

UNIVERSITI PUTRA MALAYSIA

SYNTHESIS AND BIOLOGICAL EVALUATION OF A SERIES OF ANALOGUES OF 2,4,6-TRIHYDROXY-3-GERANYLACETOPHENONE, AN ANTI-INFLAMMATORY NATURAL PRODUCT COMPOUND

NG CHEAN HUI

IB 2017 8



SYNTHESIS AND BIOLOGICAL EVALUATION OF A SERIES OF ANALOGUES OF 2,4,6-TRIHYDROXY-3-GERANYLACETOPHENONE, AN ANTI-INFLAMMATORY NATURAL PRODUCT COMPOUND



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

January 2017

COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia

DEDICATION



This thesis is dedicated to my beloved family, Professor and friends

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

SYNTHESIS AND BIOLOGICAL EVALUATION OF A SERIES OF ANALOGUES OF 2,4,6-TRIHYDROXY-3-GERANYLACETOPHENONE, AN ANTI-INFLAMMATORY NATURAL PRODUCT COMPOUND

By

NG CHEAN HUI

January 2017

Chair : Prof Khozirah Binti Shaari, PhD Faculty : Institute of Bioscience

The natural product molecule 2,4,6-trihydroxy-3-geranylacetophenone (tHGA) isolated from the medicinal plant *Melicope ptelefolia* was shown to exhibit potent lipoxygenase (LOX) inhibitory activity. It is known that LOX plays an important role in inflammatory response as it catalyzes the oxidation of unsaturated fatty acids, such as linoleic acid to form hydroperoxides that are potent proinflammatory mediators. The search for selective LOX inhibitors may provide new therapeutic approach for inflammatory diseases. Previous studies reported that tHGA was an effective LOX inhibitor and was able to control airway-hyper-responsiveness in an acute model of murine asthma. However, the structure-activity relationship (SAR) of this group of compounds is still unknown. Herein, we report the synthesis of tHGA analogues using simple Friedel-Craft acylation, direct *C*-alkylation and methylation reactions with the objective of obtaining a better insight into the structure-activity relationships of the compounds.

 \bigcirc

A total of seventeen synthetic analogues of tHGA were synthesized and evaluated for their soybean 15-LOX inhibitory activity, while three of them are new compounds. Modifications were made on the acyl moiety, alkyl moiety, and also the important hydroxyl group of phloroglucinol structural core. The combination of both electrophilic substitution on the phloroglucinol compound and nucleophilic substitution on the acylphloroglucinol derivatives gave tHGA analogues. *In vitro* soybean 15-LOX inhibiting activity was measured using spectrophotometric method. All the synthesized analogues showed potent to moderate soybean 15-LOX inhibitory activity in a dose-dependent manner (IC₅₀ = 10.31–95.38 μ M), the most active being compound **18e** (IC₅₀ value of 10.31 μ M ± 1.5) with the longest aliphatic chain on the acyl substituent. Interestingly, four target compounds **18c** (IC₅₀ value of 12.32 μ M ± 0.6), **18d** (IC₅₀ value of 15.26 μ M ± 0.5), **18e** (IC₅₀ value of 10.31 μ M ± 1.5) and **18g** (IC₅₀ value of 15.20 μ M ± 1.2) exhibited better 15-LOX inhibition than tHGA (8) where improvement in activities range from approximately 30-50%. The SAR study revealed that the presence of a short, branched acyl substituent and the introduction of a cyclohexyl ring were less favourable for LOX inhibitory activity when compared to aliphatic acyl substituent. On the other hand, the introduction of a planar aromatic ring in the acyl substituent was found to improve the inhibitory activity. The results of the simple SAR study suggest that a longer, aliphatic and aromatic acyl substituent is favourable for better inhibitory action.

Kinetic inhibition assay showed that both of the most active compound **18e** and tHGA (**8**) are competitive inhibitors. Molecular docking studies (cDOCKER) and molecular dynamic (MD) simulation (GROMACs) revealed that hydrophobic interactions were the main driving force for the binding interactions of the active analogues with the target protein. Analogues with the larger lipophilic nature had better binding affinity as compared to others. Besides, the binding interaction with one crucial amino acid residue (His499) involved in iron chelation for the target enzyme correlates well with the kinetic assay's result. Therefore, our findings support that these geranylated acylphloroglucinol compounds have promising potential as lead compounds for the design of new anti-inflammatory drugs or Non-Steroidal Anti-inflammatory Drugs (NSAIDs). The combination of both the bioassay results and *in silico* studies has reinforced the crucial structural features that are involved in the inhibitory activity which is important information for structure-based drug design.

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

SINTESIS DAN PENILAIAN AKTIVITI BIOLOGI BAGI SATU SIRI ANALOG 2,4,6-TRIHIDROKSI-3-GERANILASETOFENON, SATU SEBATIAN SEMULA JADI BAGI ANTI-RADANG

Oleh

NG CHEAN HUI

Januari 2017

Pengerusi : Prof Khozirah Binti Shaari, PhD Fakulti : Institut Biosains

2,4,6-trihidroksi-3-geranil-asetofenon (tHGA) merupakan molekul semula jadi yang dipencilkan daripada tumbuhan ubatan *Melicope ptelefolia* dan telah menpamerkan aktiviti perencatan enzim lipoksigenas (LOX) yang poten. Enzim LOX dikenali memainkan peranan penting dalam tindak balas keradangan kerana ia memangkinkan pengoksidaaan asid lemak tak tepu, seperti asid linoleik, untuk membentuk hidroperoksida yang merupakan perantara pro-radang yang poten. Pencarian perencat LOX terpilih dapat memberikan pendekatan terapeutik baru untuk penyakit radang. Pengajian sebelum ini melaporkan bahawa tHGA adalah perencat LOX yang berkesan dan dapat mengawal hiper-responsif bagi saluran udara dalam model tikus asma yang runcing. Namun begitu, hubungkait struktur-aktiviti (SAR) bagi kumpulan sebatian ini masih tidak diketahui. Di sini, kita melaporkan sintesis untuk beberapa sebatian analog tHGA dengan menggunakan tindak balas pengasilan Friedel-Craft yang mudah, diikuti dengan tindakbalas pengalkilan-C dan pemetilan dengan tujuan untuk mendapatkan gambaran yang lebih baik mengenai SAR sebatian.

Sebanyak tujuh belas analog tHGA telah disintesis dan diuji dengan aktiviti perencatan 15-LOX kacang soya, manakala tiga daripada mereka adalah sebatian baru. Pengubahsuaian telah dibuat pada kumpulan asil, alkil, dan juga hidroksil pada teras struktur sebatian floroglusinol. Penggabungan kedua-dua gantian elektrofilik pada sebatian floroglusinol dan gantian nukleofilik pada terbitan asilfloroglusinol menghasilkan analog tHGA. Aktiviti perencatan 15-LOX kacang soya *in vitro* diukur dengan menggunakan cara spektrofotometrik. Semua analog yang disintesis menunjukkan aktiviti yang poten dan sederhana dalam aktiviti perencatan 15-LOX kacang soya secara bersandarkan dos (IC₅₀ = 10.31-95.38 μ M). Sebatian **18e** (nilai IC₅₀ 10.31 μ M ± 1.5) merupakan sebatian yang paling aktif dengan rantai alifatik

terpanjang di gantian asil. Secara menariknya, empat sebatian sasaran iaitu **18c** (nilai IC₅₀ 12.32 μ M ± 0.6), **18d** (nilai IC₅₀ 15.26 μ M ± 0.5), **18e** (nilai IC₅₀ 10.31 μ M ± 1,5) dan **18g** (nilai IC₅₀ 15.20 μ M ± 1.2) menunjukkan perencatan 15-LOX yang lebih baik daripada tHGA (**8**) di mana penambahbaikan aktiviti adalah sekitar 30-50%. Kajian SAR mendedahkan bahawa penggantian dengan kumpulan asil yang pendek, yang bercabang dan mempunyai kumpulan sikloheksil adalah kurang baik untuk aktiviti perencatan LOX berbanding dengan penggantian dengan kumpulan asil alifatik. Manakala, penggantian kumpulan asil dengan kumpulan aromatik yang satar didapati dapat mempertingkatkan aktiviti perencatan. Secara amnya, kajian SAR ini menunjukkan bahawa kumpulan asil yang panjang, alifatik dan aromatik memberikan aktiviti perencatan yang lebih baik.

Bioasai perencatan aktiviti kinetik menunjukkan bahawa kedua-dua sebatian iaitu **18e** yang merupakan sebatian yang paling aktif dan tHGA (**8**) adalah perencat kompetitif. Kajian dok molekul (cDOCKER) dan simulasi dinamik molekul (MD) (GROMACs) menunjukkan bahawa interaksi hidrofobik adalah penyebab utama yang mendorong interaksi yang mengikat analog aktif dengan protein sasaran. Analog dengan sifat yang lebih lipofilik mempunyai potensi mengikat yang lebih tinngi berbanding dengan yang lain. Selain itu, interaksi mengikat dengan satu baki asid amino penting (His499) yang terlibat dalam kelatan besi untuk enzim sasaran berhubung kait dengan keputusan asai kinetik. Oleh itu, penemuan kajian ini menyokong bahawa sebatian geranil asilfloroglusinol mempunyai potensi yang menggalakkan sebagai sebatian utama untuk reka bentuk ubat-ubatan anti-radang baru atau ubat anti-radang bukan steroid (NSAIDs). Penggabungan kedua-dua keputusan bioasai dan pengajian *in silico* telah memperkuatkan ciri-ciri struktur yang penting bagi reka bentuk ubat berdasarkan struktur.

ACKNOWLEDGEMENTS

This dissertation arose as a research project to fulfil part of the requirement for the degree of Doctor of Philosophy in Medicinal Chemistry. During this time, I have worked with a great number of people who have contributed in various ways to my research. It is a pleasure to express my gratitude to all of those who have helped me directly and indirectly.

First and foremost, I would like to express my sincere gratitude to my supervisor, Prof. Dr. Khozirah Binti Shaari for being such a wonderful person in sacrificing her precious time to help me with constructive comments, valuable suggestions and continuous encouragement.

Besides, I would like to thank my co-supervisors Assoc. Prof. Dr. Faridah Binti Abas and Dr. Lam Kok Wai for their insightful comments, teaching, and guidance in the laboratory experiments and analysis. Their invaluable efforts are crucial for the success of my studies. Special thanks goes to Assoc. Prof. Dr. Intan Safinar Binti Ismail, Dr. Fadzureena Jamaludin and Dr. Radhakrishnan Narayanaswamy for their precious help and suggestion in my research studies.

I extend all my sincere sense of gratitude to all the staff from Laboratory of Natural Products (Institute of Bioscience, UPM), for their superior help and guidance in any aspect during the project completion. I would also like to thank Malaysian Ministry of Higher Education (KPT) for providing the scholarship throughout my studies, Malaysian Ministry of Science, Technology, and Environment (MOSTI) for providing the research grant under eScience grant scheme (02-01-04-SF1593), Forest Research Institute Malaysia (FRIM) for soybean 15-LOX inhibition assay facilities and also Computer-Aided-Design & Drafting laboratory in UKM (KL) for molecular dynamic simulation facilities.

Not forgetting my lovely friends and colleagues especially Mr. Pang Xiang Yang, Mr. Kamal Rullah, Mr. Mohd. Fadhlizil Fasihi Bin Mohd Aluwi, Ms. Siti Nur Aisyah Mohd Hashim, Dr. Leong Sze Wei, Dr. Siti Munirah Binti Mohd Faudzi, Ms. Naveena Reddy Kalidas, Mr. Rameshkumar Santhanam, Mr. Karthi Vashan, Mr. Saminathan Poothan Mookiah for their encouragement and support during my hard time. Specially thanks to Mr. Kamal Rullah, Mr. Mohd. Fadhlizil Fasihi Bin Mohd Aluwi who have inspired my interest on molecular docking simulation through their respective research and studies.

Lastly, special thanks to my family members for their support, love, patience and encouragement. Their support and encouragements throughout my PhD life were precious which I cannot repay.



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

Khozirah Shaari, PhD

Professor Institute of Biosains Universiti Putra Malaysia (Chairman)

Faridah Abas, PhD

Associate Professor Faculty of Food Science and Technology Universiti Putra Malaysia (Member)

Lam Kok Wai, PhD

Senior Lecturer Faculty of Pharmacy Universiti Kebangsaan Malaysia (Member)

ROBIAH BINTI YUNUS, PhD Professor and Dean School of Graduate Studies Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature:	Date:	
-		

Name and Matric No.: Ng Chean Hui GS33674

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature:	
Name of Chairman	
of Supervisory	
Committee:	Prof. Dr. Khozirah Shaari
Signature:	
Name of Member	
of Supervisory	
Committee:	Dr. Faridah Abas
Signature:	
Name of Member of Supervisory	
Committee:	Dr. Lam Kok Wai

TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	ii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
DECLARATION	viii
LIST OF TABLES	xii
LIST OF FIGURES	xiv
LIST OF SCHEMES	xviii
LIST OF APPENDICES	xix
LIST OF ABBREVIATIONS	xxii

CHAPTER

1	INTRODUCTION	
T	1 1 General Introductions	1
	1.2 Objectives of Research	3
2	LITERATURE REVIEW	
	2.1 Inflammation	4
	2.2 The Arachidonic Acid Metabolism	5
	2.3 The Lipoxygenase Enzyme and how it Functions	7
	2.4 In Silico Methodologies in Drug Discovery Research	11
	2.5 Examples of Lipoxygenase Inhibitors	14
	2.6 The Discovery of 2,4,6-trihydroxy-3-geranylacetophenone	21
	(tHGA)	

3 METHODOLOGY

3.1 General Instrumentations	29
3.2 Chromatographic Methods	29
3.3 Solvents	29
3.4 Synthesis of 2,4,6-trihydroxy-3-geranylacetophenone	29
(tHGA) analogues	
3.5 Synthesis and Spectral Data of synthetic analogues	33
3.6 Bioassay Procedures	48
3.7 Computational Studies	49

4

SYNTHESIS AND BIOLOGICAL EVALUATION OF 2,4,6-TRIHYDROXY-3-GERANYLACETOPHENONE (THGA) ANALOGUES

4.1 Synthesis of 2,4,6-trihydroxy-3-geranylacetophenone (tHGA)	52
Analogues	
4.2 Anti-inflammatory Activity of 2,4,6-trihydroxy-3-	123
geranylacetophenone (tHGA) Analogues	

5	COMPUTATIONAL STUDIES ON THE LIPOXYGENASE INHIBITORY ACTIVITY OF 2,4,6-TRIHYDROXY-3- GERANYLACETOPHENONE (THGA) ANALOGUES 5.1 Ligand-Enzyme Interaction Studies 5.2 ADMET Analysis 5.3 TOPKAT Analysis	129 162 165
6	CONCLUSIONS AND RECOMMENDATIONS 6.1 Conclusions 6.2 Some Recommendations for Future Work	167 168
REFI BIOD LIST	ERENCES ENDICES ATA OF STUDENT OF PUBLICATIONS	170 182 251 252

LIST OF TABLES

Table		Page
1	NMR data for 2,2-dimethyl-1-(2,4,6-trihydroxyphenyl)propane-1-	56
2	one (1/a) NMR data for 2-methyl-1-(2.4.6-trihydroxyphenyl)propan-1-one	60
2	(17b) $(2, 1, 0, $	00
3	NMR data of 1-(2,4,6-trihydroxyphenyl)propan-1-one (17c), 1-	64
	(2,4,6-trihydroxyphenyl)butan-1-one (17d) and 1-(2,4,6-	
	trihydroxyphenyl)pentan-1-one (17e)	
4	NMR data for Cyclohexyl-(2,4,6-trihydroxyphenyl)methanone	75
5	(1/1) NMR data for $(F)_3$ -Phenyl-1- $(2/4)$ 6-tribydroxynhenyl)prop-2-en-	79
5	1-one $(17g)$	1)
6	NMR data for (E) -1-(3-(3,7-dimethylocta-2,6-dienyl)-2,4,6-	85
	trihydroxyphenyl)propan-1-one (15)	
7	NMR data for (E) -1- $(3-(3,7-dimethylocta-2,6-dienyl)-2,4,6-$	86
	trihydroxyphenyl)propan-1-one (18c)	_
8	NMR data for (E) -1-(3-(3,7-dimethylocta-2,6-dienyl)-2,4,6-	86
0	trihydroxyphenyl)butan-1-one (18d) NMP data for (F) 1 (2 (2.7 dimethyloate 2.6 dianyl) 2.4.6	07
9	tribydroxyphenyl)pentan-1-one (18e)	07
10	NMR data for 2-methyl-1-(2.4.6-trihydroxy-3-(3-methylbut-2-	87
10	envl)phenvl)propan-1-one (18f)	07
11	NMR data for (<i>E</i>)-1-(3-(E)-3,7-dimethylocta-2,6-dienyl)-2,4,6-	88
	trihydroxyphenyl)-3-phenylprop-2-en-1-one (18g)	
12	NMR data for 2-methyl-1-(2,4,6-trihydroxy-3-(3-methylbut-2-	96
10	enyl)phenyl)propan-1-one (19b)	07
13	NMR data for 1-(2,4,6-trinydroxy-3-(3-methylbut-2-	97
14	NMR data for $1_{(2,4,6,trihydroxy,3_{(3,methylbut,2_{(3,meth$	97
11	envl)phenvl)butan-1-one (19d)	71
15	NMR data for 1-(2,4,6-trihydroxy-3-(3-methylbut-2-	98
	enyl)phenyl)pentan-1-one (19e)	
16	NMR data for Cyclohexyl-(2,4,6-trihydroxy-3-(3-methylbut-2-	98
17	enyl)phenyl)methanone (19f)	00
17	NMR data for (<i>E</i>)-3-phenyl-1-(2,4,6-trihydroxy-3-(3-methylbut-2-	99
18	NMP data for $1(24.6 tribudrovy 3.(3 methylbut 2)$	00
10	envl)phenvl)ethanone (19h)))
19	NMR data for (E)-1-(3-(3.7-dimethylocta-2.6-dienyl)-2-hydroxy-	114
-	4,6-dimethoxyphenyl)pentan-1-one (20e)	
20	NMR data for (E)-1-(3-(3,7-dimethylocta-2,6-dienyl)-2-hydroxy-	115
_	4,6-dimethoxyphenyl)ethanone (20h)	
21	NMR data for 1-(2-hydroxy-4,6-dimethoxy-3-(3-methylbut-2-	116
22	enyl)phenyl)pentan-1-one (21e)	116
LL	invir uata for 1-(2-flydroxy-4,0-dimetnoxy-3-(3-methylbut-2- envl)phenvl)ethanone (21h)	110
	chyr)phonyr)ethanolle (2111)	

- Anti-inflammatory activities of phloroglucinol, 2,4,6- 124
 trihydroxyacetophenone, acylphloroglucinol intermediates (17b-g),
 tHGA analogues (15, 18c-g, 19b-h, 20-21e, 20-21h), tHGA (8) and
 NDGA on soybean 15-LOX enzyme
- LogP values for acylphloroglucinol intermediates (**17b-g**), tHGA 126 analogues (**15, 18c-g, 19b-h, 20-21e, 20-21h**) and tHGA (**8**)
- 25 K_m (Michaelis-Menten constant), V_{max} (maximum enzyme 128 velocity) and K_i (Inhibitor constant) for the inhibition of soybean 15-LOX by the tested compounds
- 26 Binding energy decomposition (kcal/mol) on a per residue basis 157 include molecular mechanic (ΔE_{MM}), polar solvation (ΔG_{PB}), non-polar solvation (ΔG_{SA}) and free binding energy (ΔG_{bind}) between the most active compound **18e** and the residues in the binding pocket of soybean LOX-1 model.
- 27 Binding energy decomposition (kcal/mol) on a per residue basis 158 include molecular mechanic (ΔE_{MM}), polar solvation (ΔG_{PB}), non-polar solvation (ΔG_{SA}) and free binding energy (ΔG_{bind}) between compound **19e** and the residues in the binding pocket of soybean LOX-1 model
- 28 Binding energy decomposition (kcal/mol) on a per residue basis 159 include molecular mechanic (ΔE_{MM}), polar solvation (ΔG_{PB}), nonpolar solvation (ΔG_{SA}) and free binding energy (ΔG_{bind}) between tHGA (8) and the residues in the binding pocket of soybean LOX-1 model
- 29 Summary of binding free energy (ΔG_{bind}) for compound **18e**, **19e** 160 and tHGA (**8**)
- 30Results of ADMET predictions on six important parameters164
- 31 Results of toxicity predictive test on six important parameters 166

LIST OF FIGURES

Figure		Page
1	Schematic flow of the drug discovery pipeline	1
2	An outline of the pathways for eicosanoid biosynthesis starting	5
	from arachidonic acid	
3	Products and enzymes of the 5-LOX pathway	6
4	Crystal structure of LOX-1 enzyme	8
5	Principle steps of LOX reaction	10
6	Oxidation of linoleic acid by LOX	11
7	Structures of curcumin (1) and it's analogue (2)	15
8	Docked poses of (A) curcumin (1) (dark grey) and (B) analogue	16
	(2) (purple) in the soybean LOX binding site	
9	Chemical structure of Diospyrin (3)	17
10	Binding mode of diospyrin (3) inside the active site of LOX	17
11	Structures of prenylated chalcones (4 and 5)	18
12	Hydrogen bonding of the compounds with 5-LOX active site	19
10	residues: (A) compound (4) and (B) compound (5)	•
13	Chemical structures of the lignans (6 and 7)	20
14	Binding orientation of (A) Lignan (6) (blue colored) (B) Lignan	20
	(7) (green colored) respectively at the substrate binding site of	
15	Soybean LOA-1 Melicone ntelefelin Chemp or Donth	21
15	Chamical structures of compounds (8 11) isolated from Malicona	$\frac{21}{22}$
10	ntalifolia	
17	Inhibition of soybean $15 - I \cap X$ by tHGA (8) in comparison with	23
17	NDGA	23
18	Inhibition of human PBML 5-LOX by tHGA (8) compared to	24
10	NDGA	21
19	The 2D representation of the docking result of tHGA (8) with the	24
	residues in the active site of 5-LOX model show by LIGPLOT	
20	Synthesis of mallotophillippens C	26
21	Acylphloroglucinols from Hypericum densiflorum	27
22	COX-1 and COX-2 enzyme inhibitory activities of compounds	27
	(12-14)	
23	Acylphloroglucinols isolated from Hypericum empetrifolium	28
24	Synthesis scheme for Friedel-Craft acylation	30
25	Synthesis scheme for Direct C-alkylation	31
26	Synthesis scheme for Methylation	32
27	IC ₅₀ values of naturally active LOX inhibitors in which the	52
	structure features is similar to that of arachidonic acid	
28	EIMS spectrum for 2,2-dimethyl-1-(2,4,6-	56
• •	trihydroxyphenyl)propane-1-one (17a)	
29	¹ H NMR spectrum for 2,2-dimethyl-1-(2,4,6-	57
20	trihydroxyphenyl)propane-1-one (17a)	F 0
30	NMR spectrum for 2,2-dimethyl-1-(2,4,6-	58
21	trinyaroxypnenyi)propane-1-one (1/a)	<u> </u>
51	LINIS spectrum for 2-methyl-1-(2,4,6-trinydroxypnenyl)propan-	60
	1-011e (1/D)	

 \bigcirc

32	¹ H NMR spectrum for 2-methyl-1-(2,4,6-	61
	trihydroxyphenyl)propan-1-one (17b)	
33	¹³ C NMR spectrum for 2-methyl-1-(2,4,6-	62
	trihydroxyphenyl)propan-1-one (17b)	
34	EIMS spectrum for 1-(2,4,6-trihydroxyphenyl)propan-1-one	65
25	(1/c) EIMS spectrum for 1 (2.4.6 tribudrowumbenyl)buten 1 one (17d)	66
55	Envis spectrum for $1-(2,4,6-trinydroxyphenyi)butan-1-one (1/u)$	00
36	EIMS spectrum for 1-(2,4,6-trihydroxyphenyl)pentan-1-one	67
	(17e)	
37	¹ H NMR spectrum for 1-(2,4,6-trihydroxyphenyl)propan-1-one	68
•	(17c)	
38	¹ H NMR spectrum for 1-(2,4,6-trihydroxyphenyl)butan-1-one	69
20		70
39	¹ H NMR spectrum for 1-(2,4,6-trinydroxypnenyl)pentan-1-one	/0
40	¹³ C NMP spectrum for 1 (2.4.6 tribudrovumbenul) monon 1 one	71
40	(17_{0})	/1
41	¹³ C NMR spectrum for 1.(24.6-trihydroxyphenyl)butan-1-one	72
71	(17d)	12
42	¹³ C NMR spectrum for 1-(2.4.6-trihydroxyphenyl)pentan-1-one	73
12	(17e)	15
43	EIMS spectrum for Cyclohexyl-(2.4.6-	75
	trihydroxyphenyl)methanone (17f)	
44	¹ H NMR spectrum for Cyclohexyl-(2,4,6-	76
	trihydroxyphenyl)methanone (17f)	
45	¹³ C NMR spectrum for Cyclohexyl-(2,4,6-	77
	trihydroxyphenyl)methanone (17f)	
46	EIMS spectrum for (E)-3-Phenyl-1-(2,4,6-	79
	trihydroxyphenyl)prop-2-en-1-one (17g)	
47	¹ H NMR spectrum for (E) -3-Phenyl-1- $(2,4,6)$ -	80
	trihydroxyphenyl)prop-2-en-1-one (17g)	
48	13 C NMR spectrum for (<i>E</i>)-3-Phenyl-1-(2,4,6-	81
10	trihydroxyphenyl)prop-2-en-1-one (17g)	0.0
49	EIMS spectrum for (E) -1-(3-(3,7-dimethylocta-2,6-dienyl)-2,4,6-	89
50	trinydroxypnenyl)propan-1-one (15)	00
50	H NMR spectrum for (E) -1- $(3-(3,)-\text{dimethylocta-}2,6-\text{dienyl})$ -	90
51	^{13}C NMP spectrum for (F) 1 (3 (3.7 dimethylocts 2.6 dionyl)	01
51	2.4.6-tribudroxynhenyl)propan 1-one (15)	91
52	HSOC NMR spectrum for $(F)_1_2(3, 37)$ -dimethylocta-2.6-	92
52	dienvl)-2.4.6-trihvdroxyphenvl)propan-1-one (15)	
53	HMBC NMR spectrum for (E) -1-(3-(3.7-dimethylocta-2.6-	93
	dienyl)-2,4,6-trihydroxyphenyl)propan-1-one (15)	
54	EIMS spectrum for 2-methyl-1-(2,4,6-trihydroxy-3-(3-	100
	methylbut-2-enyl)phenyl)propan-1-one (19b)	
55	¹ H NMR spectrum for 2-methyl-1-(2,4,6-trihydroxy-3-(3-	101
	methylbut-2-enyl)phenyl)propan-1-one (19b)	

56	¹³ C NMR spectrum for 2-methyl-1-(2,4,6-trihydroxy-3-(3-	102
	methylbut-2-enyl)phenyl)propan-1-one (19b)	
57	HSQC spectrum for 2-methyl-1-(2,4,6-trihydroxy-3-(3-	103
	methylbut-2-enyl)phenyl)propan-1-one (19b)	
58	HMBC spectrum for 2-methyl-1-(2,4,6-trihydroxy-3-(3-	104
	methylbut-2-enyl)phenyl)propan-1-one (19b)	
59	EIMS spectrum for 1-(2,4,6-trihydroxy-3-(3-methylbut-2-	106
	enyl)phenyl)ethanone (19h)	
60	¹ H NMR spectrum for 1-(2,4,6-trihydroxy-3-(3-methylbut-2-	107
	enyl)phenyl)ethanone (19h)	
61	¹³ C NMR spectrum for 1-(2,4,6-trihydroxy-3-(3-methylbut-2-	108
	enyl)phenyl)ethanone (19h)	
62	HSOC spectrum for 1-(2,4,6-trihydroxy-3-(3-methylbut-2-	109
	enyl)phenyl)ethanone (19h)	
63	HMBC spectrum for 1-(2.4.6-trihydroxy-3-(3-methylbut-2-	110
	envl)phenvl)ethanone (19h)	
64	Intramolecular bonding of alkylated acylphloroglucinol	112
01	derivatives	112
65	EIMS spectrum for (E) -1-(3-(3.7-dimethylocta-2.6-dienyl)-2-	117
00	hydroxy-4.6-dimethoxyphenyl)pentan-1-one (20e)	
66	¹ H NMR spectrum for (F) -1- $(3-(3,7-dimethylocta-2,6-dienyl)-2-$	118
00	hydroxy-4 6-dimethoxyphenyl)pentan-1-one (20e)	110
67	13 C NMR spectrum for (F)-1-(3-(3.7-dimethylocta-2.6-dienyl)-2-	119
07	hydroxy-4 6-dimethoxyphenyl)pentan-1-one (20e)	117
68	HSOC spectrum for $(E)_1(3,(3,7-\text{dimethylocta}_2,6-\text{dienyl})_2$	120
00	hydroxy-4 6-dimethoxyphenyl)pentan-1-one (20e)	120
69	HMBC spectrum for (E)-1-(3-(3.7-dimethylocta-2.6-dienyl)-2-	121
0)	hydroxy-4 6-dimethoxyphenyl)pentan-1-one (20e)	121
70	Does response curve for compound 180 and tHCA (8)	125
70	Lineweaver Burk plot of 13 HPOD generation by soubean LOX	123
/1	1 in the presence of compound 18 (A) and tHGA (8) (B) at	127
	The the presence of compound for (A) and thore (b) (b) at 25° C pH 9.0	
72	Three-dimensional (3-D) docking model of hinding interaction of	131
12	the compound with amino acid residues: (A) Compound 18 e: (B)	151
	tHGA (8): (C) Compound 18g	
73	(A) Three-dimensional (3-D) docking model of the superimposed	133
	structures of the most active compound 18e with the (9Z.11E)-	
	13(R)-hydroperoxy-9.11-octadecadienoic acid (13-HPOD) as	
	LOX substrate; (B) Two-dimensional (2-D) diagram of binding	
	interaction between the most active compound 18e with amino	
	acid residues of soybean LOX-3; (C) Two-dimensional (2-D)	
	diagram of binding interaction between 13-HPOD with amino	
	acid residues of soybean LOX-3	
74	Three-dimensional (3-D) docking model of the superimposed	134
	structures of the active compound 18g with the most active	
	compound 18e and also the (9Z, 11E)-13(R)-hydroperoxy-9,11-	

octadecadienoic acid (13-HPOD) as LOX substrate.

	75	Three-dimensional (3-D) docking model of binding interaction of the NDGA with amino acid residues	135
	76	Alignment of soybean LOX-3 (11K3) and soybean LOX-1 sequences showing 73.13 % identities	137
	77	Ramachandran plot of LOX-1 model produced by PROCHECK (A) after homology modeling, (B) after MD simulation for compound 18e , (C) after MD simulation for compound 19e and, (D) after MD simulation for compound tHGA (8)	138
	78	The Fe atom and its ligands including the imidazole N-atoms of three histidine residues (His499, His504 and His690) and the carboxylate oxygen of the C-terminal Asn694 for soybean LOX-1 enzyme	140
	79	The 2D representation of the docking result of compound 18e with the residues in the active site of soybean LOX-1 model	141
	80	Three-dimensional (3-D) diagram of binding interactions of the most active compound 18e with the adjacent amino acid residues	142
	81	The 2D representation of the docking result of compound 19e with the residues in the active site of soybean LOX-1 model	143
	82	Three-dimensional (3-D) diagram of binding interactions of compound 19e with the adjacent amino acid residues	144
	83	The 2D representation of the docking result of tHGA (8) with the residues in the active site of soybean LOX-1 model	145
	84	Three-dimensional (3-D) diagram of binding interactions of the tHGA (8) with the adjacent amino acid residues	145
	85	(A) Total RMSD evolution; (B) The variation of the total energy in investigated system; (C) Time dependence of the temperature along the simulation time	146
	86	The 2D representation of the MD simulation result of compound 18e (snapshots taken during 5-10ns)	148
	87	The 3D representation of the MD simulation result of compound 18e (snapshots taken during 5-10ns)	149
	88	The 2D representation of the MD simulation result of compound 19e (snapshots taken during 5-10ns)	150
	89	The 3D representation of the MD simulation result of compound 19e (snapshots taken during 5-10ns)	151
	90	The 2D representation of the MD simulation result of tHGA (8) (snapshots taken during 5-10ns)	152
	91	The 3D representation of the MD simulation result of tHGA (8) (snapshots taken during 5-10ns)	153
	92	Stereoview of the binding site of compound 18e after simulation	155
	93	Stereoview of the binding site of compound 19e after simulation	155
	94	Stereoview of the binding site of tHGA (8) after simulation	156
	95	Topliss tree diagram for the complete docking analysis	161
	96	Recommended structures for highly active LOX inhibitors	168

LIST OF SCHEMES

Scheme		Page
1	General Reaction Scheme for Friedel-Craft Acylation	30
2	General Reaction Scheme for Direct C-Alkylation	31
3	General Reaction Scheme for Methylation	32
4	Reaction Mechanism of the Friedel-Craft Acylation	53
5	Reaction Scheme for the Synthesis of Acylphloroglucinols	54
6	Reaction Mechanism of the Direct C-Alkylation	82
7	Reaction Scheme for Direct <i>C</i> -Alkylation of Acylphloroglucinols	83
8	Reaction Mechanism for the <i>O</i> -Methylation Reaction	111
9	Reaction Scheme for Methylation of the tHGA Analogues	112



LIST OF APPENDICES

Append	lix	Page
A1	EIMS spectrum for (E)-1-(3-(3,7-dimethylocta-2,6-dienyl)-	182
	2,4,6-trihydroxyphenyl)propan-1-one (18c)	
A2	EIMS spectrum for (E)-1-(3-(3,7-dimethylocta-2,6-dienyl)-	183
	2,4,6-trihydroxyphenyl)butan-1-one (18d)	
A3	EIMS spectrum for (<i>E</i>)-1-(3-(3,7-dimethylocta-2,6-dienyl)-	184
	2,4,6-trihydroxyphenyl)pentan-1-one (18e)	
A4	EIMS spectrum for (<i>E</i>)-cyclohexyl-(3-(3,7-dimethylocta-2,6-	185
	dienyl)-2,4,6-trihydroxyphenyl)methanone (18f)	
A5	EIMS spectrum for (E) -1- $(3-(E)$ -3,7-dimethylocta-2,6-	186
	dienvl)-2.4.6-trihvdroxyphenvl)-3-phenvlprop-2-en-1-one	
	(18g)	
A6	EIMS spectrum for 1-(2.4.6-trihydroxy-3-(3-methylbut-2-	187
	envl)phenvl)propan-1-one (19c)	10,
Α7	EIMS spectrum for 1-(2.4.6-trihydroxy-3-(3-methylbut-2-	188
	envl)phenvl)butan-1-one (19d)	100
Δ8	FIMS spectrum for $1-(2.4.6-trihydroxy-3-(3-methylbut-2-$	189
110	envl)phenvl)pentan_1-one (19a)	107
٨٥	FIMS spectrum for Cyclobavyl (2.4.6 tribydrovy 3.(3	100
	methylbut_2_enyl)methanone (10f)	170
A 10	EIMS spectrum for (F) 3 phenyl 1 (2.4.6 tribudrovy 3.(3)	101
AIU	Elivis spectrum for (E) -3-pitchyl-1- $(2,4,0$ -ulliydroxy-3- $(3-$	191
A 11	ED(S construction for (E) + (2, (2, 7, dimethylocto 2, 6, dianyl)) 2	102
AII	Envis spectrum for (E) -1- $(3-(3,7-a))$ environmentation (20b)	192
A 10	FING second seco	102
A12	Elwis spectrum for 1-(2-nydroxy-4,6-dimetnoxy-3-(3-	193
110	ED (2	104
AI3	EIMS spectrum for 1-(2-nydroxy-4,6-dimethoxy-3-(3-	194
	methylbut-2-enyl)phenyl)ethanone (21h)	105
BI	¹ H NMR spectrum for (E) -1- $(3-(3,7)$ -dimethylocta-2,6-	195
	dienyl)-2,4,6-trihydroxyphenyl)propan-1-one (18c)	10.4
B 2	¹ H NMR spectrum for (E) -1- $(3-(3,7)$ -dimethylocta-2,6-	196
	dienyl)-2,4,6-trihydroxyphenyl)butan-1-one (18d)	
B3	¹ H NMR spectrum for (E) -1- $(3-(3,7-dimethylocta-2,6-$	197
	dienyl)-2,4,6-trihydroxyphenyl)pentan-1-one (18e)	
B4	¹ H NMR spectrum for (<i>E</i>)-cyclohexyl-(3-(3,7-dimethylocta-	198
	2,6-dienyl)-2,4,6-trihydroxyphenyl)methanone (18f)	
B5	¹ H NMR spectrum for (E) -1- $(3-(E)$ -3,7-dimethylocta-2,6-	199
	dienyl)-2,4,6-trihydroxyphenyl)-3-phenylprop-2-en-1-one	
	(18 g)	
B6	¹ H NMR spectrum for 1-(2,4,6-trihydroxy-3-(3-methylbut-2-	200
	enyl)phenyl)propan-1-one (19c)	
B7	¹ H NMR spectrum for 1-(2,4,6-trihydroxy-3-(3-methylbut-2-	201
	enyl)phenyl)butan -1-one (19d)	
B8	¹ H NMR spectrum for 1-(2,4,6-trihydroxy-3-(3-methylbut-2-	202
	enyl)phenyl)pentan-1-one (19e)	

B9	¹ H NMR spectrum for Cyclohexyl-(2,4,6-trihydroxy-3-(3-	203
	methylbut-2-enyl)phenyl)methanone (19f)	
B10	¹ H NMR spectrum for (<i>E</i>)-3-phenyl-1-(2,4,6-trihydroxy-3-(3-	204
	methylbut-2-enyl)phenyl)prop-2-en-1-one (19g)	
B11	¹ H NMR spectrum for (E) -1- $(3-(3,7-dimethylocta-2,6-$	205
	dienyl)-2-hydroxy-4,6-dimethoxyphenyl)ethanone (20h)	
B12	¹ H NMR spectrum for 1-(2-hydroxy-4,6-dimethoxy-3-(3-	206
	methylbut-2-enyl)phenyl)pentan-1-one (21e)	
B13	¹ H NMR spectrum for 1-(2-hydroxy-4,6-dimethoxy-3-(3-	207
	methylbut-2-enyl)phenyl)ethanone (21h)	
C1	13 C NMR spectrum for (E)-1-(3-(3.7-dimethylocta-2.6-	208
• •	dienvl)-2.4.6-trihvdrox vphenvl)propan-1-one (18c)	
C2	13 C NMR spectrum for (E)-1-(3-(3.7-dimethylocta-2.6-	209
02	dienvl)-2.4 6-trihydroxyphenvl)butan-1-one (18d)	207
C3	13 C NMR spectrum for (F)-1-(3-(3.7-dimethylocta-2.6-	210
05	dienvl)-2 4 6-trihydroxyphenvl)pentan-1-one (18e)	210
C_{1}	¹³ C NMR spectrum for (<i>F</i>)-cycloheyyl-(3-(3-7-dimethylocta-	211
04	2.6 dianyl) 2.4.6 tribydroxynhanyl)mathanona (18f)	211
C5	^{13}C NMP spectrum for (F) 1 (3 (F) 3.7 dimethylosta 2.6	212
CJ	dianyl) 2.4.6 tribydroxynhanyl) 3 phanylprop 2 an 1 one	212
	(18_{α})	
C (13 C NMD spectrum for 1 (2.4.6 tribudroux 2.(2 mothulbut 2	212
Co	C NMR spectrum for 1-(2,4,0-thinydroxy-3-(3-methylout-2-	215
07	¹³ C N (D) (19) (19) (19) (19)	014
C7	NMR spectrum for 1-(2,4,6-trinydroxy-3-(3-methylbut-2-	214
C 0	enyl)pnenyl)butan -1-one (19d)	015
68	NMR spectrum for 1-(2,4,6-trinydroxy-3-(3-methylbut-2-	215
C 0	enyl)phenyl)pentan-1-one (19e)	01.6
09	¹³ C NMR spectrum for Cyclohexyl-(2,4,6-trihydroxy-3-(3-	216
C 10	methylbut-2-enyl)phenyl)methanone (191)	017
C10	¹³ C NMR spectrum for (E) -3-phenyl-1- $(2,4,6$ -trihydroxy-3-	217
C (1)	(3-methylbut-2-enyl)phenyl)prop-2-en-1-one (19g)	• • •
CII	¹³ C NMR spectrum for (E) -1- $(3-(3,7-d)$ in the spectrum for (E) -1- $(3-(3,7-d)$ in the spectrum for (E) -1- $(3-(3,7-d))$ in the spectrum for (E) -1-	218
	dienyl)-2-hydroxy-4,6-dimethoxyphenyl)ethanone (20h)	
C12	¹³ C NMR spectrum for 1-(2-hydroxy-4,6-dimethoxy-3-(3-	219
	methylbut-2-enyl)phenyl)pentan-1-one (21e)	•••
C13	¹³ C NMR spectrum for 1-(2-hydroxy-4,6-dimethoxy-3-(3-	220
	methylbut-2-enyl)phenyl)ethanone (21h)	
D1	HSQC spectrum for (E) -1- $(3-(3,7-dimethylocta-2,6-dienyl)$ -	221
	2,4,6-trihydroxyphenyl)propan-1-one (18c)	
D2	HSQC spectrum for (E) -1- $(3-(3,7-dimethylocta-2,6-dienyl)$ -	222
	2,4,6-trihydroxyphenyl)butan-1-one (18d)	
D3	HSQC spectrum for (E) -1- $(3-(3,7-dimethylocta-2,6-dienyl)$ -	223
	2,4,6-trihydroxyphenyl)pentan-1-one (18e)	
D4	HSQC spectrum for (E)-cyclohexyl-(3-(3,7-dimethylocta-	224
	2,6-dienyl)-2,4,6-trihydroxyphenyl)methanone (18f)	
D5	HSQC spectrum for (<i>E</i>)-1-(3-(<i>E</i>)-3,7-dimethylocta-2,6-	225
	dienyl)-2,4,6-trihydroxyphenyl)-3-phenylprop-2-en-1-one	
	(18g)	

D6	HSQC spectrum for 1-(2,4,6-trihydroxy-3-(3-methylbut-2-	226
D7	HSOC spectrum for 1 (24.6 tribudrovy 3 (3 methylbut 2	227
DT	envl)phenvl)butan -1-one (19d)	221
D8	HSOC spectrum for 1-(2.4.6-trihydroxy-3-(3-methylbut-2-	228
-	enyl)phenyl)pentan-1-one (19e)	-
D9	HSQC spectrum for (E)-3-phenyl-1-(2,4,6-trihydroxy-3-(3-	229
	methylbut-2-enyl)phenyl)prop-2-en-1-one (19g)	
D10	HSQC spectrum for (E)-1-(3-(3,7-dimethylocta-2,6-dienyl)-	230
	2-hydroxy-4,6-dimethoxyphenyl)ethanone (20h)	
D11	HSQC spectrum for 1-(2-hydroxy-4,6-dimethoxy-3-(3-	231
	methylbut-2-enyl)phenyl)ethanone (21h)	
E1	HMBC spectrum for (E)-1-(3-(3,7-dimethylocta-2,6-dienyl)-	232
	2,4,6-trihydroxyphenyl)propan-1-one (18c)	
E2	HMBC spectrum for (E)-1-(3-(3,7-dimethylocta-2,6-dienyl)-	234
	2,4,6-trihydroxyphenyl)butan-1-one (18d)	
E3	HMBC spectrum for (<i>E</i>)-1-(3-(3,7-dimethylocta-2,6-dienyl)-	236
	2,4,6-trihydroxyphenyl)pentan-1-one (18e)	
E4	HMBC spectrum for (E)-cyclohexyl-(3-(3,7-dimethylocta-	238
	2,6-dienyl)-2,4,6-trihydroxyphenyl)methanone (18f)	240
ES	HMBC spectrum for (E) -1-(3-(E)-3,7-dimethylocta-2,6-	240
	dienyi)-2,4,6-trinydroxypnenyi)-3-pnenyiprop-2-en-1-one	
EC	(18g) UMDC spectrum for 1 (2.4 6 tribudroup 2 (2 methodbut 2	242
E0	nvibc spectrum for 1-(2,4,0-timydroxy-5-(5-methylbut-2-	242
F7	HMBC spectrum for 1.(2.4.6.trihydroxy.3.(3.methylbut-2.	244
	envl)phenvl)butan -1-one (19d)	244
F8	HMBC spectrum for 1-(2.4.6-trihydroxy-3-(3-methylbut-2-	245
Lo	envl)phenvl)pentan-1-one (19e)	210
E9	HMBC spectrum for Cyclohexyl-(2.4.6-trihydroxy-3-(3-	246
_,	methylbut-2-enyl)phenyl)methanone (19f)	
E10	HMBC spectrum for (E)-3-phenyl-1-(2,4,6-trihydroxy-3-(3-	247
	methylbut-2-enyl)phenyl)prop-2-en-1-one (19g)	
E11	HMBC spectrum for (<i>E</i>)-1-(3-(3,7-dimethylocta-2,6-dienyl)-	248
	2-hydroxy-4,6-dimethoxyphenyl)ethanone (20h)	
E12	HMBC spectrum for 1-(2-hydroxy-4,6-dimethoxy-3-(3-	249
	methylbut-2-enyl)phenyl)pentan-1-one (21e)	
E13	HMBC spectrum for 1-(2-hydroxy-4,6-dimethoxy-3-(3-	250
	methylbut-2-enyl)phenyl)ethanone (21h)	

LIST OF ABBREVIATIONS

AA	Arachidonic acid
AB	Aerobic biodegradability
	Absorption, distribution, metabolism, excretion, and toxicity
ADMET	prediction
AlogP	Estimated lipophilicity
AM	Ames mutagenicity
AS	Aqueous solubility
А	Alpha
Å	Angstrom
BALF	Bronchoalveolar lavage fluid
BBB	Blood brain barrier
В	Beta
CDCl ₃	Deuterated chloroform
CD ₃ OD	Deuterated methanol
CHARMm	Chemistry at HARvard Macromolecular Mechanics
cLogP	Estimated hydrophilicity
COX	Cyclooxygenase
CYP2D6	Cytochrome P ₄₅₀ 2D6
cysLTs	Cysteinyl leukotriene
¹³ C	Carbon-13
Δ	Chemical shift
°C	Degree in Celsius
DIP	Direct Inlet Probe
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
D	Doublet
Dd	Doublet of doublets
ddd	Doublet of doublets
Dq	Doublet of quartets
EIMS	Electron Ionization Mass Spectrometry
ΔE_{MM}	Molecular Mechanic Energy
FLAP	Five-Lipoxigenase-Activating Protein
GC-MS	Gas-chromatogrpahy-mass-spectrometer
GROMACs	GROningen MAchine for Chemical Simulations
G	Gram
ΔG_{bind}	Free binding interaction energy
ΔG_{PB}	Polar solvation energy
ΔG_{SA}	Non-polar solvation energy
5-HETE	5-hydroxyeicosatetraenoic acid
HIA	Human Intestinal Absorption
HMBC	Heteronuclear Multiple Bond Correlation

13-HPOD	13-Hydroperoxide
5-HpETE	5-hydroperoxyeicosatetraenoic acid
HPODEs	Hydroperoxyoctadecadienoic acid
HSQC	Heteronuclear Single Quantum Coherence
$^{1}\mathrm{H}$	Ptoron-1
IC ₅₀	Half maximal inhibitory concentration
IgE	Immunoglobulin E
IL	Interleukin
J	<i>J</i> -coupling constant
kcal/mol	Kilocalorie per mole
kDa	Kilo Dalton
K_i	Michaelis constant
K_m	Michaelis concentration
LOX	Lipoxygenase
LTC ₄	Leukotriene C ₄
1	Litre
MD	Molecular dynamic
MM-GBSA	Molecular Mechanics- Generalized Born
MHz	Megahertz
mmLOX	Mackerel muscle LOX
MM-PBSA	Molecular Mechanics-Poisson-Boltzmann
m	Multiplet
mg	Milligram
mL	Microliters
μg	Microgram
μM	Micro molar
NCE	New Chemical Entities
NDGA	Nordihydroguaiaretic
NMR	Nuclear Magnetic Resonance
NSAIDs	Non-Steroidal-Anti-Inflmmatory-Drugs
ns	Nanosecond
OI	Ocular Irritancy
PAMPs	Pathogen-Associated Molecular Patterns
PBML	Peripheral Blood Mononuclear Leukocytes
PDB	Protein Data Bank
PG	Prostaglandin
PGE_2	Prostaglandin E ₂
PPB	Plasma Protein Binding
PSA	Polar Surface Area
π	Pi
PUFAs	Poly-Unsaturated Fatty Acid
QSAR	Quantitative Structure Activity Relationship
RC	Rodent carcinogenicity

RMSD	Root-mean square derivation
S	Singlet
SAR	Structure Activity Relationship
SI	Skin Irritancy
SS	Skin Sensitization
Т	Triplet
tHGA	2,4,6-trihydroxy-3-geranylacetophenone
Th ₂	T-helper 2
TLC	Thin Layer Chromatography
TLRs	Toll-Like Receptors
TOPKAT	TOxicity Prediction by Komputer Assisted Technology
UV	Ultraviolet
V _{max}	Maximal Velocity
WHO	World Health Organization

C

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Drug discovery and development is an important activity to combat diseases especially those of unmet clinical needs. Furthermore, for some diseases the drugs in clinical use have been found to have serious side effects or the drugs have been rendered ineffective due to development of resistance of the causative agent. Therefore, the task of discovering and developing safe and more effective drugs become more pressing. The World Health Organization (WHO) identified that 11% of the 252 drugs discovered in the twenty-first century and considered as basic or essential were exclusively of flowering plant origin (Veeresham, 2012). In many areas of drug discovery research, the influence of natural products is very clear as can be seen from the high number of 'natural product mimics' approved as drugs for many diseases (Newman and Cragg, 2007). The drug discovery process involves the identification of lead and its target, synthesis, characterization, screening and assay for therapeutic efficacy. The average time required to bring a drug to the market ranges from 10-15 years at an average cost of U\$\$ 897 million to U\$\$ 1.9 billion (Giersiefen et al., 2003). Figure 1 shows the schematic flow of a drug discovery pipeline.



Figure 1: Schematic flow of the drug discovery pipeline (Prakash and Devangi, 2010)

Rational drug design efficiently guided medicinal chemists in lead identification to rapidly synthesize a large number of potential pharmacologically active compounds. Lead identification combines the knowledge and skills from the field of cheminformatics, molecular modeling and structural bioinformatics and understanding of the physicochemical properties of the three-dimensional molecule. Meanwhile, lead optimization aims to improve the effectiveness, diminish toxicity, or increase absorption for enhancing the most promising compounds (Adam, 2005). Although lead optimization is a time-consuming and costly step which often becomes a tight bottleneck in the drug discovery process, it is a key step in turning a biologically active chemical into an effective and safe drug. Thus, lead optimization is an essential step in the drug discovery process (Prakash and Devangi, 2010).



Structure-Activity-Relationship (SAR) is the relationship between the biological activity of a molecule and its chemical or three-dimensional structural features. The physiological action of a molecule is a function of its chemical constitution, thus the analysis of SAR enables the determination of the chemical groups that are responsible for inducing a target pharmacological activity. This allows the medicinal chemist to modify the potency of a bioactive compound (typically a drug) by inserting or substituting new chemical groups into the bioactive compound and test the modification for their biological effects (Kalyani *et al.*, 2013). Further refinement of the method enabled mathematical relationships between the chemical structure and the biological activity, known as quantitative structure-activity relationship (QSAR), to be built. QSAR can be considered as the method of trying to build a model to understand why some compound interacts and others do not (Prakash and Devangi, 2010).

Natural products containing a phloroglucinol core have been reported to have interesting biological properties (Chung, 1995). In an earlier study on the antiinflammatory properties of the medicinal plant *Melicope ptelefolia*, a simple compound containing the phloroglucinol structural-core, 2,4,6-trihydroxy-3 geranylacetophenone (tHGA), was identified as one of the bioactive principles of the plant (Suryati, 2005; Khozirah *et al.*, 2006). Initially, this compound was found to exert a dose-dependent inhibition against soybean 15-lipoxygenase (15-LOX) with an IC₅₀ value of 20 μ M. Subsequently, this compound was shown to exert a dose-dependent inhibition of cysteinyl leukotriene secretion from activated macrophage cells. Further exploration of both the chemistry and pharmacology of tHGA revealed that tHGA inhibited human 5-lipoxygenase (5-LOX) and both cyclooxygenase isoforms (COX-1 and COX-2), albeit with greater selectivity towards COX-2 (Khozirah *et al.*, 2006; Khozirah *et al.*, 2011).

When used in an acute model of murine asthma, tHGA was found to be as effective as Zileuton, a clinically used 5-LOX inhibitor. The compound was able to control airway hyper-responsiveness to methacholine challenge, and reduce pulmonary cellular infiltration, goblet cell metaplasia, cytokine (IL-4, IL-5, IL-13) and cysteinyl leukotriene secretions as well as reduce systemic IgE concentrations (Khozirah *et al.*, 2006; Khozirah *et al.*, 2011; Ismail *et al.*, 2012). These interesting biological activities of tHGA have prompted this study to synthesize several synthetic analogues of the compound by varying the substituents and to re-evaluate them for any improvement in their anti-inflammatory activity against LOX. In summary, tHGA is an effective LOX inhibitor and able to control airway hyper-responsiveness in acute model of murine asthma, however, the SARs for this groups of compounds is still unkown. A better insight into the SARs is important for designing a better drug.

1.2 Objectives of Research

On going effort to develop a better lead compound than tHGA, the studies of SARs of the compounds generated substantial interest because it is believed to be essential for a better drug design. With the assistance from the *in silico* studies, a better insight about the SARs of the compounds will help to identify important structural features that influence the ligand-protein interactions between the compounds and the enzyme. Our goal of present study is to synthesize several synthetic analogues of tHGA and to re-evaluate them for any improvement in their anti-inflammatory activity against LOX.

The specific objectives of the present study are:

- 1. To synthesize a series of analogues of tHGA.
- 2. To determine the 15-LOX inhibitory activity of the synthesized analogues.
- 3. To determine the structure-activity relationships of the synthetic analogues with regards to their 15-LOX inhibition.
- 4. To determine the ligand-receptor interactions of tHGA analogues with 15-LOX enzyme via *in silico* studies.
- 5. To predict the pharmacological effect of tHGA analogues by using ADMET and TOPKAT analysis.

REFERENCES

- Abas, F., Shaari, K., Israf, D. A., Syafri, S., Zainal, Z. and Lajis, N.H., 2010. LC– DAD–ESI-MS analysis of nitric oxide inhibitory fractions of tenggek burung (Melicope ptelefolia Champ. ex Benth.). *Journal of food composition and analysis 23*, 107-112.
- Abdellatif, K.R., Dong, Y., Chen, Q.H., Chowdhury, M.A. and Knaus, E.E., 2007. Novel (E)-2-(aryl)-3-(4-methanesulfonylphenyl) acrylic ester prodrugs possessing a diazen-1-ium-1, 2-diolate moiety: Design, synthesis, cyclooxygenase inhibition, and nitric oxide release studies. *Bioorganic & Medicinal Chemistry* 15, 6796-6801.
- Adam, M., 2005. Integrating research and development: the emergence of rational drug design in the pharmaceutical industry. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences* 36, 513-537.
- Alavijeh, M.S., Chishty, M., Qaiser, M.Z. and Palmer, A.M., 2005. Drug metabolism and pharmacokinetics, the blood-brain barrier, and central nervous system drug discovery. *NeuroRx* 2, 554-571.
- Aparoy, P., Reddy, R.N., Guruprasad, L., Reddy, M.R. and Reddanna, P., 2008. Homology modeling of 5-lipoxygenase and hints for better inhibitor design. *Journal of Computer-aided Molecular Design* 22, 611-619.
- Arnold, K., Bordoli, L., Kopp, J. and Schwede, T., 2006. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics* 22, 195-201.
- Barnes, N.C., Piper, P.J. and Costello, J.F., 1984. Comparative effects of inhaled leukotriene C₄, leukotriene D₄, and histamine in normal human subjects. *Thorax* 39, 500-504.
- Benkert, P., Biasini, M. and Schwede, T., 2011. Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics* 27, 343-350.
- Bhattacharjee, R., Devi, A. and Mishra, S., 2015. Molecular docking and molecular dynamics studies reveal structural basis of inhibition and selectivity of inhibitors EGCG and OSU-03012 toward glucose regulated protein-78 (GRP78) overexpressed in glioblastoma. *Journal of Molecular Modeling* 21, 1-17.
- Biasini, M., Bienert, S., Waterhouse, A., Arnold, K., Studer, G., Schmidt, T., Kiefer, F., Cassarino, T.G., Bertoni, M., Bordoli, L. and Schwede, T., 2014. SWISS-

MODEL: modelling protein tertiary and quaternary structure using evolutionary information. *Nucleic Acids Research*, gku340.

Bisgaard, H., 1984. Leukotrienes and prostaglandins in asthma. Allergy 39, 413-420.

- Bohr, G., Gerhäuser, C., Knauft, J., Zapp, J. and Becker, H., 2005. Antiinflammatory Acylphloroglucinol Derivatives from Hops (Humulus 1 upulus). *Journal of natural products* 68, 1545-1548.
- Buccellati, C., Rossoni, G., Bonazzi, A., Berti, F., Maclouf, J., Folco, G. and Sala, A., 1997. Nitric oxide modulation of transcellular biosynthesis of cysleukotrienes in rabbit leukocyte-perfused heart. *British Journal of Pharmacology* 120, 1128-1134.
- Calder, P.C., 2009. Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. *Biochimie* 91, 791-795.
- Charlier, C. and Michaux, C., 2003. Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs. *European Journal of Medicinal Chemistry* 38, 645-659.
- Chedea, V.S. and Jisaka, M., 2011. Inhibition of Soybean Lipoxygenases-Structural and Activity Models for the Lipoxygenase Isoenzymes Family. INTECH Open Access Publisher.
- Chedea, V.S., Pintea, A., Bunea, A., Braicu, C., Stanila, A. and Socaciu, C., 2014. Physalis alkekengi carotenoidic extract inhibitor of soybean lipoxygenase-1 activity. *BioMed research international* 2014.

Chembioinformatics on Web, n.d. Retrieved from http://www.molinspiration.com/.

- Chen, X., Wang, S., Wu, N. and Yang, C.S., 2004. Leukotriene A4 hydrolase as a target for cancer prevention and therapy. *Current cancer drug targets* 4, 267-283.
- Choe, E. and Min, D.B., 2009. Mechanisms of antioxidants in the oxidation of foods. *Comprehensive Reviews in Food Science and Food Safety* 8, 345-358.
- Choi, J., Chon, J.K., Kim, S. and Shin, W., 2008. Conformational flexibility in mammalian 15S-lipoxygenase: Reinterpretation of the crystallographic data. *Proteins: Structure, Function, and Bioinformatics* 70, 1023-1032.
- Chung, K.F., 1995. Leukotriene receptor antagonists and biosynthesis inhibitors: Potential breakthrough in asthma therapy. *European Respiratory Journal* 8, 1203–1213.

- Ciochina, R. and Grossman, R.B., 2006. Polycyclic polyprenylated acylphloroglucinols. *Chemical reviews* 106, 3963-3986.
- Crockett, S.L., Wenzig, E.M., Kunert, O. and Bauer, R., 2008. Anti-inflammatory phloroglucinol derivatives from *Hypericum empetrifolium*. *Phytochemistry Letters* 1, 37-43.
- Cummings, M.D., DesJarlais, R.L., Gibbs, A.C., Mohan, V. and Jaeger, E.P., 2005. Comparison of automated docking programs as virtual screening tools. *Journal of Medicinal Chemistry* 48, 962-976.
- Dahlén, S.E., Hedqvist, P., Hammarström, S. and Samuelsson, B., 1980. Leukotrienes are potent constrictors of human bronchi. *Nature* 288, 484-486.
- Dahlén, S.E., 2006. Treatment of asthma with antileukotrienes: first line or last resort therapy?. *European Journal of Pharmacology* 533, 40-56.
- Dainese, E., Sabatucci, A., van Zadelhoff, G., Angelucci, C.B., Vachette, P., Veldink, G.A., Agro` A.F. and Maccarrone, M., 2005. Structural stability of soybean lipoxygenase-1 in solution as probed by small angle X-ray scattering. *Journal* of molecular biology 349, 143-152.
- Das, D., 1993. Enzymes. In Biochemistry (8th ed.). Calcutta: Academic Publishers.
- Di, L. and Kerns, E.H., 2006. Biological assay challenges from compound solubility: strategies for bioassay optimization. *Drug Discovery Today* 11, 446-451.
- Di, L. and Kerns, E.H., 2015. Drug-like properties: concepts, structure design and methods from ADME to toxicity optimization. Academic Press.
- Durrant, J.D. and McCammon, J.A., 2011. Molecular dynamics simulations and drug discovery. *BMC Biology* 9, 1-9.
- Faudzi, S.M., Leong, S.W., Abas, F., Aluwi, M.M., Rullah, K., Lam, K.W., Ahmad,
 S., Tham, C.L., Khozirah, S. and Lajis, N.H., 2015. Synthesis, biological evaluation and QSAR studies of diarylpentanoid analogues as potential nitric oxide inhibitors. *MedChemComm* 6, 1069-1080.
- Feussner, I. and Wasternack, C., 2002. The lipoxygenase pathway. *Annual Review of plant biology* 53, 275-297.
- Friscić, T., Drab, D.M. and MacGillivray, L.R., 2004. A test for homology: photoactive crystalline assemblies involving linear templates based on a homologous series of phloroglucinols. *Organic letters* 6, 4647-4650.
- Giersiefen, H., Hilgenfeld, R. and Hillisch, A., 2003. Modern methods of drug discovery. In *Modern methods of drug discovery* (pp. 1-18). Birkhäuser Basel.

- Gurenlian, J.R., 2009. Inflammation: the relationship between oral health and systemic disease. *Dental Assistant (Chicago, III.: 1994)* 78, 8-10.
- Hammes, G.G., 2002. Multiple conformational changes in enzyme catalysis. *Biochemistry* 41, 8221-8228.
- Henry, G.E., Campbell, M.S., Zelinsky, A.A., Liu, Y.B., Bowen-Forbes, C.S., Li, L., Nair, M.G., Rowley, D.C. and Seeram, N.P., 2009. Bioactive Acylphloroglucinols from *Hypericum densifolium*. *Phytotherapy Research* 23, 1759-1762.
- Hess, B., Bekker, H., Berendsen, H. J. and Fraaije, J.G., 1997. LINCS: a linear constraint solver for molecular simulations. *Journal of Computational Chemistry* 18, 1463-1472.
- Hess, B., Kutzner, C., Van Der Spoel, D. and Lindahl, E., 2008. GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation. *Journal of Chemical Theory and Computation* 4, 435-447.
- Hofsäß, C., Lindahl, E. and Edholm, O., 2003. Molecular dynamics simulations of phospholipid bilayers with cholesterol. *Biophysical journal* 84, 2192-2206.
- Homeyer, N. and Gohlke, H. 2012. Free energy calculations by the molecular mechanics Poisson– Boltzmann surface area method. *Molecular Informatics* 31, 114-122.
- Iikubo, K., Ishikawa, Y., Ando, N., Umezawa, K. and Nishiyama, S., 2002. The first direct synthesis of α-mangostin, a potent inhibitor of the acidic sphingomyelinase. *Tetrahedron Letters* 43, 291-293.
- Ismail, N., Jambari, N.N., Zareen, S., Akhtar, M.N., Khozirah, S., Zamri-Saad, M., Tham, C.L., Sulaiman, M.R., Lajis, N.H. and Israf, D.A., 2012. A geranyl acetophenone targeting cysteinyl leukotriene synthesis prevents allergic airway inflammation in ovalbumin-sensitized mice. *Toxicology and Applied Pharmacology* 259, 257–262.
- Kalyani, G., Sharma, D., Vaishnav, Y. and Deshmukh, V.S., 2013. A review on drug designing, methods, its application and prospects. *International Journal of Pharmaceutical Research and Development (IJPRD)* 5, 15-30.
- Karim, A., Shahrim, M., Nasouddin, S.S., Othman, M., Mohd Adzahan, N., Hussin, S.R. and Khozirah, S., 2011. Consumers knowledge and perception towards *Melicope ptelefolia* (Daun Tenggek Burung): a preliminary qualitative study. *International Food Research Journal* 18, 1481-1488.
- Katsori, A.M., Chatzopoulou, M., Dimas, K., Kontogiorgis, C., Patsilinakos, A., Trangas, T. and Hadjipavlou-Litina, D., 2011. Curcumin analogues as

possible anti-proliferative & anti-inflammatory agents. *European Journal of Medicinal Chemistry* 47, 2722-2735.

- Kelavkar, U.P., Cohen, C., Kamitani, H., Eling, T.E. and Badr, K.F., 2000. Concordant induction of 15-lipoxygenase-1 and mutant p53 expression in human prostate adenocarcinoma: correlation with Gleason staging. *Carcinogenesis* 21, 1777-1787.
- Khozirah, S., Safri, S., Abas, F., Lajis, N.H. and Israf, D.A., 2006. A geranylacetophenone from the leaves of *Melicope ptelefolia*. *Natural Product Research* 20, 415–419.
- Khozirah, S., Suppaiah, V., Lam, K.W., Stanslas, J., Tejo, B.A., Israf, D.A., Abas, F., Ismail, I.S., Shuaib, N.H., Zareen, S. and Lajis, N.H., 2011. Bioassay-guided identification of an anti-inflammatory prenylated acylphloroglucinol from *Melicope ptelefolia* and molecular insights into its interaction with 5lipoxygenase. *Bioorganic & Medicinal Chemistry* 19, 6340–6347.
- Kollman, P.A., Massova, I., Reyes, C., Kuhn, B., Huo, S., Chong, L., Lee, M., Lee, T., Duan, Y., Wang, W., Donini, O., Cieplak, P., Srinivasan, J., Case, D.A. and Cheatham, T.E., 2000. Calculating structures and free energies of complex molecules: combining molecular mechanics and continuum models. *Accounts of Chemical Research* 33, 889-897.
- Kontoyianni, M., McClellan, L.M. and Sokol, G.S., 2004. Evaluation of docking performance: comparative data on docking algorithms. *Journal of Medicinal Chemistry* 47, 558-565.
- Krogsgaard-Larsen, P., Liljefors, T. and Madsen, U., 2006. *Textbook of drug design & discovery- Third Edition*. CRC Press.
- Kubinyi, H., 1998. Structure-based design of enzyme inhibitors and receptor ligands. *Curr Opin Drug Discov Devel* 1, 4-15.
- Kubo, I., Shimizu, K. and Ha, T.J., 2013. *Molecular design of soybean lipoxygenase inhibitors based on natural products*. INTECH Open Access Publisher.
- Kuhn, H., Saam, J., Eibach, S., Holzhütter, H.G., Ivanov, I. and Walther, M., 2005. Structural biology of mammalian lipoxygenases: enzymatic consequences of targeted alterations of the protein structure. *Biochemical and Biophysical Research Communications* 338, 93-101.
- Kulkarni, A.P., 2001. Lipoxygenase-a versatile biocatalyst for biotransformation of endobiotics and xenobiotics. *Cellular and Molecular Life Sciences CMLS* 58, 1805-1825.

- Kuntz, I.D., Blaney, J.M., Oatley, S.J., Langridge, R. and Ferrin, T.E., 1982. A geometric approach to macromolecule-ligand interactions. *Journal of Molecular Biology* 161, 269-288.
- Kwon, H.S., Cho, S.J., Ha, T.J., Harikishore, A., Yoon, H.S., Park, K.H., Kim, S.I. and Jang, D. S., 2014. Lipoxygenase Inhibitory Effects of Dibenzylbutane Lignans from the Seeds of *Myristica fragrans* (Nutmeg). *Notes* 35, 3095.
- Laidlaw, T.M. and Boyce, J. A., 2012. Cysteinyl leukotriene receptors, old and new; implications for asthma. *Clinical & Experimental Allergy* 42, 1313-1320.
- Lam, B.K., Penrose, J.F., Freeman, G.J. and Austen, K.F., 1994. Expression cloning of a cDNA for human leukotriene C₄ synthase, an integral membrane protein conjugating reduced glutathione to leukotriene A₄. *Proceedings of the National Academy of Sciences* 91, 7663-7667.
- Laskowski, R.A., MacArthur, M.W., Moss, D.S. and Thornton, J.M., 1993. PROCHECK: a program to check the stereochemical quality of protein structures. *Journal of Applied Crystallography* 26, 283-291.
- Laskowski, R.A., Rullmann, J.A.C., MacArthur, M.W., Kaptein, R. and Thornton, J.M., 1996. AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. *Journal of Biomolecular NMR* 8, 477-486.
- Lee, Y.R., Li, X., and Kim, J.H., 2008. Concise Total Synthesis of Biologically Interesting Mallotophilippens C and E. *The Journal of organic chemistry* 73, 4313-4316.
- Lengauer, T. and Rarey, M., 1996. Computational methods for biomolecular docking. *Current Opinion in Structural Biology* 6, 402-406.
- Lewis, R.A., Austen, K.F. and Soberman, R.J., 1990. Leukotrienes and other products of the 5-lipoxygenase pathway: biochemistry and relation to pathobiology in human diseases. *New England Journal of Medicine* 323, 645-655.
- Liavonchanka, A. and Feussner, I., 2006. Lipoxygenases: occurrence, functions and catalysis. *Journal of plant physiology* 163, 348-357.
- Loiseau, J., Vu, B.L., Macherel, M.H. and Le Deunff, Y., 2001. Seed lipoxygenases: occurrence and functions. *Seed Science Research* 11, 199-211.
- Maccarrone, M., van Aarle, P.G., Veldink, G.A. and Vliegenthart, J.F., 1994. In vitro oxygenation of soybean biomembranes by lipoxygenase-2. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1190, 164-169.

- Maccarrone, M., Melino, G. and Finazzi-Agro, A., 2001. Lipoxygenases and their involvement in programmed cell death. *Cell Death and Differentiation* 8, 776-784.
- Machado, F.S., Mukherjee, S., Weiss, L.M., Tanowitz, H.B. and Ashton, A.W., 2011. Bioactive lipids in Trypanosoma cruzi infection. *Advances in parasitology* 76, 1.
- Malde, A.K., Zuo, L., Breeze, M., Stroet, M., Poger, D., Nair, P.C., Oostenbrink, C. and Mark, A.E., 2011. An automated force field topology builder (ATB) and repository: version 1.0. *Journal of Chemical Theory and Computation* 7, 4026-4037.
- Maltby, N.H., Taylor, G.W., Ritter, J.M., Moore, K., Fuller, R.W. and Dollery, C.T., 1990. Leukotriene C₄ elimination and metabolism in man. *Journal of Allergy and Clinical Immunology* 85, 3-9.
- Mammino, L. and Kabanda, M.M., 2007. Model structures for the study of acylated phloroglucinols and computational study of the caespitate molecule. *Journal of Molecular Structure: THEOCHEM* 805, 39-52.
- Marti-Renom, M.A., Stuart, A.C., Fiser, A., Sánchez, R., Melo, F. and Šali, A., 2000. Comparative protein structure modeling of genes and genomes. *Annual Review of Biophysics and Biomolecular Structure* 29, 291-325.
- McConkey, B.J., Sobolev, V. and Edelman, M., 2002. The performance of current methods in ligand-protein docking. *CURRENT SCIENCE-BANGALORE* 83, 845-856.
- Meng, X.Y., Zhang, H.X., Mezei, M. and Cui, M., 2011. Molecular docking: a powerful approach for structure-based drug discovery. *Current Computer-aided Drug Design* 7, 146-157.
- Minor, W., Steczko, J., Stec, B., Otwinowski, Z., Bolin, J.T., Walter, R. and Axelrod,
 B., 1996. Crystal structure of soybean lipoxygenase L-1 at 1.4 Å resolution.
 Biochemistry 35, 10687-10701.
- Narayanaswamy, R., Lam, K.W. and Ismail, I.S., 2013. Molecular docking analysis of natural compounds as Human neutrophil elastase (HNE) inhibitors. *Journal of Chemical and Pharmaceutical Research* 5, 337-341.
- Newman, D.J. and Cragg, G.M., 2007. Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products* 70, 461-477.
- Patrick, G.L., 2005. An Introduction to Medicinal Chemistry 3th Edition. Oxford University Press.

- Pidgeon, G.P., Lysaght, J., Krishnamoorthy, S., Reynolds, J.V., O'Byrne, K., Nie, D. and Honn, K.V., 2007. Lipoxygenase metabolism: roles in tumor progression and survival. *Cancer and Metastasis Reviews* 26, 503-524.
- Pontiki, E.A. and Hadjipavlou-Litina, D.J., 2003. Synthesis, Antioxidant and Antiinflammatory Activity of Novel Arylacetic and Aryl-hydroxamic Acids. *Arzneimittelforschung* 53, 780-785.
- Pontiki, E.A. and Hadjipavlou-Litina, D.J., 2006. Antioxidant and anti-inflammatory activity of aryl-acetic and hydroxamic acids as novel lipoxygenase inhibitors. *Medicinal Chemistry* 2, 251-264.
- Pontiki, E.A. and Hadjipavlou-Litina, D.J., 2007. Synthesis of phenyl-substituted amides with antioxidant and antiinflammatory activity as Novel Lipoxygenase Inhibitors. *Medicinal Chemistry* 3, 175-186.
- Pontiki, E. and Hadjipavlou-Litina, D., 2008. Lipoxygenase inhibitors: a comparative QSAR study review and evaluation of new QSARs. *Medicinal Research Reviews* 28, 39-117.
- Prakash, N. and Devangi, P., 2010. Drug Discovery. Journal of Antivirals & Antiretrovirals 2, 63-68.
- Predictive Toxicology in Discovery Studio, n.d. Retrieved from http://accelrys.com/products/datasheets/ds_topkat.pdf.
- Prigge, S.T., Boyington, J.C., Gaffney, B.J. and Amzel, L.M., 1996. Structure conservation in lipoxygenases: Structural analysis of soybean lipoxygenase-1 and modeling of human lipoxygenases. *Proteins: Structure, Function, and Bioinformatics* 24, 275-291.
- Pugazhendhi, D. and Umamaheswari, T.S., 2013. Insilico Methods in Drug Discovery – A Review. International Journal of Advanced Research in Computer Science and Software Engineering 3, 680-683.
- Rainsford, K.D., Ying, C. and Smith, F., 1996. Effects of 5-Lipoxygenase Inhibitors on Interleukin Production by Human Synovial Tissues in Organ Culture: Comparison with Interleukin-1-synthesis Inhibitors. *Journal of Pharmacy and Pharmacology* 48, 46-52.
- Reddy, N.P., Aparoy, P., Reddy, T.C.M., Achari, C., Sridhar, P.R. and Reddanna, P., 2010. Design, synthesis, and biological evaluation of prenylated chalcones as 5-LOX inhibitors. *Bioorganic & Medicinal Chemistry* 18, 5807-5815.
- Reininger, W. and Hartl, A., 1977. U.S. Patent No. 4,053,517. Washington, DC: U.S. Patent and Trademark Office.
- Richard, M.C., 2005. Bioinformatics in Computer-Aided Drug Design. BeyeNETWORK.

- Robinson, D.S., Wu, Z., Domoney, C. and Casey, R., 1995. Lipoxygenases and the quality of foods. *Food Chemistry* 54, 33-43.
- Rullah, K., Aluwi, M.F.F.M., Yamin, B.M., Bahari, M.N.A., Leong, S.W., Ahmad, S., Abas, F., Ismail, N.H., Jantan, I. and Lam, K.W., 2014. Inhibition of prostaglandin E₂ production by synthetic minor prenylated chalcones and flavonoids: Synthesis, biological activity, crystal structure, and in silico evaluation. *Bioorganic & Medicinal Chemistry Letters* 24, 3826-3834.
- Saim, J.M., Akram, M., Halima, N., Khan, U., Asif, M.H., Osama, A., Tasneem, Q. and Mohiuddin, E., 2013. Hepatotoxicity: mini review. *Comprehensive Research Journals of Biological Science* 1, 001-005.
- Sala, A., Voelkel, N., Maclouf, J. and Murphy, R.C., 1990. Leukotriene E₄ elimination and metabolism in normal human subjects. *Journal of Biological Chemistry* 265, 21771-21778.
- Sala, A., Rossoni, G., Buccellati, C., Berti, F., Folco, G. and Maclouf, J., 1993. Formation of sulphidopeptide-leukotrienes by cell-cell interaction causes coronary vasoconstriction in isolated, cell-perfused heart of rabbit. *British Journal of Pharmacology* 110, 1206-1212.
- Sala, A., Aliev, G.M., Rossoni, G., Berti, F., Buccellati, C., Burnstock, G., Folco, G. and Maclouf, J., 1996. Morphological and functional changes of coronary vasculature caused by transcellular biosynthesis of sulfidopeptide leukotrienes in isolated heart of rabbit. *Blood* 87, 1824-1832.
- Schmid, N., Eichenberger, A.P., Choutko, A., Riniker, S., Winger, M., Mark, A.E. and van Gunsteren, W.F., 2011. Definition and testing of the GROMOS force-field versions 54A7 and 54B7. *European Biophysics Journal* 40, 843-856.
- Setty, B.N., Werner, M.H., Hannun, Y.A. and Stuart, M.J., 1992. 15-Hydroxyeicosatetraenoic acid-mediated potentiation of thrombin-induced platelet functions occurs via enhanced production of phosphoinositidederived second messengers--sn-1, 2-diacylglycerol and inositol-1, 4, 5trisphosphate. *Blood* 80, 2765-2773.
- Shahlaei, M., Madadkar-Sobhani, A., Mahnam, K., Fassihi, A., Saghaie, L. and Mansourian, M., 2011. Homology modeling of human CCR5 and analysis of its binding properties through molecular docking and molecular dynamics simulation. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1808, 802-817.
- Siedow, J.N., 1991. Plant lipoxygenase: structure and function. *Annual Review of Plant Biology* 42, 145-188.
- Singleton, V.L. and Kratzer, F.H., 1973. Plant phenolics. *Toxicants occurring naturally in foods*, 309-345.

- Skrzypczak-Jankun, E., McCabe, N.P., Selman, S.H. and Jankun, J., 2000. Curcumin inhibits lipoxygenase by binding to its central cavity: theoretical and X-ray evidence. *International Journal of Molecular Medicine* 6, 521-527.
- Skrzypczak-Jankun, E., Bross, R.A., Carroll, R.T., Dunham, W.R. and Funk, M.O., 2001. Three-dimensional structure of a purple lipoxygenase. *Journal of the American Chemical Society* 123, 10814-10820.
- Skrzypczak-Jankun, E., Borbulevych, O.Y., Zavodszky, M.I., Baranski, M.R., Padmanabhan, K., Petricek, V. and Jankun, J., 2006. Effect of crystal freezing and small-molecule binding on internal cavity size in a large protein: X-ray and docking studies of lipoxygenase at ambient and low temperature at 2.0 Å resolution. Acta Crystallographica Section D: Biological Crystallography 62, 766-775.
- Skrzypczak-Jankun, E., Chorostowska-Wynimko, J., Selman, S.H. and Jankun, J., 2007. Lipoxygenases-a challenging problem in enzyme inhibition and drug progression and survival. *Current Enzyme Inhibition* 3, 119-132.
- Smith, K.A., 2010. Structure and synthesis of phloroglucinol derivatives from *Hypericum roeperianum*. (Doctoral dissertation). University of KwaZulu-Natal, South Africa.
- Solomons, T.W.G. and Fryhle, C.B., 2004. Organic Chemistry, 8th (pp. 828-829, 1014). John Wiley and Sons Inc., Hoboken, NJ.
- Speca, S., Giusti, I., Rieder, F. and Latella, G., 2012. Cellular and molecular mechanisms of intestinal fibrosis. *World J Gastroenterol* 18, 3635-61.
- Spiliotopoulos, D., Spitaleri, A. and Musco, G., 2012. Exploring PHD fingers and H3K4me0 interactions with molecular dynamics simulations and binding free energy calculations: AIRE-PHD1, a comparative study. *PloS one* 7, e46902.
- Steinhilber, D. and Hofmann, B., 2014. Recent Advances in the Search for Novel 5-Lipoxygenase Inhibitors. *Basic & Clinical Pharmacology & Toxicology* 114, 70-77.
- Suh, M.E., Park, S.Y. and Lee, H.J., 2002. Comparison of QSAR methods (CoMFA, CoMSIA, HQSAR) of anticancer 1-N-substituted imidazoquinoline-4, 9dione derivatives. *BULLETIN-KOREAN CHEMICAL SOCIETY* 23, 417-422.
- Sultana, C., Shen, Y., Rattan, V. and Kalra, V.K., 1996. Lipoxygenase metabolites induced expression of adhesion molecules and transendothelial migration of monocyte-like HL-60 cells is linked to protein kinase C activation. *Journal of Cellular Physiology* 167, 477-487.
- Suryati, S., 2005. Phytochemicals and Anti-Inflammatory Activity of *Melicope ptelefolia* Champ Ex Benth. (Master's Thesis). Universiti Putra Malaysia, Malaysia.

- Tilley, S.L., Coffman, T.M. and Koller, B.H., 2001. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *The Journal of Clinical Investigation* 108, 15-23.
- Tiwari, B., Pratapwar, A.S., Tapas, A.R., Butle, S.R. and Vatkar, B.S., 2010. Synthesis and antimicrobial activity of some chalcone derivatives. *Int. J. ChemTech Res* 2, 499-503.
- Trevor, A.J., Katzung, B.G. and Masters, S.B., 1995. *Katzung & Trevor's Review of Pharmacology*. United States of America: McGraw-Hill Companies.
- Uddin, G., Rauf, A., Arfan, M., Rehman, T.U., Khan, A.Z., Ali, G., Rehman, B. and Zia-ul-Haq, M., 2013. Molecular docking of Diospyrin as a LOX inhibitory compound. *Journal of Saudi Chemical Society*.
- Van De Waterbeemd, H., Smith, D.A., Beaumont, K. and Walker, D.K., 2001. Property-based design: Optimisation of drug absorption and pharmacokinetics. *Journal of Medicinal Chemistry* 44, 1313-1333.
- Van De Waterbeemd, H. and Gifford, E., 2003. ADMET in silico modelling: towards prediction paradise?. *Nature reviews Drug discovery* 2, 192-204.
- Veeresham, C., 2012. Natural products derived from plants as a source of drugs. *Journal of Advanced Pharmaceutical Technology & Research* 3, 200.
- Wadood, A., Ahmed, N., Shah, L., Ahmad, A., Hassan, H. and Shams, S., 2013. Insilico drug design: An approach which revolutionarised the drug discovery process. *OA Drug Design & Delivery* 1, 1-4.
- Wandzik, I., 2006. Current molecular docking tools and comparisons thereof. *MATCH* 55, 271-278.
- Wang, J., Morin, P., Wang, W. and Kollman, P.A., 2001. Use of MM-PBSA in reproducing the binding free energies to HIV-1 RT of TIBO derivatives and predicting the binding mode to HIV-1 RT of efavirenz by docking and MM-PBSA. *Journal of the American Chemical Society* 123, 5221-5230.
- Wang, B., Yang, L.P., Zhang, X.Z., Huang, S.Q., Bartlam, M. and Zhou, S.F., 2009. New insights into the structural characteristics and functional relevance of the human cytochrome P₄₅₀ 2D6 enzyme. *Drug Metabolism Reviews* 41, 573-643.
- Widdicombe, J.H. and Wine, J.J., 2015. Airway gland structure and function. *Physiological reviews* 95, 1241-1319.
- Wong-ekkabut, J. and Karttunen, M., 2016. The good, the bad and the user in soft matter simulations. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1858, 2529-2538.

- Wu, G., Robertson, D.H., Brooks, C.L. and Vieth, M., 2003. Detailed analysis of grid-based molecular docking: A case study of CDOCKER—A CHARMmbased MD docking algorithm. *Journal of Computational Chemistry* 24, 1549-1562.
- Yang, Y.S., Zhang, F., Tang, D.J., Yang, Y.H. and Zhu, H.L., 2014. Modification, Biological Evaluation and 3D QSAR Studies of Novel 2-(1, 3-Diaryl-4, 5-Dihydro-1 H-Pyrazol-5-yl) Phenol Derivatives as Inhibitors of B-Raf Kinase. *PloS one 9*, e95702.
- Zhang, Y.Y., Lind, B., Rådmark, O. and Samuelsson, B., 1993. Iron content of human 5-lipoxygenase, effects of mutations regarding conserved histidine residues. *Journal of Biological Chemistry* 268, 2535-2541.
- Zhu, J., Kilty, I., Granger, H., Gamble, E., Qiu, Y.S., Hattotuwa, K., Elston, W., Liu, W.L., Oliva, A., Pauwels, R.A. and Kips, J.C., 2002. Gene expression and immunolocalization of 15-lipoxygenase isozymes in the airway mucosa of smokers with chronic bronchitis. *American Journal of Respiratory Cell and Molecular Biology* 27, 666-677.

Re: Permission to use my article as a part of my thesis work - Reg		
Molecules Editorial Office Fri 8/79, 11-48 AM You v	۹	← Reply
You forwarded this message on 9/9/2016 5:07 PM		
Dear Dr. Ng,		
Thank you very much for your inquiry regarding the copyright of material that has been published in our journal.		
Since the article you asked about is licensed under a Creative Commons-BV license, you can freely use the paper including the figures and tables accordingly - if the authors of the article created it themselves - of course under the premise that you cite the article when using the figure.		
If the figure is cited in the article to be from another source and not from the authors of the article, though, you need to ask for the permission to use the figure with the other source since they hold the copyright (and not the authors).		

Copyright permission letter