CysLT) dan nitrikoksida (NO) dalam makrofaj mencit, seperti AGP. Namun, DDAG didapati gagal untuk mempamerkan aktiviti sedemikian. SRS27 yang didapati kurang toksik berbanding dengan AGP, menunjukkan pengubahsuaian kimia yang mudah ke atas DDAG menghasilkan sebatian yang mengandungi aktiviti terhadap CysLT dan NO.
seperti AGP dan mengekalkan profil ketoksikan seperti DDAG. Menariknya, analog lain seperti SRS28, SRS49, SRS76 dan SRS83 dengan pengubahsuaian kimia pada nombor karbon yang sama iaitu 3 dan 19 dalam DDAG didapati tidak menunjukkan aktiviti perencatan sintesis CysLT dan NO.

Selanjutnya, potensi anti-inflamasi oleh SRS27 dikaji dalam model asma mencit diaruh dengan ovalbumin (OVA). SRS27 yang diberikan secara profilaktik menunjukkan penurunan ketara parameter asma. Sebatian ini yang diberikan secara intraperitonium pada dos 3 mg/kg sebanyak 2 kali sehari selama 3 hari berturut-turut telah mengurangkan jumlah sel yang diaruh oleh OVA seperti makrofaj, eosinofil, limfosit, dan neutrofil, serta sitokin inflamasi seperti IL-4, IL-5, IL-13 dan eotaxin dalam ceair bronchoalveolar (BAL lavage). Sebatian ini juga merencatkan penghasilan IgE. Disamping itu, ia juga dapat merencat rembesan lendir dan ekspresi pengantara inflamasi seperti TNF-α, PKM-1, Muc5ac, RANTES, IL-33 dan iNOS. Daripada sudut mekanisme, sebatian tersebut menghalang transloksi nukleus NF-κB p65 di dalam paru-paru. Selaras dengan pemerhatian ini, p65 NF-κB nuclear translokasi juga didapati direncatkan oleh sebatian tersebut di dalam sel kanser paru-paru (A549). Perencatan ini bukan terhasil akibat daripada ketoksikan sel dimana kiraan darah periferi dalam mencit BALB/C normal yang dirawat dengan 3 mg/kg SRS27 adalah tidak terjejas. Hasil kajian ketoksikan akut menggunakan model mencit telah menyokong idea ini dengan membuktikan SRS27 sebagai satu sebatian yang selamat seperti DDAG. Kajian farmakokinetik pula menunjukkan bahawa dos tunggal 3 mg/kg SRS27 mempunyai jangka separuh hayat yang agak singkat (4.8 min) tetapi mampu mencapai jual kepekatan maksima darah sebanyak 13 - 19 μM yang boleh dikaitkan dengan aktiviti anti-asma in vitro. SRS27 merupakan analog DDAG yang pertama diuji positif dalam model asma mencit dimana ia berpotensi dijadikan sebagai sebatian prototaip dan templat untuk penambahbaikan sebagai sebatian profilaktik yang berpotensi untuk mengawal asma pada masa hadapan.
ACKNOWLEDGEMENTS

First and foremost, I would like to take this opportunity to dedicate and express my sincere gratitude to my project supervisor, Prof. Dr. Johnson Stanslas for his constant and valuable guidance, encouragement, advice and remarkable understanding and knowledge throughout the course of my study.

In addition, my sincere appreciation goes to Assoc. Prof. Dr. Fred Wong Wai-Shiu and Prof. Dr. Shiran Md Sidek for their advices during the course of this project. I would like to specially thank Assoc. Prof. Dr. Fred Wong Wai-Shiu for his kind and generous hospitality during my one and half years of attachment in his lab. I would like to extend my appreciation to my fellow labmates from NUS, Fera Goh Yiqian, Alan Koh Hock Meng, Guan Shou Ping, Cherng Chang, Chan Tze Khee and David Chan for their ideas, help, troubleshooting and companionship during my attachment. Special thanks to my fellow friends, Kong Li Ren, Fhu Chee Wai, Robin Lim and Woo Chern Chiu for their hospitality and advices in ensuring my attachment ended with fruitful outcomes.

My heartfelt thanks and gratitude goes to all members of CRDD, especially Dr. Sreenivasa Rao Sagineedu for the synthesis work of andrographolide analogues; Ben Wong for his assistance and valuable advice; Dr. Rafid Salim Jabir for helping me with the statistical analysis. Special thanks to Velan Suppaiah, Wong Mei Szin, Ethel Jeyaraj, Noorlela Ramli, Michelle Wong, Cik Ruhaidah Ramli. I thank you for your assistance and contributions in different ways throughout my journey of completion of this project.

I am also grateful and honoured to have been awarded with a tutor contract by Universiti Putra Malaysia and KPT scholarship by Malaysia Ministry of Higher Education.

Lastly, my true admiration and heartiest appreciation goes out to my wife, Elaine Goh Hui Ling; father, Lim Mun Teck; mother, Yeow Lay Yoong; my brother, Lim Jun Hoh; my late grandmother, Tan Boon Lan; and those who are special to me, for their endless supports and concerns as well as their unrelenting love and understanding throughout the years of my study. I am forever indebted to my wife and family, for their presence, it would have been impossible for me to have this project completed. Therefore, this thesis is dedicated to them.
I certify that a Thesis Examination Committee has met on 2 June 2014 to conduct the final examination of Lim Chee Woei on his thesis entitled “3,19-Diacetyl-14-Deoxy-11,12-Didehydroandrographolide (SRS27) Antagonises Inflammatory Response and Oxidative Stress in In Vitro and In Vivo Asthma Models” 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.

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xxv</td>
</tr>
</tbody>
</table>

## CHAPTER 1 INTRODUCTION

1.1 Background of the Study
1.2 Problem Statements
1.3 Significance of the Study
1.4 Hypothesis
1.5 Objectives of the Study
   1.5.1 Main Objective
   1.5.2 Specific Objectives

## CHAPTER 2 LITERATURE REVIEW

2.1 Asthma
2.2 Prevalence of Asthma
2.3 Pathophysiology of Allergic Asthma
   2.3.1 Th2 Inflammatory Pathway
   2.3.2 Th1 Cells
   2.3.3 Eosinophilic Airway Inflammation
   2.3.4 Airway Hyper-responsiveness
   2.3.5 Airway Remodelling
   2.3.6 Airway Mucus Hyper-secretion
   2.3.7 Elevated Serum Level IgE
   2.3.8 Nuclear Factor-KappaB in Asthma
   2.3.9 Cysteinyl Leukotriene in Allergic Asthma
   2.3.10 Nitric Oxide in Asthma
2.4 Current Treatment for Allergic Asthma
   2.4.1 β2-adrenoceptor agonists
   2.4.2 Anticholinergic Agents
   2.4.3 Phosphodiesterase Inhibitors
   2.4.4 Corticosteroids
   2.4.5 Mast Cell Stabilizing Drugs
   2.4.6 Drugs Targeting IgE
   2.4.7 Allergen Immunotherapy
   2.4.8 Cytokine-based Immunotherapy
   2.4.9 Drugs Targeting Leukotriene
2.5 Pharmacological Activities of New Compounds from *Andrographis paniculata*
   2.5.1 Andrographolide and 14-deoxy-11,12-didehydroandrographolide
   2.5.2 Andrographolide Analogues


3 IN VITRO ANTI-NITRIC OXIDE AND ANTI-CYSTEINYL LEUKOTRIENE SYNTHESIS ACTIVITIES OF ANDROGRAPHOLIDE AND ITS DERIVATIVES

3.1 Introduction 28
3.2 Materials 30
  3.2.1 Compounds Isolation and Synthesis 30
  3.2.2 Cell Lines 30
  3.2.3 Reagents and Chemicals 30
  3.2.4 Tissue Culture Materials 30
  3.2.5 Animals 31
  3.2.6 Instrumentations 31
3.3 Methods 31
  3.3.1 Cell Culture 31
  3.3.2 Plating 32
  3.3.3 Cryogenic Preservation and Recovery 32
  3.3.4 Compounds Dilution and Preparation 32
  3.3.5 In vitro cytotoxicity assay (MTT assay) 33
  3.3.6 Griess Assay 33
  3.3.7 Induction and Harvesting of Mouse Peritoneal Macrophages 33
  3.3.8 Plating of Mouse Peritoneal Macrophages and Treatment 34
  3.3.9 Cysteinyl Leukotriene Measurement 34
  3.3.10 Structure-Property Analysis of DDAG Derivatives 35
  3.3.11 Statistical Analysis 35
3.4 Results 35
  3.4.1 Cysteinyl Leukotriene 35
  3.4.2 Nitric Oxide 38
  3.4.3 Toxicity profile of AGP, DDAG and SRS27 41
  3.4.4 Structure-Property Analysis of DDAG Derivatives 44
3.5 Discussion 46
3.6 Conclusion 49

4 IN VITRO INHIBITORY ACTIVITY OF SRS27 AGAINST NF-κB SIGNALLING PATHWAY

4.1 Introduction 50
4.2 Materials 51
  4.2.1 Compounds Isolation and Synthesis 51
  4.2.2 Cell Lines 51
  4.2.3 Reagents and Chemicals 51
  4.2.4 Tissue Culture Materials 51
  4.2.5 Instrumentations 51
4.3 Methods 52
  4.3.1 Cell Culture 52
  4.3.2 Drug Treatment and TNF-α stimulation 52
  4.3.3 Biological Response Study 52
    4.3.3.1 RT-PCR 52
    4.3.3.2 Polymerase chain reaction 53
    4.3.3.3 Gel electrophoresis 53
4.3.4 Protein Study
  4.3.4.1 Total protein extraction 53
  4.3.4.2 Nuclear protein extraction 54
  4.3.4.3 Western Blot 54
  4.3.4.4 NF-κB Transcription Factor Assay (Trans-AM Assay) 55

4.4. Results
  4.4.1 Effects of andrographolide analogues on the NF-κB-activated biological response 55
  4.4.2 Effects of andrographolide analogues on DNA-binding activity 57
  4.4.3 Effects of andrographolide analogues on TNF-α-induced NF-κB activation 58

4.5 Discussion 60
4.6 Conclusion 62

5 MOUSE ASTHMA MODEL
5.1 Introduction 64
5.2 Materials 66
  5.2.1 Compounds Isolation and Synthesis 66
  5.2.2 Animals 66
  5.2.3 Chemicals and Reagents 66
  5.2.4 Laboratories Wares and Consumables 67
  5.2.5 Instrumentations 67
5.3 Methods 67
  5.3.1 In vivo preparation of SRS27 solution 67
  5.3.2 Mouse Asthma Model 68
    5.3.2.1 Systemic Sensitization 68
    5.3.2.2 Airway Challenge 68
    5.3.2.3 Administration of SRS27 to Mice 68
    5.3.2.4 Serum Collection 69
    5.3.2.5 Bronchoalveolar Lavage (BAL) Fluid Collection 69
    5.3.2.6 Lung Sample Collection 69
    5.3.2.7 Total Cell Count 70
    5.3.2.8 Differential Cell Count 70
  5.3.3 Histology 70
    5.3.3.1 Periodic Acid Fluorescent Staining 71
    5.3.3.2 Haematoxylin and Eosin Staining 71
    5.3.3.3 Analysis and Scoring Criteria 71
  5.3.4 Enzyme-Linked Immunosorbent Assay (ELISA) 72
    5.3.4.1 Cytokine and chemokine levels in BAL fluid 72
    5.3.4.2 Immunoglobulin E (IgE) level in serum 72
  5.3.5 Reverse Transcription–Polymerase Chain Reaction (RT-PCR) 73
    5.3.5.1 RNA extraction 73
    5.3.5.2 Reverse transcription 73
    5.3.5.3 Polymerase Chain Reaction 73
    5.3.5.4 Gel electrophoresis 74
  5.3.6 Measurement of Airway Hyper-responsiveness (AHR) 74
  5.3.7 Detection of Nuclear Protein Expression in Lung 75
Samples
5.3.7.1 Nuclear protein extraction 75
5.3.7.2 NF-κB Transcription Factor Assay (TransAM Assay) 76
5.3.7.3 Western Blot 76
5.3.8 Statistical Analysis 76

5.4 Results
5.4.1 Effects of SRS27 on OVA-induced inflammatory cell recruitment in BAL fluid 77
5.4.2 Effects of SRS27 on airway cell infiltration and airway mucus production 82
5.4.3 Effects of SRS27 on cytokine levels in BAL fluid and serum IgE levels 85
5.4.4 Effects of SRS27 on lung mRNA expression of inflammatory markers 90
5.4.4.1 Effects of SRS27 on mRNA expression of chitinases in lung tissue 91
5.4.4.2 Effects of SRS27 on mRNA expression of adhesion molecules in lung tissue 92
5.4.4.3 Effects of SRS27 on mRNA expression of pro-inflammatory mediators in lung tissue 93
5.4.4.4 Effects of SRS27 on mRNA expression of cytokines in lung tissue 95
5.4.4.5 Effects of SRS27 on mRNA expression of chemokines in lung tissue 96
5.4.4.6 Effects of SRS27 on mRNA expression of oxidative stress markers in lung tissue 97
5.4.5 Effects of SRS27 on OVA-induced AHR in mice 99
5.4.6 Effects of SRS27 on the NF-κB signalling pathway in Lung Tissues 101

5.5 Discussion
5.6 Conclusion

6 PHARMACOKINETIC PROFILE OF SRS27
6.1 Introduction 113
6.2 Materials
6.2.1 Compounds Isolation and Synthesis 114
6.2.2 Animals 114
6.2.3 Chemicals and Reagents 114
6.2.4 Laboratories Wares and Consumables 114
6.2.5 Instrumentations 114
6.3 Methods
6.3.1 Preparation of SRS27 for in vivo Pharmacokinetic Study 114
6.3.2 Administration of SRS27 to Mice 114
6.3.3 Sample Collection and Handling 114
6.3.4 Extraction Procedure 115
6.3.5 HPLC Analysis 115
6.3.6 Calibration Curve and Method Validation 115
6.3.7 Pharmacokinetic Analysis of SRS27 116
6.3.8 Lipinski Rule of Five
6.3.9 Statistical Analysis

6.4 Results
6.4.1 Method Development and Validation
6.4.2 Pharmacokinetic Profile of SRS27 in Mouse
6.4.3 Lipinski’s rule

6.5 Discussion
6.6 Conclusion

7  ACUTE TOXICITY PROFILE OF SRS27
7.1 Introduction
7.2 Materials
7.2.1 Compounds Isolation and Synthesis
7.2.2 Animals
7.2.3 Chemicals and Reagents
7.2.4 Laboratories Wares and Consumables
7.2.5 Instrumentations
7.3 Methods
7.3.1 Peripheral Cell Count
7.3.2 AST, ALT and Creatinine levels
7.3.3 Acute Toxicity of SRS27 in Mice
7.3.4 Histopathological Evaluation of Liver, Lung, Kidney and Ovary
7.3.5 Statistical Analysis
7.4 Results
7.4.1 Peripheral Cell Count
7.4.2 AST, ALT and Creatinine level
7.4.3 Body and Organ Weight Changes
7.4.4 Histological Changes
7.5 Discussions
7.6 Conclusion

8  GENERAL DISCUSSION AND CONCLUSION
8.1 General Discussion
8.2 Conclusion
8.3 Future Research

REFERENCES
APPENDICES
BIODATA OF STUDENT
LIST OF PUBLICATIONS
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Molecular properties of DDAG and its derivatives</td>
<td>45</td>
</tr>
<tr>
<td>4.1</td>
<td>Primers used to study biological response in A549 cells</td>
<td>53</td>
</tr>
<tr>
<td>5.1</td>
<td>Primers used to study biological response in mouse lung samples.</td>
<td>73</td>
</tr>
<tr>
<td>6.1</td>
<td>Pharmacokinetic parameters calculation.</td>
<td>116</td>
</tr>
<tr>
<td>6.2</td>
<td>Intra-day and Inter-day precision.</td>
<td>118</td>
</tr>
<tr>
<td>6.3</td>
<td>Comparison of pharmacokinetic parameters of SRS27, AGP and DDAG.</td>
<td>120</td>
</tr>
<tr>
<td>6.4</td>
<td>Table summarises the pharmaceutical properties of AGP, DDAG and SRS27 according to Lipinski’s rule.</td>
<td>121</td>
</tr>
<tr>
<td>7.1</td>
<td>Scoring system employed for histopathological evaluation of organs.</td>
<td>127</td>
</tr>
<tr>
<td>7.2</td>
<td>Scores of liver, kidney, lung, spleen and ovary toxicity of mice treated with various concentrations of SRS27 compared with those of the control group.</td>
<td>133</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>World map of the prevalence of clinical asthma in 2004.</td>
<td>4</td>
</tr>
<tr>
<td>2.2</td>
<td>Ranking of the prevalence of current asthma symptoms in childhood with positive response to clinical asthma seen by country in 2004</td>
<td>5</td>
</tr>
<tr>
<td>2.3</td>
<td>The initiation of allergic airway inflammation by Th2 cell-mediated response.</td>
<td>7</td>
</tr>
<tr>
<td>2.4</td>
<td>NF-κB signalling pathway and its activated downstream genes.</td>
<td>12</td>
</tr>
<tr>
<td>2.5</td>
<td>The synthesis scheme of CysLT.</td>
<td>13</td>
</tr>
<tr>
<td>2.6</td>
<td>The role of CysLT in asthma pathogenesis.</td>
<td>14</td>
</tr>
<tr>
<td>2.7</td>
<td>The production of NO in the lung airway of an asthmatic patient.</td>
<td>15</td>
</tr>
<tr>
<td>2.8</td>
<td>Chemical structures of AGP, DDAG and its derivatives.</td>
<td>24</td>
</tr>
<tr>
<td>3.1</td>
<td>Single concentration effect of various AGP analogues on CysLT released from calcium ionophore stimulated mouse macrophages and cell viability of macrophages</td>
<td>36</td>
</tr>
<tr>
<td>3.2A</td>
<td>Dose-response effects of SRS27, DDAG, AGP and NDGA on CysLT released from calcium ionophore stimulated mouse macrophages</td>
<td>37</td>
</tr>
<tr>
<td>3.2B</td>
<td>Viability of calcium ionophore-stimulated mouse macrophages upon treatment with increasing concentration of SRS27, DDAG, AGP and NDGA</td>
<td>37</td>
</tr>
<tr>
<td>3.3A</td>
<td>Effects of AGP, DDAG, SRS27, SRS28, SRS49, SRS76 and SRS83 on NO production in RAW 264.7 stimulated by IFN-γ</td>
<td>39</td>
</tr>
<tr>
<td>3.3B</td>
<td>Effects of test agents on RAW 264.7 cell viability after 24 hr exposure to 10 U of IFN-γ</td>
<td>40</td>
</tr>
<tr>
<td>3.4A</td>
<td>Dose-response cytotoxic effects of AGP, DDAG and SRS27 on the viability of RAW264.7</td>
<td>41</td>
</tr>
<tr>
<td>3.4B</td>
<td>Dose-response cytotoxic effects of AGP, DDAG and SRS27 on the viability of PC3</td>
<td>42</td>
</tr>
<tr>
<td>3.4C</td>
<td>Dose-response cytotoxic effects of AGP, DDAG and SRS27 on the viability of A549</td>
<td>42</td>
</tr>
</tbody>
</table>
3.4D Dose-response cytotoxic effects of AGP, DDAG and SRS27 on the viability of MCF-7

3.4E Dose-response cytotoxic effects of AGP, DDAG and SRS27 on the viability of HCT116

3.4F Dose-response cytotoxic effects of AGP, DDAG and SRS27 on the viability of VERO

4.1 Effects of SRS27 on the NF-κB-activated biological response

4.2 DNA-binding activity of p65 nuclear factor-κB in nucleus of A549 cells stimulated with TNF-α for 5 min in the presence or absence of 30 µM of SRS27 was determined using a TransAM p65 transcription factor ELISA kit.

4.3 Effects of SRS27 on TNF-α-induced NF-κB activation

4.4 Semi-quantitative analysis of three independent experiments of bands from figure 4.3

4.5 Immunoblotting of p65 level in nuclear extracts of A549 cells stimulated with TNF-α for 5 min in the presence and absence of 30 µM SRS27

4.6 Semi-quantitative analysis of three independent experiments of bands from figure 4.5.

4.7 Proposed anti-inflammatory mechanism of action of AGP, DDAG and SRS27.

5.1 Allergen aerosol delivery system.

5.2 Animal sensitization, challenge and treatment schedule.

5.3 Lung lobes in mouse.

5.4 The FinePointe™ system (Buxco Research Systems, Wilmington, NC, USA).

5.5 Light micrographs of cell population in a saline-challenged mouse and OVA-challenged mouse stained with Liu staining.

5.6 Total and different inflammatory cell counts in BAL fluid of OVA-sensitized and saline-challenged (Saline) and OVA-sensitized and OVA-challenged (OVA) mice.

5.7 Light micrographs of cell population in BAL fluid of DMSO-treated OVA-challenged mice and OVA-challenged mice
treated with 0.1 mg/kg, 0.3 mg/kg, 1.0, 3.0 mg/kg of SRS27.

5.8 Total and differential cell counts were performed on BAL fluid obtained from vehicle control (3% DMSO) mice, and four drug treatment groups (0.1 0.3, 1.0 and 3.0 mg/kg SRS27) 24 hours after the last aerosol challenge.

5.9 Representative photomicrographs of lung sections of saline control mice, OVA-challenged mice, OVA-challenged and DMSO-treated mice, and OVA-challenged and 3.0 mg/kg SRS27-treated mice 24 hours after the last aerosol challenge.

5.10 Mean scores of inflammatory cell infiltration in three different lung samples of respective lung sections of Figure 5.9.

5.11 Representative fluorescent photomicrographs of saline control mice, OVA-challenged mice, OVA-challenged and DMSO-treated mice, and OVA-challenged and 3.0 mg/kg SRS27-treated mice 24 hours after the last aerosol challenge.

5.12 Mean scores of mucus production in three different lung samples of respective lung sections of Figure 5.11.

5.13A Level of IL-4 in BAL fluid of saline control mice, OVA-challenged mice, DMSO-treated mice, and 0.1, 0.3, 1.0, and 3.0 mg/kg SRS27-treated mice measured using ELISA.

5.13B Level of IL-5 in BAL fluid of saline control mice, OVA-challenged mice, DMSO-treated mice, and 0.1, 0.3, 1.0, and 3.0 mg/kg SRS27-treated mice measured using ELISA.

5.13C Level of IL-13 in BAL fluid of saline control mice, OVA-challenged mice, DMSO-treated mice, and 0.1, 0.3, 1.0, and 3.0 mg/kg SRS27-treated mice measured using ELISA.

5.13D Level of Eotaxin in BAL fluid of saline control mice, OVA-challenged mice, DMSO-treated mice, and 0.1, 0.3, 1.0, and 3.0 mg/kg SRS27-treated mice measured using ELISA.

5.14 Level of IFN-γ in BAL fluid of saline control mice, OVA-challenged mice, DMSO-treated mice, and 0.1, 0.3, 1.0, and 3.0 mg/kg SRS27-treated mice measured using ELISA.

5.15 Levels of isoprostane and 8-OH-2-deoxyguanosine in BAL fluid of saline control mice, OVA-challenged mice, DMSO-treated mice, and 0.1, 0.3, 1.0, and 3.0 mg/kg SRS27-treated mice measured using ELISA.
5.16 Levels of total IgE and OVA-specific IgE in mouse serum of saline control mice, OVA-challenged mice, DMSO-treated mice, and 0.1, 0.3, 1.0, and 3.0 mg/kg SRS27-treated mice measured using ELISA.

5.17 (A) Representative images of polymerase chain reaction products of chitinases. (B) Mean relative intensity of bands of chitinases against saline group quantified using Image J.

5.18 (A) Representative images of polymerase chain reaction products of adhesion molecules. (B) Mean relative intensity of bands of adhesion molecules against saline group quantified using Image J.

5.19 (A) Representative images of polymerase chain reaction products of inflammatory mediators. (B) Mean relative intensity of bands of inflammatory mediators against saline group quantified using Image J.

5.20 (A) Representative images of polymerase chain reaction products of cytokines. (B) Mean relative intensity of bands of cytokines against saline group quantified using Image J.

5.21 (A) Representative images of polymerase chain reaction products of chemokines. (B) Mean relative intensity of bands of chemokines against saline group quantified using Image J.

5.22 (A) Representative images of polymerase chain reaction products of oxidative stress markers. (B) Mean relative intensity of bands of oxidative stress markers against saline group quantified using Image J.

5.23 Airway resistance and dynamic compliance of mechanically ventilated mice (Saline= saline control mice, OVA= OVA-challenged mice, 3% DMSO= DMSO-treated mice and SRS27= 3.0 mg/kg SRS27-treated mice) in response to increasing concentrations of methacholine (0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg).

5.24 Representative images of immunoblotting of p65 level in lung nuclear protein lysate and Intensity of bands against saline group quantified using Image J.

5.25 Nuclear binding activity of NF-κB in lung tissue.

6.1 Representative chromatogram of (A) SRS27 (100µM) and (B) DDAG (50 µM) in mobile phase (50% ACN and 50% H2O).

6.2 The mean plasma concentration–time profile of SRS27 after administration of a single i.p. bolus dose of 3 mg/kg to female
Balb/C mice.

7.1A Peripheral blood leukocytes analysis of haematological blood profile obtained from normal control, vehicle control, and SRS27-treated non-asthmatic BALB/C mice.

7.1B Peripheral blood leukocytes analysis of haematological blood profile obtained from normal control, vehicle control, and SRS27-treated non-asthmatic BALB/C mice.

7.2 AST and ALT level in serum of control and mice treated with 3 mg/kg of SRS27 measured during the toxicity study.

7.3 Creatinine level in serum of control, DMSO (3%) and mice treated with 3 mg/kg of SRS27 measured during the toxicity study.

7.4 Percentage normalised body weight changes relative to day 0 of control and mice treated with various doses of SRS27 during the toxicity study.

7.5 Relative organ weight of control mice and mice treated with various doses of SRS27 measured at the end of the toxicity study.

7.6 Photomicrograph of liver sections of vehicle control mice and mice treated with various doses of SRS27.

7.7 Photomicrograph of lung sections of vehicle control mice and mice treated with various doses of SRS27.

7.8a Photomicrograph of kidneys of vehicle control mice and mice treated with various doses of SRS27.

7.8b Photomicrograph of kidneys of vehicle control mice and mice treated with various doses of SRS27.

7.8c Photomicrograph of kidney section of one mouse treated with 6 mg/kg of SRS27.

7.9 Photomicrographs of ovaries of vehicle control mice and mice treated with various doses of SRS27.

8.1 Proposed anti-inflammatory mechanism of action of AGP, DDAG and SRS27
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-LOX</td>
<td>5-Lipoxygenase</td>
</tr>
<tr>
<td>8-OHdG</td>
<td>8-Oxo-2'-deoxyguanosine</td>
</tr>
<tr>
<td>A549</td>
<td>Adenocarcinomic human alveolar basal epithelial cells.</td>
</tr>
<tr>
<td>AChE</td>
<td>Acetylcholinesterase</td>
</tr>
<tr>
<td>AGP</td>
<td>Andrographolide</td>
</tr>
<tr>
<td>AHR</td>
<td>Airway hyperresponsiveness</td>
</tr>
<tr>
<td>AHU</td>
<td>Animal Holding Unit</td>
</tr>
<tr>
<td>AIRIAP</td>
<td>Asthma Insights and Reality in Asia Pacific</td>
</tr>
<tr>
<td>AKT</td>
<td>Protein Kinase B</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine transaminase</td>
</tr>
<tr>
<td>Al(OH)₃</td>
<td>Aluminium hydroxide</td>
</tr>
<tr>
<td>AMCase</td>
<td>Acidic mammalian chitinase</td>
</tr>
<tr>
<td>AMV</td>
<td>Avian myeloblastosis virus</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AP</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>AP-1</td>
<td>Activator protein</td>
</tr>
<tr>
<td>ASM</td>
<td>Airway smooth muscle</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate transaminase</td>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under curve</td>
</tr>
<tr>
<td>B₂₀</td>
<td>Maximum binding well</td>
</tr>
<tr>
<td>BAL</td>
<td>Bronchoalveolar lavage</td>
</tr>
<tr>
<td>BALB/C</td>
<td>Albino, laboratory-bred strain mice</td>
</tr>
<tr>
<td>Baso</td>
<td>Basophils</td>
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<tr>
<td>BCA</td>
<td>Bicinchoninic acid</td>
</tr>
<tr>
<td>Bcl-3</td>
<td>B-cell lymphoma 3-encoded</td>
</tr>
<tr>
<td>BEAS-2B</td>
<td>Human lung epithelial cells</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
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<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>Calcium ion</td>
</tr>
<tr>
<td>CARE</td>
<td>Centre for Animal Resources</td>
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<tr>
<td>CD4+</td>
<td>Cluster of differentiation 4</td>
</tr>
<tr>
<td>CDER</td>
<td>Centre for Drug Evaluation and Research</td>
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<tr>
<td>Cdyn</td>
<td>Dynamic compliance</td>
</tr>
<tr>
<td>CHMP</td>
<td>Committee for Medicinal Products for Human Use</td>
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<tr>
<td>CI</td>
<td>Clearance</td>
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<td>Cmax</td>
<td>Maximum concentration</td>
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<td>CNS</td>
<td>Central nervous system</td>
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<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
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<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
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<td>COX</td>
<td>Cyclooxygenases</td>
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<td>CS</td>
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<td>CVM</td>
<td>Centre for Veterinary Medicine</td>
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<tr>
<td>CXC</td>
<td>α-chemokines</td>
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<tr>
<td>CysLT</td>
<td>Cysteinyl leukotriene</td>
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<tr>
<td>DDAG</td>
<td>14-deoxy-11,12-didehydroandrographolide</td>
</tr>
<tr>
<td>DEPC</td>
<td>Diethylpyrocarbonate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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<tr>
<td>DMEM</td>
<td>Dulbecco's Modified Eagle's medium</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>D PX</td>
<td>Digital Picture Exchange</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>ECL</td>
<td>Enhanced chemiluminescence</td>
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<tr>
<td>ECP</td>
<td>Eosinophil cationic proteins</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
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<tr>
<td>ELISA</td>
<td>Enzyme immunoassay</td>
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<td>EPO</td>
<td>Eosinophil peroxidase</td>
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<td>Eos</td>
<td>Eosinophils</td>
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<tr>
<td>ERK</td>
<td>Extracellular signal-related kinase</td>
</tr>
<tr>
<td>E-selectin</td>
<td>Endothelial selectin</td>
</tr>
<tr>
<td>FBS</td>
<td>Foetal bovine serum</td>
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<tr>
<td>FceR</td>
<td>High affinity IgE receptor</td>
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<td>FLAP</td>
<td>5-lipoxygenase activating protein</td>
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<td>Foxa2</td>
<td>Forkhead box α-2</td>
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<td>GATA3</td>
<td>Trans-acting T-cell-specific transcription factor</td>
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<td>GCCP</td>
<td>Good cell culture practice</td>
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<td>GPCR s</td>
<td>G protein coupled receptors</td>
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<td>G-CSF</td>
<td>Granulocyte colony stimulating factor</td>
</tr>
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<td>GINA</td>
<td>Global Initiative for Asthma</td>
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<tr>
<td>GM-CSF</td>
<td>Granulocyte macrophage colony stimulating factor</td>
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<td>GR</td>
<td>Glucocorticoid receptors</td>
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<td>Human colon cancer cell line</td>
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<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<td>H₂O₂</td>
<td>Hydrogen peroxide</td>
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<td>H₃PO₄</td>
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<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<td>HRP</td>
<td>Horseradish peroxidase</td>
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<td>IACUC</td>
<td>Institutional Animal Care and Use Committee</td>
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<td>ICAM-1</td>
<td>Intercellular Adhesion Molecule 1</td>
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<td>ICS</td>
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<td>IgA</td>
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<td>IGF</td>
<td>Insulin-like growth factor</td>
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<tr>
<td>IkB</td>
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<td>IKK-α</td>
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<td>IKK-β</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
</tr>
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<td>i.p.</td>
<td>Intraperitoneal</td>
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<tr>
<td>JAK</td>
<td>Janus kinase</td>
</tr>
<tr>
<td>JNK</td>
<td>c-Jun N-terminal kinases</td>
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<tr>
<td>kDA</td>
<td>Kilo Dalton</td>
</tr>
<tr>
<td>$K_d$</td>
<td>Elimination rate constant</td>
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<td>LABA</td>
<td>Long-acting β2-adrenoceptor agonists</td>
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<td>L-NAME</td>
<td>L-NG-Nitroarginine Methyl Ester</td>
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<tr>
<td>Log P</td>
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<td>Major basic protein</td>
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<td>MCF7</td>
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<td>Mch</td>
<td>Acetyl-β-methylcholine chloride</td>
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<td>Matrix metalloproteinase</td>
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<td>Major histocompatibility complex</td>
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<td>Mucin</td>
</tr>
<tr>
<td>Muc5ac</td>
<td>Gel-forming mucin</td>
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<td>Muc5b</td>
<td>Gel-forming mucin</td>
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<td>Neoandrographolide</td>
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<td>Nordihydroguaiaretic acid</td>
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<td>NF-kappa-B essential modulator</td>
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<td>NF-κB</td>
<td>Nuclear factor kappa B</td>
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<td>NLS</td>
<td>Nuclear localisation signal</td>
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<td>NOS</td>
<td>Nitric oxide synthase</td>
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<td>NSB</td>
<td>Non-specific binding</td>
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<td>National University of Singapore</td>
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<td>Peroxynitrite</td>
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<td>Ovalbumin</td>
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<td>P</td>
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<td>$\Delta P/V$</td>
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<td>PAGE</td>
<td>Polyacrylamide gel electrophoresis</td>
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<td>PBS</td>
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<td>PBS containing 0.1% Tween 20</td>
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<td>Polymerase chain reaction</td>
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<td>Platelet-derived growth factor</td>
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<td>Phosphatidylinositol 3-kinase</td>
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<td>Protein Kinase A catalytic subunit</td>
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<tr>
<td>PKC</td>
<td>Protein kinase C</td>
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<td>Description</td>
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<tr>
<td>PVDF</td>
<td>Polyvinylidene difluoride</td>
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<td>RANTES</td>
<td>Regulated on Activation, Normal T Cell Expressed and Secreted</td>
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<td>RAW 264.7</td>
<td>Mouse leukaemic monocyte macrophage cell line</td>
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<td>RBM</td>
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<td>RBL-2H3</td>
<td>Rat basophilic leukaemia</td>
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<td>Airway resistance</td>
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<td>Ribonucleic acid</td>
</tr>
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<td>RNasin</td>
<td>Ribonuclease inhibitor</td>
</tr>
<tr>
<td>RNAse</td>
<td>Ribonuclease</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive nitrate species</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
</tr>
<tr>
<td>RT</td>
<td>Retention time</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse transcriptase polymerase chain reaction</td>
</tr>
<tr>
<td>RTV</td>
<td>Real-Time Viewing</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>ROW</td>
<td>Relative organ weight</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>RPMI</td>
<td>Roswell Park Memorial Institute medium</td>
</tr>
<tr>
<td>SABA</td>
<td>Short-acting beta2-agonists</td>
</tr>
<tr>
<td>SCG</td>
<td>Sodium cromoglicate</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulphate</td>
</tr>
<tr>
<td>SIT</td>
<td>Allergen immunotherapy</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>SRS27</td>
<td>3,19-Diacetyl-14-deoxy-11,12-didehydroandrographolide</td>
</tr>
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<td>SRS28</td>
<td>19-Acetyl-14-deoxy-11,12-didehydroandrographolide</td>
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<td>3,19-Dipropionyl-14-deoxy-11,12-didehydroandrographolide</td>
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<td>3,19-Benzylidene-14-deoxy-11,12-didehydroandrographolide</td>
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<td>SRS83</td>
<td>3,19-(4-Chlorobenzylidene)-14-deoxy-11,12-didehydroandrographolide</td>
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<td>SRS88</td>
<td>3,19-(4-Acetamidobenzylidene)andrographolide</td>
</tr>
<tr>
<td>STAT</td>
<td>Signal transducer and activator of transcription</td>
</tr>
<tr>
<td>T1/2</td>
<td>Half-life</td>
</tr>
<tr>
<td>TA</td>
<td>Total activity well</td>
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<tr>
<td>TAE</td>
<td>Tris-acetate-EDTA</td>
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<tr>
<td>T-bet</td>
<td>T box transcription factor</td>
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<tr>
<td>TDZD-8</td>
<td>4-benzyl-2-methyl-1,2,4-thiazadizolidine-3,5-dione</td>
</tr>
<tr>
<td>TEMED</td>
<td>N, N', N''-tetramethylethylenediamine</td>
</tr>
<tr>
<td>TGFB-β</td>
<td>Transforming growth factor beta</td>
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<tr>
<td>Th0</td>
<td>Naïve T-Helper cells</td>
</tr>
<tr>
<td>Th1</td>
<td>T-helper cells 1</td>
</tr>
<tr>
<td>Th2</td>
<td>T-helper cells 2</td>
</tr>
<tr>
<td>T max</td>
<td>Time to achieve C max</td>
</tr>
<tr>
<td>TMB</td>
<td>3,3',5,5'-tetramethylbenzidine</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor alpha</td>
</tr>
<tr>
<td>TNF-R</td>
<td>Tumour necrosis factor receptor</td>
</tr>
<tr>
<td>TRCP</td>
<td>β-transducin repeat-containing protein</td>
</tr>
<tr>
<td>T regs</td>
<td>Regulatory T cells</td>
</tr>
</tbody>
</table>
TSLP  Thymic stromal lymphopoi etin
TTBS  Tris-Buffered Saline and Tween 20
Tween 20  Polyox yethylene sorbitan monola urate
 URTI  Acute upper respiratory tract infection
US  United States of America
USD  United States Dollar
UV  Ultraviolet
ΔV/ΔP  Change in volume of the lung produced by a change in pressure across the lung
V  Volt
VCAM-1  Vascular cell adhesion protein 1
Vd  Volume of distribution
VEGF  Vascular endothelial growth factor
VERO  African green monkey kidney epithelial cells
VLA  Very late antigen
WBC  White blood cells
Ym-2  Chitinase 3-like 4
YKL-40  Chitinase 3-like 1
CHAPTER 1
INTRODUCTION

1.1 Background of the Study
Asthma is one of the most common chronic diseases, affecting both children and adults worldwide, with increasing prevalence over the past 40 years. The reasons for this dramatic increase in asthma are not yet clear and are likely to include multiple contributing factors. There are currently 300 million people worldwide estimated to be affected by asthma (Masoli et al., 2004). For decades, the number of asthmatic cases has been increasing significantly. Considering population growth and increased urbanisation, the number of asthmatic patients is expected to rise to 400 million by the year 2025. It is estimated that 1 out of 250 deaths worldwide is due to asthma attacks (Peters et al., 2006).

1.2 Problem Statements
Asthma is underdiagnosed and undertreated, but the symptoms usually respond well and can be controlled with inhaled corticosteroids (CS) and a short- or long-acting β2-agonist (Braman, 2006). Glucocorticoids are the most effective anti-inflammatory drugs available for the treatment of many chronic inflammatory and immune diseases, including asthma, rheumatoid arthritis, inflammatory bowel disease and autoimmune diseases (Barnes, 2010). In Malaysia, the most preferred medications for ‘first-line’, ‘second-line’ and ‘third-line’ treatment for asthma by government doctors were inhaled short-acting beta2-agonists (SABAs), CS, and leukotriene antagonist, respectively, whereas private doctors preferred oral SABAs, inhaled CS, and oral CS, respectively (Loh and Wong, 2005). However, CS suffers from unwanted side effects and a significant proportion of patients are steroid-resistant (Adcock and Lane, 2003; Barnes, 2010). Steroid-resistant patients have symptoms consistent with asthma and show very poor or no response at all to high doses of inhaled or even systemic corticosteroids (Barnes, 2010). In addition to resistance, there has been increasing concern over the adverse effects of CS, such as osteoporosis, glaucoma, growth retardation in children and poor wound healing effects (Schacke et al., 2002). Add-on therapies for asthma such as long-acting reliever inhalers, leukotriene receptor antagonists and theophylline were invented to attenuate asthma via other approaches; however, the effects of these treatments are not as efficacious as those of steroids.

There has been a sharp increase in economic burden associated with asthma over the last 40 years, particularly in children. Asthma can involve costs related to health care use and morbidity, including missed work and school days. The large asthma burden and continued adverse outcomes present an on-going public health challenge, including the effort to enhance the uptake of underutilised management strategies to control symptoms (Akinbami et al., 2011). Approximately 300 million people worldwide currently have asthma, and its prevalence increases by 50% every decade. Although asthma is most common in developed (westernised) countries, it is becoming increasingly common in developing countries like Malaysia, which is most likely related to the increased urbanisation of communities. Despite the constant publications and guidelines from the National Asthma Education and Prevention Program to educate the public awareness on
the disease, asthma still remains poorly controlled, with annual estimated costs of up to 56 billion USD (Spangler, 2012).

1.3 Significance of the Study
With the in-depth knowledge of asthma available, many new therapies for asthma have been developed. Current leading therapies for asthma rely mainly on ICS and LABAs. Despite being the most effective treatment for asthma, ICSs cause several systemic side effects. Add-on therapies that have been introduced over the past decade also either pose noticeable side effects (in the case of omalizumab) or have a narrow therapeutic index (in the case of theophylline). Thus, there is always an urgency to discover and develop a more potent and yet safer treatment for asthma. Searching for an alternative for many inflammatory diseases in natural products is the current trend, as many of the plants have yet to be exploited and studied (Lim et al., 2012).

The bioactive compounds from the famous Asian herb *Andrographis paniculata* have been studied for their anticancer and anti-inflammatory properties for more than a decade (Lim et al., 2012). The herb contains two main diterpenoid constituents named andrographolide (AGP) and 14-deoxy-11,12-didehydroandrographolide (DDAG). Both of the compounds were found to exhibit antiasthma effects by inhibiting inflammatory responses in an allergic mouse asthma model (Bao et al., 2009; Guan et al., 2011). In the present investigation, the anti-inflammatory effects and the involved pathways of a DDAG analogue which possess improved drug-like properties compared with the parent compound as new antiasthma agents were investigated.

1.4 HYPOTHESIS
The new semi-synthetic andrographolide derivatives have anti-asthma activity through their inhibitory effect on NF-κB and related pathways.

1.5 OBJECTIVES OF THE STUDY

1.5.1 Main Objective:
The aim of the study is to investigate the potential of DDAG analogues which possess improved drug-like properties compared with the parent compound as new antiasthma agents.

1.5.2 Specific Objectives
a) To determine the inhibition of cysteinyl leukotriene and nitric oxide synthesized *in vitro* by andrographolide derivatives in mouse macrophage models.
b) To determine the effect of SRS27 on cell signalling pathway in A549 cells associated with anti-asthmatic effect.
c) To determine the efficacy of SRS27 in a mouse asthma model.
d) To evaluate toxicity and pharmacokinetics of SRS27 in mice.
REFERENCES


536 is mediated by multiple protein kinases including IκB kinase (IKK)-α, IKKβ, IKKε, TRAF family member-associated (TANK)-binding kinase 1 (TBK1), and an unknown kinase and couples p65 to TATA-binding protein-associated factor II31-mediated interleukin-8 transcription. *Journal of Biological Chemistry.* 279: 55633-55643.


compounds of Andrographis paniculata (King of bitters) extract. International Immunopharmacology. 11(1): 79-84.


Yang, D., Zhang, W., Song, L., Guo, F. (2013). Andrographolide Protects against Cigarette Smoke-Induced Lung Inflammation through Activation of Heme Oxygenase-1. Journal of Biochemical and Molecular Toxicology. 27(5): 259-265.


