BIO-PROSPECTION OF NATURAL PRODUCTS AND SYNTHESIS OF BIOACTIVE MOLECULES FOR LEAD DEVELOPMENT AND GENERATION OF NOVEL SYNTHETIC METHODOLOGIES

THESIS

SUBMITTED TO UNIVERSITY OF KASHMIR, SRINAGAR FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN CHEMISTRY 2013

BY SALEEM FAROOQ

BIO-ORGANIC CHEMISTRY DIVISION INDIAN INSTITUTE OF INTEGRATIVE MEDICINE (CSIR), JAMMU-180 001 INDIA

DEPARTMENT OF CHEMISTRY UNIVERSITY OF KASHMIR, SRINAGAR -190 006 INDIA
ACKNOWLEDGEMENTS

Thanks are due to the elegance of Almighty Allah who blessed me with complete determination and perseverance to accomplish this vital part of my academic career. His endowment of courage upon me has made it easy and smooth; otherwise I would have hardly reached to its culmination.

I feel inadequacy of words to express my deep and wholehearted gratitude with a bouquet of sincere thanks to my supervisor, Dr. S. Koul who really has mentored me in a consummate way and has beautifully coloured the sketch of my raw and fragile thoughts in both practical and moral sense. Without his everlasting direction and arduous efforts, my endeavours may not have blossomed. I will surely remain indebted to for his novel and everlasting ideas, decisive insights, priceless and exciting suggestions, interesting, motivative and prolific discussions, and incessant encouragements throughout the course that brought out a number of brain-child’s in me regarding the field of chemistry. I feel very much delighted to mention him as my supervisor.

I owe my indebtedness to the spectacular and cheerful suggestions of my supervisor, Prof. M. A. Qureshi. My research work kept marching to the fore in a rationalized manner with the inspiration, importunate guidance, gracious behaviour and incalculable blessings of his lavishness. His novel ideas, critical insights, long-time research expertise, moral support, fruitful discussions and motivating approach have remained the backbone of my feat. He has always remained a hearken listener of all the issues related to my research work. Without this kind approach offered by him, it would have been difficult to pursue my work in such a healthy way. I feel blessed to have him as my supervisor.

I am highly grateful to Dr. Ram A. Vishwakarma, Director, Indian Institute of Integrative Medicine, Canal Road, Jammu for giving me an opportunity to prove my worth in this prestigious Institute. I also extend my thanks to him for providing the entire requisite facilities to carry out my research work.

I would like to express profound regards and thanks to Prof. Peerzada, Head Department of Chemistry, University of Kashmir for his constant help, support and encouragement. I would also like to thank former head Prof. Khaliq-uz-Zaman Khan for his timely help and support. Warm and sincere regards are also due to all my teachers and colleagues in the Department of Chemistry, University of Kashmir for their encouragement and sincere advices during my research period. Special thanks are due to Mr. Zahoor Ahmad for his kind assistance.
I will always be grateful to Dr. S. C. Taneja for his prolonged inspiration, unrelenting guidance and healthy wishes which he offered during my research period.

I would like to thank Dr. G. L. Koul whose incomparable and cheerful advices along with time long research expertise has helped a great deal in accomplishing this task.

I also acknowledge my thanks to Dr. P. L. Sangwan whose research experience nourished my spirit of sincere and dedicated approach towards this field.

I also thank Dr. A. K. Tripathi, Dr. Bhahwal Ali Shah, Dr. D. Mukherjee, Sh. Budh Singh, and Sh. Samar Singh for their wholehearted assistance and congenial company that shares a good proportion in completion of my work.

With deep respect I owe my thanks to Mr. Abdul Rouf and Mr. Shakeel-ur-Rahman (I.I.I.M, Srinagar) who shared their expertise with me and also helped me in grooming up during the tenure of my research work. Their priceless help in compiling the research work is worth mentioning.

It is pleasant and joyful to pass on vote of thanks towards my colleagues, Dr. Niranjan Thota, Dr. V. Reddy, Dr. Khalid, Mr. Mushtaq, Mr. Brijesh, Miss. Renu, Mr. Imran Khan, Mr. Praveen, Mr. Rajeshvar Reddy, Mr. Nisar and Mr. Alamgir without whose timely advices, moral and physical support, inexplicable encouragements, and endless help it might have been difficult to move over the crusts and troughs of this tough, albeit wonderful period.

My thanks are also due to the members of Bio-organic Chemistry Division: Mr. Subhash Chander, Mr. Nirmal, Mr. Subhash and Smt. Asha Ji for their humble attitude and positive cooperation in many dimensions, in and out of the lab.

I am deeply satisfied with the members of Instrumentation Division, especially Dr. R. K, Khajuria, Mr. D. K, Sultan, Smt. Basant Purnima, Mr. Gunju, Dr. Arvind, Mrs. Deepika Singh, Er. Rajnesh Anand, Dr. Ajay Gupta and Mr. Parshotam for facilitating the service necessary for my research work.

I also extend my thanks to Dr. Javid Ahmad Bandey (Assistant Professor, National Institute of Technology, Hazratbal, Srinagar) for remaining my research partner. His timely advices and modest approach owes a great deal to my achievements.

I feel pleasure to mention the part of my research work which I have carried out in collaboration with the Cancer Pharmacology Division. I am highly grateful to the kindness of Dr. A. K, Saxena, Head Cancer Pharmacology Division for allowing me to do part of my research work in collaboration with them. Thanks are also to Dr. Abid Hamid Dar for
making the work possible. Furthermore, I am pleased with Aashiq Hussain and Dev Priya for carrying out the work possible with fertile results which really added a good percentage to my work proposal.

Thanks are also to Dr. Inshad Ali Khan, Head Clinical Microbiology Division, and his students for helping me in performing some biological aspect of my research work.

I am grateful to Dr. Baldev Singh and his student, Bashir Ahmad Dar for carrying out the necessary part of my research work which we accomplished in collaboration.

I am happy with the obedient nature of trainees who have worked with me. Among others humble approach and dedication of Jagjit Kaur and Vijay Kumar Tripathi is worth mentioning.

Very special thanks go to my friends: Mr. Nisar-ul-Ashraf, Mr. Hilal, Mr. Naiem, Mr. Shahzad, Mr. Sajad, Mr. Mudassir, Mr. Reyaz, Mr. Sanjay, Mr. Manjeet, Mr. Bilal Rah, Mr. Bashir A. Bhat, Mr. Verma, Mr. Mudassir Amin, Mr. Showkat Gani, Mr. Naveed Afzal and Mr. Zahoor Ahmad.

Last, but not the least, I am greatly indebted to my whole family. Words will never suffice the limit of expressing the greatest gratitude to my parents, Farooq Ahmad Mir and Nassema Begum to whom I owe my very existence. Without their whole-time and dimensionless support in each and every possible manner, it was merely possible to reach to this juncture of my life. With their eternal love, graceful blessings and constant encouragement I have been able to fill-up the pros and cons of this tedious job to a greater extent.

Saleem Farooq
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>Ac₂O</td>
<td>Acetic Anhydride</td>
</tr>
<tr>
<td>Ar</td>
<td>Aryl</td>
</tr>
<tr>
<td>CDCl₃</td>
<td>Deutrated chloroform</td>
</tr>
<tr>
<td>COSY</td>
<td>Correlation Spectroscopy</td>
</tr>
<tr>
<td>d</td>
<td>Doublet</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-Diazabicyclo[5,4,0]-undec-7-ene</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>dd</td>
<td>Doublet of doublet</td>
</tr>
<tr>
<td>DIE</td>
<td>Diisopropylether</td>
</tr>
<tr>
<td>DMAP</td>
<td>N,N-dimethylamino pyridine</td>
</tr>
<tr>
<td>DME</td>
<td>DCM-Methanol Extract</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethyl formamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulphoxide</td>
</tr>
<tr>
<td>ESI-MS</td>
<td>Electron spray ionisation- mass spectroscopy</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography- mass spectroscopy</td>
</tr>
<tr>
<td>h</td>
<td>Hours</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant</td>
</tr>
<tr>
<td>μl</td>
<td>Microlitre</td>
</tr>
<tr>
<td>M⁺</td>
<td>Molecular ion peak</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>mmol</td>
<td>Millimole</td>
</tr>
<tr>
<td>mp</td>
<td>Melting point</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>PE</td>
<td>Petroleum ether Extract</td>
</tr>
<tr>
<td>PI3Ks</td>
<td>Phosphoinositide-3-kinase</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>PP</td>
<td><em>Prangos Pabularia</em></td>
</tr>
<tr>
<td>PTEN</td>
<td>Phosphatase and tensin homolog</td>
</tr>
<tr>
<td>q</td>
<td>Quartet</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>rpm</td>
<td>Rotations per minute</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>Reverse Phase HPLC</td>
</tr>
<tr>
<td>s</td>
<td>Singlet</td>
</tr>
<tr>
<td>t</td>
<td>Triplet</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethyl silane</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
</tbody>
</table>
Declaration

I hereby declare that the thesis entitled “Bio-prospection of natural products and synthesis of bioactive molecules for lead development and generation of novel synthetic methodologies” is being submitted for the award of Ph.D degree to the University of Kashmir, Srinagar. The work has been carried out under the joint supervision of Dr. S. Koul (Chief Scientist, IIIM, Jammu) and Prof. M. A. Qurishi (Department of Chemistry, University of Kashmir). All ideas and references have been duly acknowledged. The investigations presented in this thesis are original and have not been submitted in part or in full for the award of any degree, diploma, associate-ship or other similar title in this or any other university.

Saleem Farooq

SUPERVISOR

Dr. S. Koul
Chief Scientist
Bio-Organic Chemistry Division,
Indian Institute of Integrative Medicine,
Canal Road, Jammu-180001, J&K.

SUPERVISOR

Prof. M. A. Qurishi
Department of Chemistry,
University of Kashmir,
Srinagar-190006, J&K.
Certificate

This is to certify that the thesis entitled “Bio-prospection of natural products and synthesis of bioactive molecules for lead development and generation of novel synthetic methodologies” submitted to University of Kashmir, Srinagar, embodies the original research work carried out by Mr. Saleem Farooq, under my supervision. The thesis is fit to be considered for the award of Ph.D. degree. The results in this thesis have not been submitted in part or full to any other University or Institute for the award of any degree.

Dr. M. A. Qurishi
(Supervisor)
Certificate

This is to certify that the Ph. D research work for the thesis entitled “Bio-prospection of natural products and synthesis of bioactive molecules for lead development and generation of novel synthetic methodologies” has been actually carried out in original by Research Scholar Mr. Saleem Farooq under my supervision. The thesis is submitted for consideration for the award of Ph. D degree to the University of Kashmir, Srinagar. The results embodied in the thesis have not been submitted to any other University or Institute for the award of any degree.

Dr. S. Koul
(Supervisor)
DEDICATED
TO MY
GRANDPARENTS
AND
PARENTS
## LIST OF CONTENTS

### Chapter 1
**Bio-prospection of Natural Products**

<table>
<thead>
<tr>
<th>Section A</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1.1 Introduction</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>1.2 Objectives of present work</strong></td>
<td>2</td>
</tr>
<tr>
<td><strong>1.3 Review of literature</strong></td>
<td>3</td>
</tr>
<tr>
<td>1.3.1 Natural Products as drugs from folklore approach and traditional systems</td>
<td>4</td>
</tr>
<tr>
<td>1.3.2 Current status of natural products</td>
<td>6</td>
</tr>
<tr>
<td>1.3.3 Important drugs from plants</td>
<td>8</td>
</tr>
<tr>
<td>1.3.4 Anti-cancer molecules from natural sources</td>
<td>11</td>
</tr>
<tr>
<td><strong>1.4 Classification</strong></td>
<td>12</td>
</tr>
<tr>
<td>1.4.1 Prangos Pabularia</td>
<td>12</td>
</tr>
<tr>
<td>1.4.2 Morphology</td>
<td>13</td>
</tr>
<tr>
<td>1.4.3 Ethnobotanical uses</td>
<td>14</td>
</tr>
<tr>
<td><strong>1.5 Phytochemistry of Prangos Pabularia</strong></td>
<td>16</td>
</tr>
<tr>
<td>1.5.1 Coumarins as bioactive molecules</td>
<td>16</td>
</tr>
<tr>
<td>1.5.2 Classification</td>
<td>17</td>
</tr>
<tr>
<td><strong>1.6 Pharmacology importance of coumarins</strong></td>
<td>18</td>
</tr>
<tr>
<td><strong>1.7 Results and Discussion</strong></td>
<td>21</td>
</tr>
<tr>
<td>1.7.1 Plant material</td>
<td>21</td>
</tr>
<tr>
<td>1.7.2 Extraction and isolation</td>
<td>22</td>
</tr>
<tr>
<td><strong>1.8 In vitro screening of the molecules</strong></td>
<td>26</td>
</tr>
<tr>
<td><strong>1.9 Standard experimental procedure</strong></td>
<td>27</td>
</tr>
<tr>
<td>1.9.1 Cell culture</td>
<td>27</td>
</tr>
<tr>
<td>1.9.2 Anti-proliferative activity</td>
<td>27</td>
</tr>
<tr>
<td><strong>1.10 Spectral data of compounds</strong></td>
<td>28</td>
</tr>
<tr>
<td>1.10.1 Spectra of some compounds</td>
<td>33</td>
</tr>
</tbody>
</table>

### Section B
**Simultaneous separation and the quantification of marker compounds**

<table>
<thead>
<tr>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1.11 Introduction</strong></td>
</tr>
<tr>
<td><strong>1.12 Objectives of the present work</strong></td>
</tr>
<tr>
<td><strong>1.13 Chromatographic techniques</strong></td>
</tr>
<tr>
<td><strong>1.14 Development of HPLC</strong></td>
</tr>
<tr>
<td><strong>1.15 Review of literature</strong></td>
</tr>
<tr>
<td>1.15.1 Analytical method development</td>
</tr>
<tr>
<td><strong>1.16 Results and discussion</strong></td>
</tr>
</tbody>
</table>
1.17. Materials and methods
1.17.1. preparations of sample and standard solutions
1.17.2. Instrumental conditions
1.17.3. Optimization of the HPLC conditions
1.17.4. Calibration
1.17.5. Validation
1.17.6. Quantification

1.18. Conclusion

1.19. Chromatograph and calibration curve of marker compounds

Chapter 2 Studies on Modification and bioevaluation of plant molecules 78-179
2.1 Introduction
2.2. Objectives of present work
2.3. Review of literature
2.3.1. Molecular mode of action of osthol
2.3.2. Total synthesis of osthol
2.4. Results and Discussion
2.5. Anti-proliferative activity
2.6. Effect on Mitochondrial membrane potential loss
2.7. Structure activity relationship
2.8. Screening of anologs for antimicrobial activity
2.9. Experimental
2.9.1. Standard experimental procedure
2.10. Spectra data of compounds
2.11. Spectra of some compounds

Chapter 3 CuCN Catalyzed One Pot Synthesis of γ-Keto Diesters: Domino Double Michael Addition Followed by Nef Reaction 180-220
Section A:
3.4.3. Reaction of different alkyl acrylates and nitroethane

3.5. Conclusion

3.6. Standard Experimental Procedure

3.7. Spectral data of some selected compounds

3.7.1. Spectras of some representative compounds

Section B: An efficient approach towards the synthesis of dihydropyrimidinones using heteropolyacid Montmorilonite-KSF catalyst

3.8. Introduction

3.9. Objectives of the present work

3.10. Review of literature

3.10.1. Mechanism

3.10.2. Alternative synthetic routes of Biginelli reaction

3.10.3. Microwave assisted synthesis

3.10.4. Asymmetric Biginelli reactions

3.10.5. Catalyst variations

3.11. Results and Discussion

3.11.1. Optimization of reaction

3.12. Synthesis of new 3,4-dihydropyrimidin-2(1H)-one derivatives

3.13. In vitro screening

3.14. Recycled use of the catalyst

3.15. Conclusions

3.16. Standard experimental procedure

3.17. Screening of synthesized molecules

3.18. Cell Culture methodology

3.19. Spectral data

3.20. Spectra of some selected compounds

Summary of Thesis

List of Publication
1.1. Introduction

For millennia man has utilized the properties of plants not just for food and shelter but also for health and well-being. Herbal extracts and preparations, for a long time the mainstay of the healer and physician’s ‘tool kit’, still comprise the major part of primary health care for 75–90% of the world’s rural population; even in relation to western medicine, plants still provide the basic raw materials for some 25% of prescription drugs.

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (UNESCO, 1996). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998). Medicine, in several developing countries, using local traditions and beliefs, is still the mainstay of health care.¹

Despite competition from other drug discovery methods, natural products are still providing their fair share of new clinical candidates and drugs. This was demonstrated recently by Newman, Cragg, and Sneader, who analyzed the number of natural product-derived drugs present in the total drug launches from 1981 to 2002.² They concluded that natural products were still a significant source of new drugs, especially in the anticancer and antihypertensive therapeutic areas. In another study,

---

Proudfoot reported that 8 out of 29 small molecule drugs launched in 2000 were derived from natural products or hormones and concluded that high-throughput screening did not have a significant impact on the derivation of these drugs. Medicinal folklore over the years has proved to be an invaluable guide in the present day screening of drugs. Many important modern drugs like digitoxin, reserpine, tubocurarine, ephedrine, ergometrine, atropine, vinblastine and aspirin have been discovered by following leads from the folk uses.\(^3^,\(^4^,\(^5^,\(^6^\)

### 1.2. Objectives of present work

As per the reviewed literature, the *Prangos pabularia* contains various categories of secondary metabolites, among which coumarins are present in bulk. The environmental stresses and ecological conditions have great impact on plants for the production of secondary metabolites.\(^7^\) Keeping this in view and the pharmaceutical importance of the plant, the main aim was to isolate new secondary metabolites from the plant *Prangos pabularia* and at the same time, to develop a RP-HPLC validated method for the separation and quantification of coumarins isolated from that plant, used as marker compounds as discussed in second section of this chapter. Isolation of osthole and other coumarins in large quantities in order to modify these compounds as discussed in chapter 2 to form more potent and less toxic molecules, as this is the main motive of our institute to generate library of molecules and explore them for several biological activities like Cytotoxic and anti-microbial activity, as Efflux pump inhibitors, Immunomodulators, Anti-oxidants etc.

---


1.3. Review of Literature

It has been reported that uses of 80% of 122 plant-derived drugs were related to their original ethnomedicinal purposes.\(^8\) This is widely accepted to be true when applied to drug discovery in ‘olden times’ before the advent of high-throughput screening and the post-genomic era: more than 80% of drug substances were natural products or inspired by a natural compound.\(^9\) It is, however, arguably still true: comparisons of the information presented on sources of new drugs from 1981 to 2007 indicate that almost half of the drugs approved since 1994 are based on natural products. Thirteen natural-product-related drugs were approved from 2005 to 2007.\(^10\) At least 13 natural product-derived drugs were approved between 2005 and 2007, and five of those, exenatide, ziconotide, ixabepilone, retapamulin, and trabectedin, represented the first members of novel classes of drugs.\(^11\)

Only a small fraction of the world’s biodiversity has been explored for drug discovery to date. There are at least 250,000 species of higher plants that exist on this planet, but merely a 5–10% of these terrestrial plants have ever been investigated. In addition, reinvestigation of previously investigated plants has continued to produce new bioactive compounds that have drug potential.\(^12\) Natural products will certainly play a crucial role in meeting this demand through the continued investigation of world’s biodiversity, much of which remains unexplored.\(^13\)

The discovery of a new drug is part structured investigation and part luck. Many discoveries start with the biological testing by pharmacologists of the potential source. These bioassays or screening programmes as they are known are used to determine the nature of the pharmacological activity of the material as well as its potency. These programmes are used extensively to discover lead compounds. Once the screening programme has identified materials of pharmacological activity of

\(^8\) Chin Y-W.; Balunas, M.J.; Chai, HB.; Kinghorn, AD.; *Drug discovery from natural sources*. AAPS. J. 2006, 8, 239.


interest, the compound responsible for this activity is isolated and used as a lead compound for production of related analogues. These analogues are produced by synthetic routes. The chosen synthetic route should be such that it is relatively easy to modify the structure of the lead compound either directly or during the course of its synthesis. This is an economic way of producing a greater range of analogues for testing and hence increasing the chance of discovering an active compound. These modifications involve changing the nature of side chains or introducing new substituents in previously unsubstituted positions.

1.3.1. Natural products as drugs from folklore approach and traditional systems

Historically, ethno-pharmacology was the origin of all medicines and plant products were the most important sources of drugs. This knowledge of drugs has accumulated over thousands of years as a result of man’s inquisitive nature, so that today we possess many effective means of ensuring health care. In pre-industrialized society and in agrarian societies, plant-derived natural products were used by indigenous population as therapies for many diseases ranging from infections to emphysema. On numerous occasions, the folklore records of many different cultures have provided leads to plants with useful medicinal properties. It has been estimated that less than 10% of World’s biodiversity has been studied seriously as source of medicines. A remarkable galaxy of pure compounds with different

pharmacological activities has been isolated from natural sources.\textsuperscript{20} A sampling of some of these natural products bearing novel chemical scaffolds are shown in Figure 1.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Figure 1}
\end{figure}

Isolation of natural products from higher plants, marine organisms and microorganisms is therefore still urgently needed, calling for state-of-the-art methodologies for separation and isolation procedures. Taking into consideration that a plant may contain hundreds to thousands of constituents, the separation and isolation process can be long and tedious. Isolation of natural products generally combines various separation techniques, which depend on the solubility, volatility and stability of the compounds to be separated. The choice of different separation steps is of great importance and an analytical-scale optimisation of the separation parameters is worthwhile. Natural products as leads to potential drugs: an old process or the new hope for drug discovery leading to advances in synthetic methodologies and to the possibility of making analogues of the original lead compound with improved pharmacological or pharmaceutical properties. Natural product scaffolds have also been well recognised as being privileged structures in terms of their ability to be the basis for successful drugs. Such scaffolds are being used as cores of compound libraries made by combinatorial techniques. There are several examples of libraries based on alkaloids, polyketides, and terpenoids.

### 1.3.2. Natural products and their derivatives as drugs-Current status of natural products research

On the basis of literature presented, an updated analysis of natural products in the drug discovery and development process, dating from January 1981 through December 2010. Chemical substances derived from animals, plants and microbes have been used to treat human disease since the dawn of medicine. Natural products (NPs) have provided many novel drug leads, one would assume that NPs would still play a pivotal role in the drug discovery strategy of big pharma companies. The investigation of natural products as source of novel therapeutics reached its peak in the Western pharmaceutical industry in the period 1970–1980, which resulted in a pharmaceutical landscape heavily influenced by non-synthetic molecules. Out of 877 small-molecules as New Chemical Entities (NCEs) introduced between 1981 and

---

2002, nearby half (49%) were natural products, semi-synthetic natural product analogues or synthetic compounds based on natural-product pharmacophores. Despite this puzzling (and seemingly disastrous) decision to significantly downplay the role of natural products in medicinal research in favor of far less validated discovery platform, a disproportionate number of new chemical entities (NCEs) approved even over past 10 years have in fact been natural products or natural product-based.25 The impact of natural products on drug development can be felt across virtually in every major therapeutic area. Facts and figures about natural products with relation to the drug discovery have been well represented in the review articles by Newman and Cragg over the period 1981 to 2008.26 (figure-2)

![Figure 2](image)

**Figure 2**

**Contribution of NPs Towards Drug Discovery:** Where N (an unmodified pure natural product); ND (a modified natural product); S (a synthetic compound with no natural product conception); S/NM (a synthetic compound showing competitive inhibition of the natural product substrate); S1 (a synthetic compound with a natural product pharmacophore); and S1/NM (a synthetic compound with a natural product pharmacophore showing competitive inhibition of the natural product substrate).

---

Chapter 1: Section A  Bio-prospection of Natural Products

It is interesting to see that, in a list of 1024 NCE’s, 6% are pure natural products (N), 27% natural modified molecules (ND), 13% contribution is from synthetic compounds showing competitive inhibition of the natural substrate (S/NM), 17% from synthetic compounds with a natural product pharmacophore (S1) or a natural product pharmacophore showing competitive inhibition of the natural product substrate (S1/NM), and only 37% are synthetic compounds that are derived of any natural product concept. In nutshell, the figure shows 63% drugs of the NCEs are from NPs, moreover 80% drugs which have been in use for cancer therapy are of natural products only. The above discussed NCEs are contributing to various classes of drugs such as antimalarials, antibiotics, anticancer and other therapeutic areas. Where ever lead compounds from synthetic libraries have failed to deliver, natural products have proven their worth.

1.3.3. Important drugs from plants

Aspirin 3 that bears a very simple chemical structure is a powerful synthetic drug used to treat a wide variety of ailments, more so as an anti-inflammatory drug, and a pain reliever. The natural product that provides the basis for aspirin is salicylic acid, which is isolated from the bark of the willow tree. Use of the willow tree for medicinal purposes dates back nearly 2500 years (to the time of the ancient Mediterranean empires). One of the side effects of salicylic acid is gastric discomfort and irritation, but a small modification in the form of the preparation of its acetyl derivative (aspirin) partially reduces the side effects to use it as a therapeutic agent. Aspirin 3 also functions as an important preventive treatment against heart disease because of its inhibition of prostaglandins, which affect the clotting of blood.

The earliest medicinal compounds to be isolated were stychnine 21, morphine 22, atropine 24 and colchicine 23. Morphine 22 was the first commercially pure isolated natural product used as an analgesic drug. It is also used to give relief from cancer pain, kidney stone pain and pain after surgery etc. Ephedrine 25 is one of the alkaloid

having same role to that of adrenaline has been used in the treatment of asthma and bronchitis for centuries in Chinese traditional system of medicine. It is also used as intravenously in the reversal of hypotension from spinal/epidural anesthesia and other hypotensive states, including overdose with ganglionic blocking agents, anti-adrenergic agents that lowers blood pressure. Piperine is least toxic among alkaloids and is used as a flavouring additive in brandy and also acts as a bio-enhancer. There are still many other alkaloids which find medicinal uses like atropine that dilates pupil and had been used in ophthalmology, cocaine is used as anesthetic in eye surgery and dentistry, papverine is used as antispasmodic for the relaxation of cardiac muscles and in the remedy of coughs etc. (Figure-3)

Figure 3: Structures of different drugs

---

Drugs derived from natural source have been used as anti-infective/anti-inflammatory, anti-cancer, analgesic, anti-malarial etc. Penicillin G \(29\), as antibiotic drug made it possible the treatment of diseases which were otherwise thought to be deadly.\(^{35}\) Later on many synthetic penicillin were also available like procaine penicillin, benzathine penicillin G, penicillin V and staphicilline. Chloromycetin \(30\) is a board spectrum antibiotic isolated from species of \textit{streptomyses}. Following the suite were the discoveries of tetracycline derivatives, chlortetracycline \(31\) and the macrolides, which are best represented by erythromycin A \(32\)^{36} opened a new era of broad spectrum antibiotics. (Figure-4).

\[\text{Figure 4}\]

Natural products have shown great promise in anti-malarial drug development. Aremisinin\(15\), has been used from last 2000 years by the Chinese to treat malaria.\(^{37}\) An extract from the bark of cinchona from the Andes of Ecuador and Peru was for three centuries the standard remedy for malaria. Quinine \(16\) was the first ever drug,

which consequently led to the development of other anti-malarial drugs such as chloroquine \textsuperscript{33}. \footnote{World Health Organization. Cancer pain relief, 2\textsuperscript{nd} Ed. Geneva, Switzerland: World Health Organization, 1996.} (Figure-5)

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Figure 5}
\end{figure}

\textbf{1.3.4. Anti-cancer molecules from natural sources}

Cancer is one of the leading death causes and now believed to be number one cause of premature deaths in industrialized nations. Taxol \textsuperscript{14}, a diterpene ester was discovered initially as minor component in the bark of the Pacific yew. Taxol \textsuperscript{14} stimulates the formation of microtubules from tubulin and stabilizes this polymer which stops cells from dividing. \footnote{Kingston, D. G. I. \textit{chem. commun.} 2001, 867.} Microtubules provide a sort of cytoskeleton for cells so that they can maintain shapes. These microtubules are constructed by the controlled polymerization of monomeric tubulin proteins. Taxol is a toxic drug and must be used with great care. Semi-synthesis of taxol from 10-deacetylbaccatin \textsuperscript{34} has allowed the synthesis of many analogs, of which docetaxel is the most prominent. \footnote{Potier, P. C. R. \textit{Acad. Agric. Fr.} 2000, 86, 179.} Its adverse effects were somehow similar to taxol itself and its mode of action is also similar to taxol \textsuperscript{14}.

The active alkaloids were isolated and were found to be anti-leukemic agents. The most prominent isolated are vinblastine \textsuperscript{17} and vincristine \textsuperscript{18}. Vinblastine \textsuperscript{17} is generally used in the treatment of metastatic testicular tumors with great care to avoid damaging extravasations. Vinblastine \textsuperscript{17} blocks cells in the M-phase. It binds to the \textgreek{b} subunit of tubulin in its dimeric form in a one to one complex, thus preventing its
polymerization into microtubules. Vincristine 18 is generally used in the treatment of lymphoblastic leukaemia and solid tumors.41 (Figure-6)

![Figure 6](image)

1.4. Classification

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-kingdom</td>
<td>Tracheobionta (Vascular Plant)</td>
</tr>
<tr>
<td>Super division</td>
<td>Spermatophyta (Seed Plant)</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliophyta (Flowering Plant)</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida (Dicotyledonous)</td>
</tr>
<tr>
<td>Sub-class</td>
<td>Asteridae</td>
</tr>
<tr>
<td>Order</td>
<td>Apiales</td>
</tr>
<tr>
<td>Family</td>
<td>Apiaceae (Umbelliferae)</td>
</tr>
<tr>
<td>Genus</td>
<td>Prangos</td>
</tr>
<tr>
<td>Species</td>
<td>P. pabularia (Lindl.)</td>
</tr>
</tbody>
</table>

1.4.1. Prangos pabularia

*Prangos pabularia* is a tall glabrous perennial herb. It belongs to family Apiaceae (Umbelliferae) commonly known as parsley family consisting mostly of aromatic

---

plants with hollow stems. Apiaceae is one of the largest families of flowering plants consisting of 3780 species with 434 genera. Genus Prangos consists of about 36 species distributed in the Mediterranean region, western and central Asia. Prangos pabularia Lindl. is the only species that occurs in India, distributed mainly in the middle Himalayan ranges, lying on the extreme north west of the country. In Jammu and Kashmir, it occurs in stony slopes in Drass area of Ladakh region. It is known as “Komal” in Hindi and “Kurangas” locally. Lindl was first to report the occurrence of this species in the North-West Himalayan range in 1825.

1.4.2. Morphology

Prangos pabularia is upto 1-2 m high and has an erect stem with large umbels of yellow flowers. Primary umbels with 5-6 linear bracts and 10-15 stout primary rays; secondary umbels each with 5-6 linear bracteoles. Leaves are quite different, pinnate, very compound, 30-45 cm. Fruit with spongy undulate wings. Fruiting umbels 10-15 cm across. Fruit 6-18 mm oblong, commissure broad, wings 3 mm, broad, ridges undulate furrows rough with corrugations, epicarp spongy. Carpels 1/2-terete, dorsally compressed, inner face nearly plane but the epicarp are thin, introflexed in a deep T-shaped groove; primary ridges large, vittae small, numerous; style bases depressed; styles short, early reflexed. Carpophore 2-partite. Seed 2-2.5 mm in diameter, dorsally compressed, inner face slightly concave with a deep narrow T-shaped groove. The flowers are hermaphrodite (have both male and female organs) and are pollinated by insects. The plant is self-fertile. The roots are tapering cylindrical with the basal end broken. At the upper end, the root possesses a few cylindrical rhizomatous branches (which pass downward into the main tap root) which are crowned by the stelar remains of the leaves, and towards the centre the whole petiole bases also remain. Externally the root is of brownish colour, internally at the centre it is pale white while light-brown to the outside.

---

The plant is used as antiseptic, aphrodisiac, dysmenorrhea, dyspepsia, laxative, stomachic, diuretic, digestive, molluscicide, insect repellent, carminative, stimulant, tonic, antiflatulent, nerve anthelmintic, antifungal, and antibacterial agents. The plant is considered as a valuable fodder for goats and sheep but is poisonous to lower animals. A decoction of the plant destroys snails. The young leaves and the flowers of the plant act as insect repellant. Fruits of the plant cause inflammation of the eyes and sometimes temporary blindness among horses. An infusion of the roots is useful in indigestion and flatulence and regularization of menstrual cycle in females. The plant flower extracts have been used as an aroma in cheese and milk products in the eastern parts of Turkey and have been also used to stop bleeding and heal scars in central Asia.

1.4.3. Ethnobotanical Uses

*Prangos* species are widely used in folk medicine as tonic, and for the treatment of flatulence, haemorrhoids, wounds and leukoplakia. In Central Asia, extracts of *Prangos* species have been used to stop bleeding and heal scars. Furthermore, *Prangos* species have been used in traditional medicine in Turkey as anthelmintic. Roots of the *Prangos* species, like those of *P. ferula* and *P. ferulago*, are used as aphrodisiacs, Antimicrobial, antioxidant and cytotoxic activities have also been reported in literature.

---


Prangos pabularia has been held in considerable repute in indigenous medicine for its roots and fruit. The roots of the plant have a bitter taste and fragrant odour and are claimed to be diuretic in action. They are used externally for the treatment of itches. An infusion of the roots is useful in indigestion and flatulence and regularization of menstrual cycle in females. The roots provide a large number of coumarin compounds, osthol being one of the major constituents, which has been found to be a potent respiratory and circulatory stimulant in experimental animals. Its respirotonic effect has been found to be more marked than of coramine, leptazol and caffeine. The fruit (seed) possesses a pleasant smell and sharp sweet taste. It is used as carminative, laxative, aphrodisiac, stimulant and tonic to liver, and acts as a promoter for the expulsion of foetus.

In ladakh region of Jammu & Kashmir, a tribal community known as Boto (The Buddhists) uses the plant for curing stomach disorders. The root infusion is given twice a day in small doses for one week or more to treat indigestion. Seeds are considered as carminative. The traditional practitioners of Ladakh who recommend these medicines are known as “Amchis” and the traditional medicine system is principally based on Tibetan System of Medicine. (Basant Ballabh & O.P. Chaurasia, Indian Journal of Traditional Knowledge). In western Ladakh, another traditional system of medicine is practiced known as “Soya-rigpa”. The healers in this system are also known as “Amchis”. The word Sowa-rigpa is a combination of two Tibetan words, i.e., Sowa meaning ‘to nourish’ and Rigpa meaning ‘science’. Although, the system originated in India, it is popularly practiced throughout Tibet, Bhutan, Mongolia, China and Nepal (Gurmet, 2004). In India, this system is practiced in Sikkim, Arunachal Pradesh, Darjeeling, Lahaul and Spiti and Ladakh (Namgyal and Phuntsog, 1990). Sowa-rigpa system of medicine was given official recognition recently (25 August, 2011), through the Indian Medicine Central Council Amendment Bill 2010. In this system the fruits of Prangos Pabularia are used as carminative, stimulant and diuretic.

---

1.5. Phytochemistry of Prangos Pabularia

*Prangos Pabularia* have been found rich source in secondary metabolites, mainly contains large number of coumarins. Chemical investigations have shown that the roots and stems of this plant contain about 30 chemical constituents which consist of coumarins of diversified structures, terpenoid and glycosides. 59,60,61

1.5.1. Coumarins as bioactive molecules

Coumarins 35 are a group of plant-derived polyphenolic compounds and they are consisted of fused benzene and a-pyrone rings. (Figure 7). They belong to the benzopyrones family and possess a wide variety of cytoprotective and modulatory functions, which may be translated to therapeutic potentials for multiple diseases. Their physicochemical properties seem to define the extent of the biological activity. The name of coumarin chemical family has been derived from *Coumarouna odorata Aube* (*Dipteryx odorata*), from which the simplest member has been isolated for the first time. It is a large group of naturally occurring compounds widely distributed in many families of plants such as *Asteraceae, Fabiaceae, Rosaceae, Rubiaceae, Solanaceae*, particularly in the *Umbelliferae* and *Rutaceae*. More than 1800 different natural coumarins have been discovered and described to date. 62

![Figure 7](image-url)


1.5.2. Classification

A feature common to most coumarins is oxygenation at C-7 position. The 7-hydroxycoumarin, commonly known as umbelliferone 36, is often regarded as the parent coumarin both structurally and biogenetically, of the more complex coumarins. Another common feature among coumarins is the presence of isoprenoid chains, frequently of one, but often of two or three units, attached to a carbon or oxygen or both. The prenyl group may be found as the simple 3-methylbut-2-enyl unit, but it is often encountered as the corresponding epoxide or vicinal glycol or in a variety of oxidized and skeletally rearranged forms. Biogenetically an additional heterocyclic ring can be formed when the prenyl group interacts with an O-phenolic group. The structural variations of this type encountered in the natural coumarins mostly include dihydrofuran, hydroxydihydropyran and their derivatives, furan and dihydropyran. Prenylation at C-6 and C-8 can lead to linear coumarins such as psoralen and xanthyletin or angular forms like angelicin and seselin respectively. (figure-8)

In this regard coumarins are classified as following:

(a) Simple, which includes coumarin, its hydroxylated, alkoxylated and alkylated derivatives and their glycosides.

(b) Furanocoumarins, including the typical linear form psoralen 37 and angular type angelicin 39.

(c) Pyranocoumarins 40, which have a six membered ring attached to the benzoid part like seselin and xanthyletin.

(d) Pyrone-ring substituted coumarins like 4-hydroxycoumarins and 3-phenylcoumarins.

Figure 8

---

1.6. Pharmacological Importance of Coumarins

Coumarins have attracted intense interest in recent years because of their diverse pharmacological properties. Coumarins, a wide family of compounds present in remarkable amounts in the nature, have been found to exhibit different biological and pharmacological significance, including anticancer,\(^{64}\) antioxidant,\(^{65}\) anti-inflammatory,\(^{66}\) antimicrobial,\(^{67}\) antiviral,\(^{68}\) and enzymatic inhibitory activities.\(^{69}\) Coumarin derivatives have also been used as anticoagulants (warfarin \(^5\), disclosed in USA 2427578). Warfarin \(^5\) does not present platelet inhibitory activity but acts as a competitive inhibitor of vitamin K in the biosynthesis of prothrombin. Warfarin \(^5\) and related 4-hydroxycoumarin containing molecules decrease blood coagulation by inhibiting vitamin K epoxide reductase, an enzyme that recycles oxidized vitamin K\(_1\) to its reduced form after it has participated in the carboxylation of several blood coagulation proteins, mainly prothrombin and factor VII. Despite being labeled as vitamin K antagonist,\(^{70}\) Novobiocin \(^42\) (an aminocoumarin antibiotic) analogues are useful in methods of treating, inhibiting and preventing cyst formation in autosomal dominant polycystic kidney diseases (ADPKD).\(^{71}\) (Figure-10)

Calanolides, a group of naturally occurring coumarins bearing the tetracyclic dipyranocoumarin skeleton. Among them (+)-Calanolide A \(^41\) the most active compound against HIV-1 replication also exhibiting activity against the AZT-resistant strain of HIV-1 and the pyridinone-resistant strain A17.\(^{72}\) (+)-calanolide A and several other structurally-related pyranocoumarins revealed that (+)-calanolide A is

---


active against a variety of Mycobacterium tuberculosis strains, including those resistant to the standard antitubercular drugs.\textsuperscript{73} (Figure-9)

![Chemical structures](image)

**Figure 9**

Coumarins with phenolic hydroxyl groups have the ability to scavenge reactive oxygen species and thus prevent the formation of 5-HETE (hydroxyeicosatetraenoic acid) and HHT (hydroxy heptadecatrienoic acid) in the arachidonic pathway of inflammation suppression.\textsuperscript{74,75,76} Osthol \textsuperscript{44}, 7-methoxy-8-(3-methyl-2-butenyl) coumarin is a coumarin derivative clinically ingested as an important component of medicinal plants and herbs\textsuperscript{77} in Tradition Chinese Medicine (TCM), and it exhibits many pharmacological and biological activities.\textsuperscript{78} *Cnidium monnieri* (L.) Cusson, a kind of plant containing high percentage of osthol \textsuperscript{44}, which has been used in China since several hundred years before as an herbal medicine to treat male sexual dysfunction. It has been proved that osthol \textsuperscript{44} has some important therapeutic function and safe-profile compared with other natural product, which makes it a very promising lead compound in drug discovery area. The studies on growth-inhibitory cytostatic activity in human cancer cell line: MCF-7 breast carcinoma cells revealed that osthol demonstrated some estrogenic activity by preventing the synthesis and

\textsuperscript{73} Xu, ZQ.; Barrow, WW.; Suling, WJ. *Bioorg. Med. Chem*. 2004, 12, 1199.
action of estrogens (ER antagonists)\textsuperscript{79} which indicated that osthol has the potential to become a breast cancer treatment reagent. It has been demonstrated that osthol effectively inhibits lung cancer cell invasion, therefore, osthol could be used as an anti-invasive bioactive ingredient that inhibits invasion by CL1-5 cells. The anti-invasive effects of osthol on CL1-5 cells might occur by inhibiting the degradation of IκBα protein expression to reduce NF-κB translocation and NF-κB DNA-binding activities, leading to the down regulation of MMP-9 expression.\textsuperscript{80} (Figure-10)

Linear furocoumarins have been reported to possess interesting biological activities.\textsuperscript{81} Some furanocoumarins from the roots of \textit{Oppopanax chironium} (L.) have been evaluated for activity related to T-cell functionality. Imperatorin \textsuperscript{45} and Heraclenin \textsuperscript{47} significantly inhibited T-cell receptor-mediated proliferation in human primary T cells in a concentration-dependent manner.\textsuperscript{82}

Many furano-coumarins are toxic and are produced by plants as a defence mechanism against various types of predators ranging from insects to mammals.\textsuperscript{83} Imperatorin \textsuperscript{45} and isoimperatorin \textsuperscript{46}, isolated from the umbelliferous Chinese crude drugs \textit{Tang-Bai-Zai} and \textit{Ashita-ba}, possess strong antitumour promoter activity in cultured cells.\textsuperscript{84} Their physiological bacteriostatic and antitumor activity makes these compounds attractive for further backbone derivatization and screening as novel

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure10.png}
\caption{Figure 10}
\end{figure}

\begin{thebibliography}{99}
\bibitem{79} Hamelers, I.; Schaik, R.; Sussenbach, J. S.; Steenbergh, P. H. \textit{Cancer Cell Int.} \textbf{2003}, 3, 10.
\end{thebibliography}
therapeutic agents. In addition it has been shown that 4-hydroxycoumarin 48 and 7-hydroxycoumarin 36 inhibit cell proliferation in gastric carcinoma. (Figure-11)

![Figure 11](image)

Naturally occurring coumarins possess a range of anticarcinogenic activities in rodent models. A recent study has shown that 7-hydroxycoumarin inhibits the release of Cyclin D1, which is over expressed in many types of cancer. Several natural 5, 6, 7-trioxygenated coumarins have the capacity to induce cell differentiation in human leukemia U-937 cells which make them potential lead compounds in cancer therapy.

Coumarins have a wide variety of uses in industry, mainly due to its strong fragrant odour. Its uses include that of a sweetener and fixative of perfumes, an enhancer of natural oils, such as lavender, a food additive in combination with vanillin, a flavour/odour stabilizer in tobaccos, an odour masker in paints and rubbers and finally it is used in electroplating to reduce the porosity and increase the brightness of various deposits such as nickel. 6-Methylcoumarin is mainly used as a flavour enhancer and 7-hydroxycoumarin in sunscreens.

1.7. Results and Discussion

1.7.1. Plant Material

The root part of Prangos pabularia was collected from Drass, Ladakh (J&K) in July 2008. After proper identification a voucher specimen (No. 33214) was deposited in the herbarium of University of Kashmir.

---

1.7.2. Extraction and isolation

This has been done as per the flow chart displayed at figure-12 (page no.24). Shade-dried and finely powdered plant material (root part, 2kg) was extracted with petroleum ether (60-80°C), DCM: Methanol (1:1) according to the NCI protocol to afford the respective extracts. The extracts thus obtained were concentrated under reduced pressure to give crude extracts *i.e.* petroleum extract (PE), DCM: Methanol (1:1) extract (DME) respectively.

The petroleum ether extract (PE), (30g) was dissolved in minimum amount of DCM and adsorbed on silica gel (60-120 mesh), to form slurry. The dried slurry was subjected to column chromatography over silica gel (60-120 mesh). The column was eluted with different percentages of petroleum ether, and ethyl acetate in different proportions and the following fractions (100 ml each) were collected: 1-260 (petroleum ether : EtOAc, 9:1) (Fraction A), 261-375 (petroleum ether : EtOAc, 8:2) (Fraction B), 376-510 (petroleum ether : EtOAc, 7:3) (Fraction C), 511-650 (petroleum ether : EtOAc, 6:4) (Fraction D), 651-700 (petroleum ether : EtOAc, 5:5) (Fraction E), 701-800 (petroleum ether : EtOAc, 3:7) (Fraction F), 800-1000 (petroleum ether : EtOAc, 1:9) and (Fraction G), 1001-1020 (EtOAc) (Fraction H).

These fractions on repeated column chromatography over silica gel (60-120 mesh), eluted with different solvents like petroleum ether, chloroform and ethyl acetate in increasing order of polarity and by recrystallization yielding seven pure compounds as osthol (PE-1 7gram) imperatorin (PE-2 5gram), isoimperatorin (PE-3 500mg), xanthotoxin (PE-4 500mg), bergapten (PE-5 2gram), merangin (PE-6 500mg), heraclenin (PE-7 100mg).

The DCM: Methanol (1:1) (DME) extract (20 g) was dissolved in minimum amount of methanol adsorbed on silica gel (60-120 mesh), to form slurry. The dried slurry was charged on silica gel (60-120 mesh) column The column was eluted with different percentages of DCM and methanol in different proportions and the following fractions (100 ml each) were collected: 1-50 (DCM : methanol, 9:1) (Fraction A), 51-100 (DCM : methanol, 7:3) (Fraction B), 101-160 (DCM : methanol, 5:5) (Fraction C), 161-200 (DCM : methanol, 3:7) (Fraction D), 200-270 (DCM : methanol, 2:8) (Fraction E), 271-300 (DCM : methanol, 1:9) (Fraction F), 301-350 (methanol)
(Fraction G), These fractions on subjecting to repeated column chromatography over silica gel (60-120 mesh), eluted with different solvents like DCM, chloroform and methanol in increasing order of polarity and by recrystallization yielding above compounds also and thirteen other pure compounds as oxypeucedanin (DME-1 37mg), psorlen (DME-2 100mg), isopsorlen (DME-3 70mg), 4-(2-hydroxy-3-methylbut-3-enyloxy)-7H-furo[3,2-g]chromen-7-one (DME-4 25mg), 4-hydroxy-9-(3-methylbut-2-enyl)-7H-furo[3,2-g]chromen-7-one (DME-5 75mg), Oxypeucedanin hydrate monoacetate (DME-6 62mg), oxypeucedanin hydrate (DME-7 2g), heraclenol (DME-8 5g), xanthotoxol (DME-9 125mg), bergaptol (DME-10 37mg), 3-tetrahydro-2,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-3-yloxy)-1-(7-oxo-7H-furo[3,2-g]chromen-4-yloxy)-3-methylbutan-2-yl acetate (DME-11 27mg), (-)4-[3-(β-D-glucopyranosyloxy)-2 hydroxy-3-methyl butoxy]-7Hfuro[3,2-g][1]benzopyran-7-one (DME-12 44mg), 7-hydroxocormarin (DME-13 3g). Flow chart depicting isolation of compounds from the root part of Prangos pabularia is shown in figure-12.
Figure 12: Flow chart depicting preparation of extracts and isolation of compounds from the root part of *Prangos pabularia*. 
The isolated compounds above were screened for their anticancer activity against various human cancer cell lines (colon, lung, prostate, and skin) at 25 and 50 µmol concentrations. Of all the test molecules, only PE-1, were able to display anticancer activity at lower dose, with best activity in terms of %age inhibition and other compounds PE-2, PE-5, DME-1 shows activity against a particular type of cell line. The results of the above study are summarized in table-1.
### 1.8. In vitro screening of the molecules on human cancer cell lines

Table - 1: Percentage Growth Inhibition at 25 µM and 500 µM of compounds isolated from *Prangos pabularia* against A-549, A-431, NCI-H322, PC-3, A-475 and Hct-116 cell lines using MTT assay.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Conc. in µM</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 µM</td>
<td>50 µM</td>
</tr>
<tr>
<td>PE-1</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td>PE-2</td>
<td>50 µM</td>
<td>61</td>
</tr>
<tr>
<td>PE-3</td>
<td>50 µM</td>
<td>31</td>
</tr>
<tr>
<td>PE-4</td>
<td>29</td>
<td>58</td>
</tr>
<tr>
<td>PE-5</td>
<td>25 µM</td>
<td>10</td>
</tr>
<tr>
<td>PE-6</td>
<td>50 µM</td>
<td>31</td>
</tr>
<tr>
<td>PE-7</td>
<td>50 µM</td>
<td>65</td>
</tr>
<tr>
<td>DME-1</td>
<td>25 µM</td>
<td>35</td>
</tr>
<tr>
<td>DME-2</td>
<td>50 µM</td>
<td>15</td>
</tr>
<tr>
<td>DME-3</td>
<td>50 µM</td>
<td>53</td>
</tr>
<tr>
<td>DME-4</td>
<td>25 µM</td>
<td>18</td>
</tr>
<tr>
<td>DME-5</td>
<td>50 µM</td>
<td>12</td>
</tr>
<tr>
<td>DME-6</td>
<td>25 µM</td>
<td>19</td>
</tr>
<tr>
<td>DME-7</td>
<td>50 µM</td>
<td>29</td>
</tr>
<tr>
<td>DME-8</td>
<td>25 µM</td>
<td>9</td>
</tr>
<tr>
<td>DME-9</td>
<td>50 µM</td>
<td>29</td>
</tr>
<tr>
<td>DME-10</td>
<td>25 µM</td>
<td>39</td>
</tr>
<tr>
<td>DME-11</td>
<td>50 µM</td>
<td>58</td>
</tr>
<tr>
<td>DME-12</td>
<td>25 µM</td>
<td>35</td>
</tr>
<tr>
<td>DME-13</td>
<td>50 µM</td>
<td>62</td>
</tr>
<tr>
<td>5FU</td>
<td>20 µM</td>
<td>83</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>10 µM</td>
<td>92</td>
</tr>
</tbody>
</table>
1.9. Standard Experimental Procedure

NMR spectras were recorded on Bruker 200, 400 and 500 MHz spectrometers with TMS as the internal standard. Chemical shifts were expressed in parts per million (δ ppm). Reagents and solvents used were mostly LR grade. Silica gel coated aluminum plates from M/s Merck were used for TLC. MS were recorded on High Resolution Mass Spectrometer MS Q-TOF LC/MS, Agilent Technologies 6540. Infrared spectra were recorded as KBr pellets in cm⁻¹ on a Hitachi 270-30 spectrophotometer. Optical rotations were measured on Perkin-Elmer 241 polarimeter at 25 °C using sodium D light. Melting points were determined on Buchi B-542 apparatus by open capillary method and are uncorrected. Chemicals were purchased from M/s Aldrich Chemicals, Mumbai. Thin layer chromatographic (TLC) plates were visualised under ultraviolet light at 254 nm for fluorescence quenching spots and at 366 nm for fluorescent spots. Cerric sulphate, sulphuric acid and FeCl₃ were used as spraying reagents to visualize the spots. Iodine was also used to detect the spots.

1.9.1. Cell culture

A-549, A-431, NCI-H322, PC-3, A-475 and Hct-116 cells were routinely maintained in RPMI 1640 (Sigma Aldrich) while MCF-7 cells was maintained in Minimum Essential Medium MEM (Sigma Aldrich) supplemented with 10% FBS (Merck) and 1% penicillin G and streptomycin (Sigma Aldrich) at 37 °C in a humidified incubator with 5% CO₂. All stock solutions of compounds were prepared in cell culture grade DMSO and stored in -20 °C. Compounds were diluted in culture media prior to use in experiments.

1.9.2. Antiproliferative activity

For antiproliferative activity were dissolved in cell culture grade DMSO. We screened all the compounds and parent molecule osthol against cancer cells such as A-549, A-431, NCI-H322, PC-3, A-475 and Hct-116 cell lines etc. Cell viability of the compounds treated cells was measured by using MTT assay. Briefly, cells (10⁴ cells/well) were cultured in 96 well tissue culture plates and treated with different concentrations of compounds for 48 h. At the end of incubation, 20 μL of MTT (2.5 mg/mL) was added to the wells and incubated for 4 h. Absorbance was recorded at 570 nm using Eliza Plate Reader.
### 1.10. Spectral data of compounds

**(PE-1) Osthol:** M.P. 83-84°C; $^1$H NMR (200 MHz, CDCl$_3$): $\delta$
- 1.67 (3H, s), 1.84 (3H, s), 3.52 (2H, d, $J=7.28$), 3.92 (3H, s),
- 5.25 (1H, t, $J=7.28$ Hz), 6.24 (1H, d, $J=9.46$ Hz), 6.84 (1H, d,
  $J=8.61$ Hz), 7.28 (1H, d, $J=9.30$ Hz), 7.62 (1H, d, $J=9.46$ Hz).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 17.93, 21.93, 25.79, 56.06, 107.36, 112.60, 112.98,
- 117.97, 121.14, 126.22, 132.63, 143.60, 152.83, 160.23, 161.39. EIMS $m/z$: 244,229,
  213, 175. IR (KBr) $\text{cm}^{-1}$: 1713, 1600, 1495, 1385, 1365.

**(PE-2) Imperatorin:** M.P. 101-102°C; $^1$H NMR (400 MHz, CDCl$_3$):
- $\delta$ 1.72 (3H, s), 1.74 (3H, s), 5.02 (2H, d, $J=7.21$ Hz),
  5.60 (1H, t, $J=7.20$ Hz), 6.37 (1H, d, $J=9.56$ Hz), 6.82 (1H, d,
  $J=2.22$ Hz), 7.36 (1H, s), 7.69 (1H, d, $J=2.3$ Hz), 7.76 (1H, d,
  $J=9.57$ Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 18.13, 25.62, 70.18,
- 106.72, 113.15, 114.71, 116.50, 119.78, 125.87, 131.69, 139.77, 143.84, 144.36,
  146.63, 148.64, 160.5. EIMS $m/z$: 270, 255, 202, 201, 173, 145. IR (KBr) $\text{cm}^{-1}$: 1718, 1603, 1493, 1385, 1361.

**(PE-3) Isoimperatorin:** M.P. 107-109°C; $^1$H NMR (400 MHz, CDCl$_3$):
- $\delta$ 1.64 (3H, s), 1.67 (3H, s), 5.02 (2H, d, $J=7.33$ Hz),
  5.61 (1H, t, $J=7.07$ Hz), 6.30 (1H, d, $J=9.60$ Hz), 7.01 (1H, d,
  $J=2.31$), 7.18 (1H, s), 7.65 (1H, d, $J=2.0$ Hz), 8.22 (1H, d,
  $J=9.60$ Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 19.13, 25.94, 70.18,
- 106.72, 113.17, 114.70, 116.51, 119.86, 125.88, 131.69, 139.75, 143.84, 144.37, 146.63, 148.64,
  161.46. MS at $m/z$: 270 (M$^+$+Na). IR (KBr) $\text{cm}^{-1}$: 1719, 1603, 1493, 1385, 1361.

**(PE-4) Xanthotoxin:** M.P. 147°C; $^1$H NMR (400 MHz, CDCl$_3$):
- $\delta$ 4.26 (3H, s), 6.31 (1H, d, $J=9.50$ Hz), 6.82 (1H, d, $J=2.32$ Hz),
  7.34 (1H, s), 7.69 (1H, d, $J=2.0$ Hz), 7.78 (1H, d, $J=9.45$ Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 61.4, 105.4, 112.9, 114.4, 116.3, 125.8, 132.5, 144.1, 142.5, 146.5, 147.4, 160.1. MS at $m/z$: 216 (M$^+$+Na). IR (KBr) $\text{cm}^{-1}$: 1710, 1610, 1498.
(PE-5) Bergapten. M.P. 186-187 °C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 3.91 (3H, s), 6.26 (1H, d, $J$=9.45 Hz), 6.93 (1H, d, $J$=2.2 Hz), 7.09 (1H, s), 7.61 (1H, d, $J$=2.2 Hz), 7.99 (1H, d, $J$=9.50 Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 59.6, 105.6, 114.7, 118.5, 121.2, 125.6, 130.3, 142.1, 145.2, 146.4, 147.3, 160.0. MS at $m/z$: 216 (M$^+$+Na). IR (KBr) $\max$ cm$^{-1}$: 1717, 1607, 1500.

(PE-6) Merangin. M.P. 98 °C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.29 (3H, s), 1.34 (3H, s), 3.04 (1H, m), 3.06 (2H, m), 3.94 (3H, s), 6.13 (1H, d, $J$=9.0 Hz), 7.12 (1H, d, $J$=8.5 Hz), 7.38 (1H, d, $J$=8.5 Hz), 7.56 (1H, d, $J$=9.0 Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 18.6, 23.1, 24.7, 55.9, 57.8, 60.9, 107.6, 112.1, 112.6, 117.0, 126.3, 134.7, 152.5, 160.3, 161.2. MS at $m/z$: 260 (M$^+$+Na). IR (KBr) $\max$ cm$^{-1}$: 1718, 1600, 1497, 1387, 1367.

(PE-7) R (+)-Heraclenin. M.P. 113-114 °C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 1.28 (3H, s), 1.34 (3H, s), 3.33 (1H, m), 4.55 (2H, m), 6.38 (1H, d, $J$=9.51 Hz), 6.83 (1H, d, $J$=2.23 Hz), 7.41 (1H, s), 7.62 (1H, d, $J$=2.33 Hz), 7.70 (1H, d, $J$=9.46 Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 18.87, 24.61, 58.16, 61.33, 72.47, 106.83, 113.90, 114.79, 116.50, 126.0, 131.44, 143.61, 144.33, 146.82, 148.31, 160.34. EIMS $m/z$: 286 [M$^+$], 202, 89 $[\alpha]_D^{25}$: +21.4°. IR (KBr) $\max$ cm$^{-1}$: 1720, 1589, 1490, 1383, 1365.

(DME-1) R (+)-Oxypeucedanin. M.P. 102-103 °C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.33 (3H, s), 1.40 (3H, s), 3.23 (1H, m), 4.42 (1H, m), 4.60 (1H, m), 6.29 (1H, d, $J$=10.12 Hz), 6.95 (1H, d, $J$=2.40 Hz), 7.18 (1H, s), 7.61 (1H, d, $J$=2.0 Hz), 8.21 (1H, d, $J$=10.0 Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 19.04, 24.61, 58.16, 61.33, 72.47, 106.83, 113.90, 114.79, 116.50, 126.0, 131.44, 143.61, 144.33, 146.82, 148.31, 160.34. EIMS $m/z$: 286, 202, 89 $[\alpha]_D^{25}$: +18.3° (c 0.23, CHCl$_3$). IR (KBr) $\max$ cm$^{-1}$: 1720, 1589, 1490, 1383, 1365.

(DME-2) 7H-furo[3,2-g]chromen-7-one (psorlen). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 6.39 (1H, d, $J$=9.57 Hz), 6.84 (1H, d, $J$=2.24 Hz), 7.49 (1H, s), 7.70 (1H, d, $J$=2.27 Hz), 7.69 (1H, s), 7.82 (1H, d, $J$=9.57 Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 99.85, 106.39, 114.62, 115.43, 119.87,
124.91, 144.11, 146.93, 152.03, 156.43, 161.07. MS at $m/z$: 186.16 (M$^+$+Na). IR (KBr) $\text{max} \text{cm}^{-1}$: 2202, 1717, 1607, 1500.

(DME-3) 2H-furo[2,3-h]chromen-2-one (isopsorlen): $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 6.40 (1H, d, $J=9.56$ Hz), 7.12 (1H, d, $J=1.76$ Hz), 7.35 (1H, d, $J=8.58$ Hz), 7.45 (1H, d, $J=8.49$ Hz), 7.70 (1H, $J=2.20$ Hz), 7.83 (1H, d, $J=9.55$ Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 103.93, 109.00, 113.65, 113.82, 116.84, 124.04, 145.10, 146.16, 148.35, 157.45, 161.43. MS at $m/z$: 186.16 (M$^+$+Na). IR (KBr) $\text{max} \text{cm}^{-1}$: 2202, 1717, 1607, 1500.

(DME-4) 4-(2-hydroxy-3-methylbut-3-enyloxy)-7H-furo[3,2-g]chromen-7-one: $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 1.83 (3H, s), 4.40 (1H, m), 4.48 (1H, m), 5.07 (1H, s), 5.20 (1H, s), 6.28 (1H, d, $J=9.71$ Hz), 6.98 (1H, d, $J=2.31$ Hz), 7.17 (1H, s), 7.60 (1H, d, $J=2.81$ Hz), 8.20 (1H, d, $J=9.83$ Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 18.74, 74.05, 75.57, 94.59, 104.78, 107.33, 112.76, 113.34, 114.11, 139.48, 143.52, 145.21, 148.61, 152.52, 158.13, 161.44. MS at $m/z$: 286.28 (M$^+$+Na). IR (KBr) $\text{max} \text{cm}^{-1}$: 3540, 2202, 1717, 1607, 1500.

(DME-5) R (+)-Oxypeucedanin hydrate monoacetate: M.P. 138$^\circ$C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 1.34 (3H, s), 1.38 (3H, s), 2.06 (3H, s), 3.71 (1H, bs), 4.59 (2H, m), 5.34 (1H, m), 6.20 (1H, d, $J=9.0$ Hz), 6.91 (1H, d, $J=2.2$ Hz), 7.07 (1H, s), 7.60 (1H, d, $J=2.2$ Hz), 8.14 (1H, d, $J=9.0$ Hz). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 21.05, 26.47, 71.38, 71.55, 94.35, 104.86, 106.77, 113.0, 113.18, 139.11, 145.10, 148.52, 152.59, 158.15, 161.14, 170.54. MS at $m/z$: 346.202 (M$^+$+Na). IR (KBr) $\text{max} \text{cm}^{-1}$: 3400, 1738, 1716, 1603, 1497, 1382, 1367, 1244.

(DME-6) 4-hydroxy-9-(3-methylbut-2-enyl)-7H-furo[3,2-g]chromen-7-one: $^1$H NMR (400 MHz, CD$_3$OD): $\delta$ 1.69 (3H, s), 1.86 (3H, s), 3.77 (2H, d, $J=6.4$ Hz), 5.16 (1H, t, $J=6.8$ Hz), 6.37 (1H, d, $J=10$ Hz), 6.96 (1H, d, $J=2.0$ Hz), 7.81 (1H, d, $J=2.4$ Hz), 8.21 (1H, d, $J=10$ Hz). $^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$ 17.99, 24.17, 24.85, 104.25, 105.50, 105.73, 109.67, 113.25, 123.01, 131.61, 138.07, 147.20, 150.27, 153.31, 161.10. MS at $m/z$: 270.28 (M$^+$+Na). IR (KBr) $\text{max} \text{cm}^{-1}$: 3280, 1718, 1600, 1497, 1382, 1364.
(DME-7) **R (+)-Oxypeucedanin Hydrate:** M.P. 138 °C; $^1$H NMR (500 MHz, CD$_3$OD): $\delta$ 1.23 (3H, s), 1.27 (3H, s), 2.52 (1H, bs), 3.04 (1H, bs), 3.85 (1H, dd, $J$=8.21 Hz), 4.45 (1H, dd, $J$=3.96 & 10.45 Hz), 4.74 (1H, dd, $J$=10.25 & 2.57 Hz), 6.37 (1H, d, $J$=9.51 Hz), 6.94 (1H, d, $J$=2.23 Hz), 7.55 (1H, s), 7.88 (1H, d, $J$=2.19 Hz), 8.03 (1H, d, $J$=9.61 Hz). $^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$ 25.15, 26.75, 72.77, 76.47, 78.30, 107.96, 114.83, 114.93, 117.92, 127.92, 133.06, 144.19, 146.71, 148.47, 149.09, 162.78. MS at $m/z$: 304 (M$^+$+Na). IR (KBr) max cm$^{-1}$: 3400, 1716, 1604, 1500, 1389, 1370.

(DME-8) **R (+)-Heraclenol:** M.P. 116 °C; $^1$H NMR (500 MHz, CD$_3$OD): $\delta$ 1.28 (3H, s), 1.31 (3H, s), 3.48 (1H, bs), 3.82 (1H, m), 4.38 (1H, m), 4.57 (1H, m), 6.26 (1H, d, $J$=9.50 Hz), 6.93 (1H, d, $J$=2.50 Hz), 7.15 (1H, s), 7.87 (1H, d, $J$=2.10 Hz), 8.40 (1H, d, $J$=9.50 Hz). $^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$ 24.83, 27.25, 72.70, 75.83, 78.14, 94.63, 106.32, 108.34, 112.94, 115.36, 141.70, 146.79, 150.76, 153.86, 159.83, 163.32. EIMS $m/z$: 304, 202; $[\alpha]_D^{25}$: +15.2 (c 0.8, CHCl$_3$). IR (KBr) max cm$^{-1}$: 3540, 1724, 1606, 1491, 1389, 1370.

(DME-9) **Xanthotoxol:** M.P. 247 °C; $^1$H NMR (200 MHz, CD$_3$OD): $\delta$ 3.54 (1H, bs), 6.35 (1H, d, $J$=9.56 Hz), 6.87 (1H, d, $J$=2.17 Hz), 7.32 (1H, s), 7.79 (1H, d, $J$=2.17 Hz), 7.98 (1H, d, $J$=9.56 Hz). $^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$ 107.86, 111.23, 114.48, 117.67, 127.27, 131.57, 140.91, 147.03, 147.12, 148.20, 163.0. MS at $m/z$: 202 (M$^+$+Na). IR (KBr) max cm$^{-1}$: 3280, 1710, 1491, 1389, 1370.

(DME-10) **Bergaptol:** M.P. 276 °C; $^1$H NMR (400 MHz, CD$_3$OD): $\delta$ 2.7 (1H, bs), 6.36 (1H, d, $J$=9.60 Hz), 6.91 (1H, d, $J$=2.0 Hz), 7.36 (1H, s), 7.84 (1H, d, $J$=2.4 Hz), 8.02 (1H, d, $J$=9.60 Hz). $^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$ 107.94, 111.23, 114.64, 117.84, 127.38, 131.83, 147.14, 148.33, 163.10. MS at $m/z$: 202 (M$^+$+Na). IR (KBr) max cm$^{-1}$: 3210, 1714, 1598, 1492.
(DME-11) 3-tetrahydro-2,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-3-yloxy)-1-(7-oxo-7H-furo[3,2-g]chromen-4-yloxy)-3-methylbutan-2-yl acetate: \(^1\)H NMR (200 MHz, CDCl\(_3\)): \(^1\)H NMR (500 MHz, CD\(_3\)OD): \(\delta\) 1.43 (6H, s), 2.04 (2H, s), 3.19-3.32 (4H, m), 3.66 (1H, m), 3.84 (1H, m), 4.52 (1H, m), 4.59 (1H, m), 4.89 (1H, m), 6.31 (1H, d, \(J\)=9.78 Hz), 7.04 (1H, d, \(J\)=2.0 Hz), 7.15 (1H, s), 7.63 (1H, d, \(J\)=2.23 Hz), 8.08 (1H, d, \(J\)=9.59 Hz). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 21.93, 24.03, 24.41, 62.09, 71.23, 71.28, 71.69, 71.75, 72.47, 72.65, 78.27, 93.96, 95.22, 104.99, 106.33, 112.73, 139.09, 145.03, 148.44, 152.47, 158.15, 161.09, 170.34. MS at \(m/z\): 508.47 (M\(^{+}\)+Na). IR (KBr)\(_{\text{max}}\) cm\(^{-1}\): 3315, 1730, 1620, 1600, 1570.

(DME-12) (-)-4-[3-(\(\beta\)-D-glucopyranosyloxy)-2 hydroxy-3-methyl butoxy]-7HFuro[3,2-g][1]benzopyran-7-one : M.P. 252 °C; \(^1\)H NMR (500 MHz, CD\(_3\)OD): \(\delta\) 1.43 (6H, s), 3.19-3.32 (4H, m), 3.66 (1H, m), 3.84 (1H, m), 4.52 (1H, m), 4.59 (1H, m), 4.89 (1H, m), 6.40 (1H, d, \(J\)=9.56 Hz), 6.96 (1H, d, \(J\)=2.22 Hz), 7.56 (1H, s), 7.90 (1H, d, \(J\)=2.2 Hz), 7.99 (1H, d, \(J\)=9.59 Hz). \(^{13}\)C NMR (100 MHz, CD\(_3\)OD): \(\delta\) 22.63, 24.83, 48.69, 61.94, 70.46, 73.98, 74.44, 75.55, 76.30, 79.56, 97.34, 107.05, 114.00, 114.15, 116.05, 126.79, 131.96, 140.77, 143.19, 145.57, 147.30, 148.13, 161.88. [\(\alpha\)]\(_D\)\(^{30}\): -30° (H\(_2\)O, c 0.5). MS at \(m/z\): 466 (M\(^{+}\)+Na). IR (KBr)\(_{\text{max}}\) cm\(^{-1}\): 3315, 1730, 1620, 1600, 1570.
1.10.1. Spectra of Some compounds

$^1$H & $^{13}$C NMR spectra of compound (PE-1)
Bio-prospection of Natural Products

$^1$H & $^{13}$C NMR spectra of compound (PE-2)
Chapter 1: Section A  

Bio-prospection of Natural Products

$^1$H & $^{13}$C NMR spectra of compound (PE-7)
Bio-prospection of Natural Products

$^1$H & $^{13}$C NMR spectra of compound (DME-1)
Bio-prospection of Natural Products

$^1$H & $^{13}$C NMR spectra of compound (DME-2)
Bio-prospection of Natural Products

$^1$H & $^{13}$C NMR spectra of compound (DME-3)
Bio-prospection of Natural Products

$^1$H & $^{13}$C NMR spectra of compound (DME-4)
Bio-prospection of Natural Products

$^1$H & $^{13}$C NMR spectra of compound (DME-5)
Bio-prospection of Natural Products

1H & 13C NMR spectra of compound (DME-7)
Bio-prospection of Natural Products

$\text{H & } ^{13}\text{C NMR spectra of compound (DME-8)}$
Bio-prospection of Natural Products

\[ ^1H \& ^{13}C \text{NMR spectra of compound (DME-9)} \]
$^1$H & $^{13}$C NMR spectra of compound (DME-10)
Bio-prospection of Natural Products

$^1$H & $^{13}$C NMR spectra of compound (DME-11)
$^1$H & $^{13}$C NMR spectra of compound (DME-12)
1.11. Introduction

Analytical chemistry is the science that is mainly concerned about determining the qualitative and quantitative chemical composition of natural and artificial materials under study. The qualitative analysis gives information about the nature of the sample and the quantitative analysis about the contents present in a sample. The instrumental methods of analysis are based upon the measurement of some physical properties of a substance in order to determine its chemical composition, quality and quantification. In this modern age of science and technology, many instrumental techniques have emerged, from which titrimetric spectrophotometric and chromatographic techniques are being most widely used for the sample analyses. The main objective in these methods is the analysis of the drug which is of interest alone in the matrix of the excipients, additive, degradation products, impurities etc, and also other drugs in case of the combination products. The advantage of these techniques is that they are simple and low cost, but the drawback is that the methods are not very sensitive. Due to the limitation of specificity, there are hardly any reports of their use for the assay of stability of samples.

1.12. Objectives of the Present Work

In our earlier studies on *Prangos pabularia*, isolation of new furanocoumarin glycosides, identification of chemical constituents from its essential oil and the thermal studies of oxypeucedanin hydrate monoacetate crystal (its possible use as a good candidate for modern optoelectronic devices)\(^1\) have been reported. Chemical

---
transformation of couramins (osthol) isolated from Prangos pabularia,\textsuperscript{2,3} for augmentation of their biological activity with optimized or minimised toxicities, better drug delivery or higher potency, being one of the major goals of our institute, isolation of chemical constituents; in particular coumarins from Prangos pabularia became a requirement. It may be pertinent to say that coumarins form an important class of compounds known for various pharmacological activities such as anti-inflammatory,\textsuperscript{29} antipyretic,\textsuperscript{4} antioxidant,\textsuperscript{5} bronchodilator,\textsuperscript{6} vasodilator,\textsuperscript{7} antiamoebic,\textsuperscript{8} antibacterial,\textsuperscript{9} antifungal\textsuperscript{10} activities. Their physiological bacteriostatic and antitumor activity makes these compounds attractive for further backbone derivatization and screening as novel therapeutic agents.\textsuperscript{11} In addition it has been shown that 4-hydroxycoumarin and 7-hydroxycoumarin inhibit cell proliferation in gastric carcinoma.\textsuperscript{12} Thus keeping in view the above said merits of natural product isolation and their synthetic modification for the development of drug like molecules, we were interested in the development of standard HPLC methods (resolution and quantification) of the natural product isolates of plant P. pabularia by using the marker compounds already explained in section A of the present chapter.

The main aim is to develop rapid RP-HPLC method for simultaneous separation and the quantification of twelve marker compounds of prangos pabularia with highest

\begin{itemize}
  \item[(a)] Koul, S. K.; Dhar, K. L.; Thakur, R.S. Phytochemistry 1979, 18, 1762.
  \item[(b)] Koul, S. K., Thakur, R. S.; Dhar, K. L. Indian J. Chem. 1979, 4, 396.
  \item[(a)] Backhouse, C. N.; Delporte, C. L.; Negrete, R.E.; Erazo, S.; Zuniga, A.; Pinto, A.; et al. J. Ethnopharmacol. 2001, 78, 27.
  \item[(a)] Piao, XL.; Park, IH.; Baek, SH.; Kim, HY.; Park, MK.; Park, JH. J. Ethnopharmacol. 2004, 93, 243.
  \item[(a)] Ramanithrasimbola, D.; Rakotondramanana, DA.; Rasoaaina, P.; Randrianantoa, A.; Ratsimamanga, S.; Plazzino, G. J. Ethnopharmacol. 2005, 102, 400.
  \item[(a)] Dongmoa, AB.; Azebaze, AGB.; Nguelefack, TB.; Ouahouod, BM.; Sontia, B.; Meyerf, M.; et al. J. Ethnopharmacol. 2007, 111, 329.
  \item[(a)] Lake, B. Food Chem Tox. 1999, 3, 412.
  \item[(a)] Stein, AC.; Alvarez, S.; Avancini, C.; Zacchino, S.; Poser, GV. J. Ethnopharmacol, 2006, 107,195.
\end{itemize}
selectivity, precision and accuracy. In literature, to best of our knowledge, it is the first report of the quantification of twelve coumarins (furano and non-furano coumarins) in a single chromatogram by using gradient RP-HPLC method. The analytical method was validated as per ICH guideline.\textsuperscript{13}

1.13. Chromatographic Techniques

Chromatography is the powerful techniques in which differential migration of components take place between two phases, one is stable which is known as stationary phase and another is movable which is known as a mobile phase.

Various chromatographic methods that have been in vogue are thin layer liquid chromatography (TLC), high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), gas chromatography (GC), capillary electrophoreses (CE). The overall goal of analytical chromatography is to achieve sufficient resolution of analytes of interest within the shortest possible time.\textsuperscript{14} Reducing the particle diameter (dp) of chromatographic packing materials is the most effective way to achieve fast and highly efficient separations.\textsuperscript{15} Major utilization/application of the above said methodologies/techniques relates to the qualitative and quantitative analysis of natural products derived from plant kingdom, microflora, higher fungi and marine sources. Because of being abundant source of structurally diverse biologically active substances and high chemical diversity, NPs derived from plants have provided important contributions to the discovery of drugs and inspiration for most of the active ingredients in medicines. The reason for this may probably be explained by the effects of evolutionary pressure to create biologically active molecules, and/or the structural similarity of protein targets across many species. Further, the large chemical diversity\textsuperscript{16} is also directly linked to a high variability of their intrinsic physicochemical properties that render their universal detection challenging. Chemical fingerprinting is an important tool used for the authentication of plant materials and products standardization, whereas biomarkers fingerprinting may be useful for the therapeutic evaluation. In recent years more people throughout

\textsuperscript{13} ICH guideline, Q2 (R1) step 4, \textit{Validation of Analytical Procedures: Text and Methodology}, \textbf{2005}.
the world are turning to the use of medicinal plant products in healthcare system. Worldwide need of alternative medicine has resulted in growth of natural product markets and interest in traditional systems of medicine. Herbal drug technology used for converting botanicals materials into medicines brings in important component of standardization and quality control with proper integration of modern scientific techniques and traditional knowledge. In order to prove the constant composition of herbal preparations, adequate analytical methods have to be applied such as photometric analysis, thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC) high performance liquid chromatography (HPLC), and gas chromatography (GC) etc.

However, due to the complex nature and inherent variability of the constituents of plant-based drugs, it is difficult to establish quality control parameter, though modern analytical techniques are expected to help in circumventing this problem. Different chromatographic techniques are most frequently used for the identification and quality control (QC) of herbal medicines or products. While there are multitudes of chromatographic techniques to achieve separation of chemical components, the common thread is the separation of compounds through the use of variations in mobile and stationary phases. These include thin layer chromatography (TLC), high-performance thin layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), and several hyphenated techniques like HPLC-MS, NMR, HPLC-MS-MS, GC-MS, etc. All these techniques can help to quantify the phytochemicals present in complex mixtures of herbal products, herbal formulations as well as in the plant extracts.


During 1970's, few consistent commercially chromatographic methods were available and most of the chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thin-layer

Chapter 1: Section B

RP-HPLC chromatography. However, most of the chromatographic techniques were not good enough for quantification of compounds and resolution between similar compounds. During this time, pressure liquid chromatography began to be used to decrease the time of flow, thus reducing the time of purification of compounds as compared to isolation by normal column chromatography. High performance liquid chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify and quantify the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as parts per trillion (ppt) may easily be identified. HPLC can be, and has been, applied to any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples and industrial chemicals.\(^\text{20}\)

High-performance liquid chromatography (HPLC) is a very powerful and versatile chromatographic technique for the separation of natural products (NPs) in complex matrices, such as crude extracts for selective detection and quantification or general profiling. The method is widespread and has been adapted to the analysis of a broad range of NPs generally without the need for complex sample preparation. The choice of the appropriate detection method in HPLC is crucial because of the diversity of NPs and the fact that there is no single technique for their efficient detection.

None of the available HPLC detectors are able to detect all the NPs in a given extract within a single analysis. This limitation is well-known in metabolomics,\(^\text{21}\) which aims at measuring all of the metabolites in an organism qualitatively and quantitatively. The complex and convoluted nature of the crude extracts analysed (from plants, marine organisms, fermentation broths, etc.) also contributes to the difficulty of NP detection. Furthermore, the analytes can be present in large or very small amounts and according to the type of study (quantification, standardisation, fingerprinting, screening, trace analysis, etc.), very sensitive and selective methods may be required for their detection. The analysis of individual NPs in complex crude extracts requires efficient separation methods prior to their detection. In this respect, high-performance liquid chromatography (HPLC) has been recognised since the early 1980’s as the most versatile technique for the efficient separation of NPs directly in crude mixtures.


without the need for complex sample preparation. HPLC has greatly developed through the years in terms of convenience, speed, choice of column stationary phases, high sensitivity and applicability to a broad variety of sample matrices and ability to hyphenate the chromatographic methods to spectroscopic detectors. From the chromatography viewpoint, the development of columns with different phase chemistry (especially reversed phase) enabled the separation of almost any type of NPs. The latest developments of HPLC, including the recent introduction of very pH-stable phases, sub-2-μm particles, and monolith columns, have considerably improved the performances of HPLC systems in terms of resolution, speed and reproducibility. Efficiencies exceeding 100,000 theoretical plates and peak capacities over 900 can be attained by coupling columns together. For the separation of crude extracts, either raw mixtures or samples enriched by extraction via simple solid phase extraction (SPE) or liquid-liquid extraction (LLE) are injected. The separations are performed mostly in reversed-phase chromatography on C-18 material with the acetonitrile-water (ACN-H₂O) or methanol-water (MeOH-H₂O) solvent system in the gradient elution mode. In order to improve the separation efficiency, various modifiers are added to the mobile phase that might strongly influence the sensitivity of detection. In multiple hyphenated systems, the presence of several different detectors online (hyphenated systems) leads to the need for an eluent composition that is compatible with all detectors. HPLC is used extensively for NP profiling and fingerprinting, for quantitative analyses, and for quality control purposes. In this context, a brief overview of the methods available for NP detection is provided here. Two main types of detectors can be defined: simple detectors used for the recording of chromatographic traces for profiling or quantification purposes (e.g., ultraviolet (UV), evaporative light scattering detectors (ELSD), electron capture detector (ECD) and detectors for hyphenated systems that generate multidimensional data (chromatographic and spectroscopic) for online identification and dereplication purposes (e.g., UV-DAD, MS, NMR).

1.15. Review of Literature

The literature reviews regarding examination of coumarins (of different plants) by means of high performance liquid chromatography (HPLC) suggest that various analytical methods are reported. This technique has shown to be a very efficient system for the separation of this class of compounds.

Waksmundzka- Hajnos & Sherma,\(^{27}\) have described the examination of a group of Linear furanocoumarins, such as psoralen, bergapten, xanthotoxin, and isopimpinellin isolated from \textit{Apium graveolens} by normal-phase HPLC equipped with a mobile phase consisting of a mixture of ethyl acetate (0.1%) and formic acid (0.1%) in chloroform, using 250nm detection wavelength.

Giannetti \textit{et al.}\(^{28}\) have reported the development and validation of a high-performance liquid chromatography method using diode array detection for the simultaneous quantification of two furocoumarins (bergapten and bergamottin) in bergamot fruits using reversed phase C18 column with mobile phase of methanol and 5% (v/v) acetic acid aqueous solution in the following gradient: 5-20% (0-13 min), 20-100% (13-25 min), 100-5% (20-30 min).

Nimisha Sharma, Arvind Negi,\(^{29}\) has reported a HPLC method for the determination and quantification of bergapten from aerial parts of \textit{P. pabularia} using \textbf{Column}: RP-18e (Merck, 5μm, 4 x 250mm.), flow rate: 0.8 ml/min, mobile Phase: ACN (B), Water (A); gradient at 254 nm detection wavelength.

Ah Yeon Park \textit{et al.}\(^{30}\) have developed a rapid and sensitive analytical method for the simultaneous determination and quantification of five coumarins in \textit{Angelica dahuricae} using reverse-phase HPLC with a C30 column and tandem mass spectrometry. The chromatographic separation performed on a Zorbax RX-C8 (2.1 × 150 mm, 5 μm) column, and a mixture of 1 mM ammonium acetate and acetonitrile


(35/65, v/v%) containing 0.1% acetic acid has been used as the mobile phase with a flow rate of 0.2 mL/min.

Waksmundzka-Hajnos et al.\textsuperscript{31} have described the method for the quantitative analysis of some coumarins from \textit{Pastinaca sativa} fruits was performed on reverse phase HPLC equipped with C-18 column and mobile phase consisting of methanol + water in gradient elution 0-10 min, 45% MeOH; 10-20 min, 45-55% MeOH; 20-30 min, 55-70% MeOH, and 30-40 min, 70% MeOH in double distilled water.

Mi-Jeong Ahn et al.\textsuperscript{32} in their report describe the development of simultaneous determination of nine coumarin compounds, on high performance liquid chromatography–diode array detector coupled with electrospray ionization/mass spectrometry (HPLC–DAD/MS) in a Korean medicinal herb (Cham-Dang-Gui, the dried root of Angelica gigas, Umbelliferae). The methanol extracts have been analyzed by HPLC using a reversed-phase C-18 column (5µm, 4.5 mm×250 mm) using a gradient acetonitrile–water solvent system at a flow rate of 1.0 ml/min.

Marian Kaminski et al.\textsuperscript{33} have developed a HPLC method for the separation and determination of selected coumarins and furanocoumarins in the crude extracts from plant tissue cultures of \textit{Ammi majus} hairy roots and \textit{Ruta graveolens} cell suspensions using mobile phase of the following solvents: A was deionized water and solvents B and C were THF and methanol, respectively. The gradient applied was as follows: 5 to 13% B within 20 min, 13 to 22% B and 5 to 7% C from 20 to 52 min, and then 65% B for 7 min with flow rate of 1.5 mL/min. The chromatographic separation was performed on 18e 5-lm column.

Manfred Kollroser et al.\textsuperscript{34} report the development of a sensitive and specific method for the simultaneous determination of coumarins: phenprocoumon, acenocoumarol, and warfarin in human plasma by HPLC-electrospray ionization tandem mass spectrometry in both clinical and forensic specimens. The separations performed on a


\textsuperscript{34} Manfred, Kollroser.; Caroline, Schober. \textit{Clinical Chemistry}, 2002, 48, 1, 84.
Symmetry C-18 column (5-µm, 150 µ 3.0 mm i.d.; Waters) at a flow rate of 0.5 mL/min with a mobile phase of acetonitrile–1 g/L formic acid (75:25 by volume).

Ting-ting Wang et al.\textsuperscript{35} have established a simple, rapid, and systematic method for preparative isolation and purification of biologically active coumarin compounds in an important traditional Chinese Medicine, Radix Angelica dahurica. HPLC has been performed with a Shimadzu LC-10AD chromatograph, an SPD-10A UV-visible detector. Compounds were separated on a 250 mm × 4.6 mm i.d., 5-µm particle, Kromasil-C18 column protected by a 5- µm particle C18 guard column. The mobile phase was 66:34 (v/v) methanol–water at a flow rate of 0.8 mL min and 254nm detection wavelength.

Yingchun Zhang et al.\textsuperscript{36} have developed a simple, rapid resolution liquid chromatography coupled with a triple quadrupole mass spectrometry (RRLC-QQQ) for the simultaneous identification and quantification of seventeen major bioactive constituents [can you list their nature] in Yuanhu Zhitong tablet (YZT), a traditional Chinese medicine. All of compounds were separated on an Agilent XDB C\textsubscript{18} column (4.6 mm × 50 mm, 1.8 µm) with linear gradient elution of acetonitrile–0.3% formic acid water (pH 2.7).

Lai-Hao et al.\textsuperscript{37} have reported HPLC method for simultaneous determination of nine coumarins and furocoumarins (psoralens) and the determination of urinary metabolites of methoxypsoralens in human urine after oral administration of Umbelliferae medicines. A Hypersil C8 (25 cm × 4.6-mm i.d.) column with a gradient of methanol and acetonitrile aqueous solution as mobile phase at flow rate of 1.0 mL/min with two-channel UV–vis absorbance detection. The eluent was monitored simultaneously both at 312 and 249 nm.

1.15.1. Analytical Method Development: 38

Methods are developed for new products when no official methods are available. Alternate methods for existing (non pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness. Trial runs are conducted, method was optimized and validated when alternate method proposed is intended to replace the existing procedure comparative laboratory data including merits / demerits are made available.

1.16. Results and Discussion

The plant material (Prangos pabularia) was collected from Drass, Ladakh (Jammu and Kashmir) in July 2008. The specimen was identified by Dr. Akhtar H. Malik, University of Kashmir (Specimen deposited under accession No. 33214 and Collection No. 1203- Javid, Kash). Chemical investigations have been carried out on the DCM: Methanol (1:1) extract of P. pabularia.

The shade dried, finely powdered plant material was extracted as per NCI (National Cancer Institute) protocol. The extract was subjected to column chromatography and eluted with solvents of increasing polarity and the fractions collected from the main column were subjected to repeated column chromatography. The compounds isolated, were purified by re-chromatography and/or by crystallization. The purified compounds (> 98% purity,) were characterized by NMR, HRMS and IR spectroscopy and also confirmed with the data given in the literature, 39,40,41 as 6-hydroxycoumarin 1, 7-hydroxycoumarin (umbelliferone) 2, heraclenol-glycoside 3, xanthotoxol heraclenol 5, oxypeucedanin hydrate 6, 8-((3,3-dimethyloxiran-2-yl)methyl)-7-methoxy-2h-chromen-2-one (merangin) 7, oxypeucedanin hydrate monoacetate 8,

xanthotoxin 9, 4-((2-hydroxy-3-methylbut-3-en-1-yl)oxy)-7h-furo[3,2-g]chromen-7-one 10, imperatorin 11, osthol 12 (Figure 1). The purity of these compounds was determined to be more than 98% by peak areas detected by HPLC. All the solvents used in the analysis were of HPLC grade and were purchased from E. Merck. All the solvents were filtered through a 0.45µm Millipore filter before use.

![Figure 1: Structure of marker compounds isolated and quantified on RP-HPLC](image)

1.17. Materials and Methods

The chromatographic system used in the present analysis for development and validation of this method consisted of RP-HPLC instrument of Agilent technologies 1200 series equipped with a quaternary pump (G13311A), Auto sampler (G1329A), Degasser (G1322A), UV detector (G1315D) and column oven (G1316B). The present analysis was carried out at room temperature on an Agilent ZORBAX Eclipse plus C\textsubscript{18} (4.6 x 250mm, 5µm). Mettler Toledo (JB1603-C/FACT) microbalance and Eppendrop micro-pipettes were used for the whole process. NMR spectra’s were recorded on Bruker 200, 400 and 500 MHz spectrometers with TMS as the internal standard. Chemical shifts were expressed in parts per million (δ ppm). All the solvents
used in the analysis were of HPLC grade and were purchased from E. Merck. Silica gel coated aluminum plates from M/s Merck were used for TLC. MS were recorded on High Resolution Mass Spectrometer MS Q-TOF LC/MS, Agilent Technologies 6540.

1.17.1. Preparation of sample and standard solutions

The sample solutions of *P. pabularia* were prepared by dissolving 10.4 mg of above mentioned extract in 2.4 ml of HPLC grade methanol and filtered before use.

The primary stock solutions of all the marker compounds were prepared by dissolving 1mg (accurately weighed) of compound in 1ml of HPLC grade methanol. These primary stock solutions were further mixed and diluted as per requirement necessary for quantification and filtered before use. The solutions were stored at -20°C when not in use and they were stable for at least six months.

1.17.2. Instrumental conditions

The RP-HPLC instrument used in the present analysis was the Agilent technologies 1200 series equipped with a quaternary pump (G13311A), Auto sampler (G1329A), Degasser (G1322A), UV detector (G1315D) and column oven (G1316B). The present analysis was carried out at room temperature on an Agilent ZORBAX Eclipse plus C\textsubscript{18} (4.6 x 250mm, 5µm). A gradient elution of eluents B (water) and C (methanol) was used for the separation of target analytes. The gradient programme was as follows: 0-5 min, 20% C; 5-10 min, 50% C; 10-15 min, 70% C; 15-18 min 80% C; 18-20 min, 90% C; 20-25 min, 95% C; and 25-30 min, 20% C. The solvent flow rate of 0.8 ml/min was maintained throughout the analysis. 10µl volume injections of sample and standard solution were used. The UV absorbance was monitored at 250nm.

1.17.3. Optimization of the HPLC conditions

Adequate resolution is necessary to acquire satisfactory quantification of compounds. In order to obtain chromatograms with baseline separation of the twelve marker compounds in *P. pabularia*, parameters such as selection of the column, mobile phase composition, conditions for gradient flow and temperature were studied using HPLC method.
Initially, different kinds of columns like ZORBAX Eclipse XDB-C\(_{18}\) (4.6 x150mm, 5\(\mu\)m), Eclipse XDB-\(C_{18}\) (9.4 X 250mm, 5\(\mu\)m) and ZORBAX Eclipse plus \(C_{18}\) (4.6 x 250mm, 5\(\mu\)m) were tested using biphasic solvent systems of (i) acetonitrile and water, (ii) acetonitrile and methanol and iii) methanol and water as the mobile phase. ZORBAX Eclipse plus \(C_{18}\) (4.6 x 250mm, 5\(\mu\)m) was found most suitable because of best resolution in comparison to the other columns and methanol and water solvent system proved to be most effective and therefore, selected for the present study. The methanol and water with a gradient program of: 0-5 min, 20% C; 5-10 min, 50% C; 10-15 min, 70% C; 15-18 min 80% C; 18-20 min, 90% C; 20-25 min, 95% C; and 25-30 min, 20% C and flow rate of 0.8ml/min provided a best baseline separation and elution of all the 12 marker compounds could be achieved in less than 30 minutes.

1.17.4. Calibration

The standard solutions containing 0.833-83.33 \(\mu\)g/ml corresponding to test ranges of twelve marker compounds were prepared from stock solutions and calibration curves were constructed and their linear ranges determined. Calibration curves were plotted by the peak area versus concentration of each analyte. The linearity was evaluated by linear regression an equation \(y = mx + c\) calculated by the least square regression method. All calibration curves showed good linearity regressions under the current chromatographic conditions (table 1). Limits of detection (LOD) and quantification (LOQ) under the present chromatographic conditions were determined on the basis of response and slope of each regression equation at a signal-to-noise ratio (S/N) of 3 and 10, respectively.
Table 1: Limits of detection (LOD) and quantification (LOQ) of marker compounds 1-12

<table>
<thead>
<tr>
<th>Marker compound</th>
<th>Calibration curve a)</th>
<th>( R^2 )</th>
<th>Linear range (µg/mL)</th>
<th>LOD(^b)) (µg/mL)</th>
<th>LOQ(^c)) (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( y = 3.31618e-005x - 0.117756 )</td>
<td>0.9999</td>
<td>0.83-83.33</td>
<td>0.065</td>
<td>0.174</td>
</tr>
<tr>
<td>2</td>
<td>( y = 4.92386e-005x - 0.464667 )</td>
<td>0.9998</td>
<td>0.83-83.33</td>
<td>0.094</td>
<td>0.261</td>
</tr>
<tr>
<td>3</td>
<td>( y = 3.31618e-005x - 0.117756 )</td>
<td>0.9999</td>
<td>0.83-83.33</td>
<td>0.073</td>
<td>0.251</td>
</tr>
<tr>
<td>4</td>
<td>( y = 6.32890e-006x - 0.237669 )</td>
<td>0.9999</td>
<td>0.83-83.33</td>
<td>0.012</td>
<td>0.042</td>
</tr>
<tr>
<td>5</td>
<td>( y = 1.00886e-005x - 0.121333 )</td>
<td>0.9999</td>
<td>0.83-83.33</td>
<td>0.020</td>
<td>0.073</td>
</tr>
<tr>
<td>6</td>
<td>( y = 8.43083e-006x - 0.619976 )</td>
<td>0.9997</td>
<td>0.83-83.33</td>
<td>0.059</td>
<td>0.177</td>
</tr>
<tr>
<td>7</td>
<td>( y = 4.62506e-005x - 0.174440 )</td>
<td>0.9997</td>
<td>0.83-83.33</td>
<td>0.382</td>
<td>1.09</td>
</tr>
<tr>
<td>8</td>
<td>( y = 1.09862e-005x - 0.212514 )</td>
<td>0.9999</td>
<td>0.83-83.33</td>
<td>0.085</td>
<td>0.234</td>
</tr>
<tr>
<td>9</td>
<td>( y = 3.95704e-005x - 0.0973815 )</td>
<td>0.9999</td>
<td>0.83-83.3</td>
<td>0.278</td>
<td>0.625</td>
</tr>
<tr>
<td>10</td>
<td>( y = 1.09242e-005x - 0.176053 )</td>
<td>0.9999</td>
<td>0.83-83.3</td>
<td>0.089</td>
<td>0.253</td>
</tr>
<tr>
<td>11</td>
<td>( y = 1.01645e-005x - 0.173190 )</td>
<td>0.9998</td>
<td>0.83-83.3</td>
<td>0.120</td>
<td>0.296</td>
</tr>
<tr>
<td>12</td>
<td>( y = 2.61995e-005x - 0.0831525 )</td>
<td>0.9992</td>
<td>0.83-83.3</td>
<td>0.162</td>
<td>0.451</td>
</tr>
</tbody>
</table>

a) The calibration curves were constructed by plotting the peak areas versus the concentration of each analyte.

b) LOD refers to the limit of detection.

c) LOQ refers to the limit of quantification.

1.17.5. Validation

The developed HPLC method was validated for its linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and stability. The intra-day and inter-day studies were carried out to check the precision of the developed assay.

The intraday variations were carried out by analyzing six replicates at three different concentrations (16.6, 41.6 and 83.3) in a day. The inter-day variations were determined by analyzing six replicates at three different concentrations over three days and the results are expressed as RSD(%) = \((SD/mean)\times100\%\) (table-2). The RSD was well below 5% indicating good precision of the developed method.
<table>
<thead>
<tr>
<th>Marker compounds</th>
<th>Amount (µg/mL)</th>
<th>Intra-day precisions (n=6)</th>
<th>Inter-day precisions (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean area</td>
<td>%RSD</td>
<td>Mean area</td>
</tr>
<tr>
<td>1.</td>
<td>16.66</td>
<td>528127.5</td>
<td>1.19</td>
</tr>
<tr>
<td></td>
<td>41.66</td>
<td>1349015.66</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>83.33</td>
<td>2624872.66</td>
<td>0.40</td>
</tr>
<tr>
<td>2.</td>
<td>16.66</td>
<td>369255</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>41.66</td>
<td>905746.33</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>83.33</td>
<td>1748411</td>
<td>0.32</td>
</tr>
<tr>
<td>3.</td>
<td>16.66</td>
<td>353819</td>
<td>2.62</td>
</tr>
<tr>
<td></td>
<td>41.66</td>
<td>1007683</td>
<td>0.616</td>
</tr>
<tr>
<td></td>
<td>83.33</td>
<td>1948529.33</td>
<td>0.157</td>
</tr>
<tr>
<td>4.</td>
<td>16.66</td>
<td>2869202.05</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>41.66</td>
<td>7436826.33</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>83.33</td>
<td>14245153</td>
<td>0.48</td>
</tr>
<tr>
<td>5.</td>
<td>16.66</td>
<td>1721615.5</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>41.66</td>
<td>4492011.66</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>83.33</td>
<td>8696901</td>
<td>0.012</td>
</tr>
<tr>
<td>6.</td>
<td>16.66</td>
<td>2061524</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>41.66</td>
<td>5472159</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>83.33</td>
<td>10471469</td>
<td>0.02</td>
</tr>
<tr>
<td>7.</td>
<td>16.66</td>
<td>354990</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>41.66</td>
<td>975354.33</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>83.33</td>
<td>1888251.33</td>
<td>0.10</td>
</tr>
<tr>
<td>8.</td>
<td>16.66</td>
<td>1514109</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>41.66</td>
<td>4058056.33</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>83.33</td>
<td>7873578</td>
<td>0.35</td>
</tr>
<tr>
<td>9.</td>
<td>16.66</td>
<td>428799</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>41.66</td>
<td>1146641</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>83.33</td>
<td>2224018.66</td>
<td>0.46</td>
</tr>
<tr>
<td>10.</td>
<td>16.66</td>
<td>1561757</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>41.66</td>
<td>4144652.33</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>83.33</td>
<td>8002167</td>
<td>0.26</td>
</tr>
<tr>
<td>11.</td>
<td>16.66</td>
<td>1666501</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>41.66</td>
<td>4465353</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>83.33</td>
<td>8569092</td>
<td>0.04</td>
</tr>
<tr>
<td>12.</td>
<td>16.66</td>
<td>618911.5</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>41.66</td>
<td>1841182.66</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td>83.33</td>
<td>3281828</td>
<td>0.31</td>
</tr>
</tbody>
</table>
Recovery studies were carried to determine accuracy of the developed assay. The known amount of investigated compound was added to the accurately weighed portion of plant extract, processed and analyzed. The recovery percentage was calculated by the formula: Recovery (%) = \frac{\text{amount found} - \text{amount present}}{\text{amount spiked}} \times 100 \text{ as shown in (Table-3).}

**Table-3: Recovery percentage of marker compounds 1-12**

<table>
<thead>
<tr>
<th>Marker compounds</th>
<th>Amount present in the extract (ng)</th>
<th>Amount added (ng)</th>
<th>Amount found (ng)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.95</td>
<td>4.3</td>
<td>6.913</td>
<td>92.16%</td>
</tr>
<tr>
<td>2</td>
<td>2.70</td>
<td>4.3</td>
<td>6.897</td>
<td>95.74%</td>
</tr>
<tr>
<td>3</td>
<td>10.83</td>
<td>4.3</td>
<td>15.121</td>
<td>99.79%</td>
</tr>
<tr>
<td>4</td>
<td>2.03</td>
<td>4.3</td>
<td>6.039</td>
<td>92.06%</td>
</tr>
<tr>
<td>5</td>
<td>18.82</td>
<td>4.3</td>
<td>22.83</td>
<td>93.25%</td>
</tr>
<tr>
<td>6</td>
<td>81.79</td>
<td>4.3</td>
<td>85.76</td>
<td>92.32%</td>
</tr>
<tr>
<td>7</td>
<td>113.09</td>
<td>4.3</td>
<td>117.29</td>
<td>97.67%</td>
</tr>
<tr>
<td>8</td>
<td>3.53</td>
<td>4.3</td>
<td>7.449</td>
<td>91.13%</td>
</tr>
<tr>
<td>9</td>
<td>8.05</td>
<td>4.3</td>
<td>12.695</td>
<td>108.02%</td>
</tr>
<tr>
<td>10</td>
<td>4.93</td>
<td>4.3</td>
<td>9.325</td>
<td>102.20%</td>
</tr>
<tr>
<td>11</td>
<td>11.93</td>
<td>4.3</td>
<td>16.075</td>
<td>96.39%</td>
</tr>
<tr>
<td>12</td>
<td>18.233</td>
<td>4.3</td>
<td>22.821</td>
<td>106.76%</td>
</tr>
</tbody>
</table>

Stability of the compounds was examined by analyzing the standard solution at 0, 2, 4, 12, 24 and 48 hrs after storage at room temperature for two days. The samples were also analyzed after storage for 3 months at 4°C and even after a storage of 5 months at -20 °C, the results obtained indicated that the solutions were stable for a at least 5
months. To test the repeatability of the method, the RSD values of retention times and peak area were determined and found to be < 0.43% and 3.1% respectively.

1.17.6. Quantification

Once developed, the analytical method was used for the simultaneous quantitative determination of 12 marker compounds in the extract of *P. pabularia* (table-4). The typical HPLC chromatogram of marker compounds and the extract sample (DCM: Methanol (1:1)) are shown in figure-2 and 3 respectively.

**Table 4**: Content of marker compounds 1-12 (%w/w) in *Prangos pabularia* determined by HPLC

<table>
<thead>
<tr>
<th>Marker compounds</th>
<th>DCM : MeOH (1:1) extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-hydroxy-coumarin (1)</td>
<td>0.123± 0.001</td>
</tr>
<tr>
<td>Umbelliferone (2)</td>
<td>0.150± 0.001</td>
</tr>
<tr>
<td>Heraclenol glycoside(3)</td>
<td>0.460± 0.002</td>
</tr>
<tr>
<td>Xanthotoxol (4)</td>
<td>0.050±0.001</td>
</tr>
<tr>
<td>Heraclenol (5)</td>
<td>0.801±0.002</td>
</tr>
<tr>
<td>Oxypeucedanin hydrate (6)</td>
<td>0.368±0.003</td>
</tr>
<tr>
<td>8-((3,3-dimethyloxiran-2-yl)methyl)-7-methoxy-2H-chromen-2-one (7)</td>
<td>0.623±0.002</td>
</tr>
<tr>
<td>Oxypeucedanin hydrate monoacetate (8)</td>
<td>0.022±0.001</td>
</tr>
<tr>
<td>Xanthotoxin (9)</td>
<td>0.325±0.001</td>
</tr>
<tr>
<td>4-((2-hydroxy-3-methylbut-3-en-1-yl)oxy)-7H-furo[3,2-g]chromen-7-one (10)</td>
<td>0.101±0.001</td>
</tr>
<tr>
<td>Imperatorin (11)</td>
<td>0.455±0.002</td>
</tr>
<tr>
<td>Osthol (12)</td>
<td>0.680±0.003</td>
</tr>
</tbody>
</table>
Figure 2: Chromatogram of marker compounds 1-12

Figure 3: Chromatogram of DCM: Methanol (1:1) extract of *Prangos pabularia*
1.18. Conclusion

In present study, a simple and accurate method developed for the simultaneous
determination of twelve marker bioactive methanol soluble compounds, 6-hydroxocouramin (1), umbelliferone (2), heraclenol glycoside(3), xanthotoxal (4), heraclenol (5), oxypeucedanin hydrate (6), 8-((3,3-dimethyloxiran-2-yl)methyl)-7-methoxy-2h-chromen-2-one (7), oxypeucedanin hydrate monoacetate (8), xanthotoxin (9), 4-((2-hydroxy-3-methylbut-3-en-1-yl)oxy)-7h-furo[3,2-g]chromen-7-one (10), imperatorin (11), osthol (12) has been successfully resolved on single chromatogram from the root
of *P. pabularia* by RP-HPLC. Through quantification of the DCM: methanol extract, heraclenol 5 and osthol 12 are found as the major constituents. These molecules are of
importance because of their anticancer and antibacterial activities. Validation results
demonstrated that the developed method is sufficiently reliable and sensitive to
evaluate the quality of *P. pabularia*. This first RP-HPLC fingerprint developed for
this plant can be helpful for the rapid analysis of its phytomolecules in various
herbs/herbal formulation/plant products.
1.19. Chromatograph and calibration curve of twelve marker compounds
Chapter 1: Section B

RP-HPLC

Umbelliferone (2)

2 (DAD: Signal A, 250 nm/Bw:4 nm)
Average RF: 4.53745e-005 RF StDev: 4.21222e-006 RF %RSD: 9.28324
Scaling: None LSQ Weighting: None Force Through Zero: Off
Replicate Mode: Replace
Fit Type: Linear
y = 4.92386e-005x - 0.464667
Goodness of fit (r^2): 0.999803

Peak 2 - ESTD - DAD: Signal A, 250 nm/Bw:4 nm

Amount (ppm)

0 25 50 75

Area

0 500000 1.0e+06 1.5e+06
Heraclenol glycoside (3)

1 (DAD: Signal A, 250 nm/Bw:4 nm)
Average RF: 3.24914e-005    RF StDev: 9.33118e-007    RF %RSD: 2.87189
Scaling: None   LSQ Weighting: None   Force Through Zero: Off
Replicate Mode: Replace
Fit Type: Linear
y = 3.31618e-005x - 0.117756
Goodness of fit (r^2): 0.999996

Heraclenol glycoside (3)
Chapter 1: Section B

Xanthotoxol (4)

4 (DAD: Signal A, 250 nm/Bw: 4 nm)
Average RF: 5.97418e-06  RF StDev: 5.23523e-007  RF %RSD: 8.76308
Scaling: None  LSQ Weighting: None  Force Through Zero: Off
Replicate Mode: Replace
Fit Type: Linear
y = 6.32804e-006x - 0.237689
Goodness of fit (r^2): 0.999943

Peak: 4 – ESTD – DAD: Signal A, 250 nm/Bw: 4 nm

Amount (ppm)
0 25 50 75
0 2.5e+06 5.0e+06 7.5e+06 1.0e+07 1.3e+07

Area
Heraclenol (5)

5 (DAD: Signal A, 250 nm/Bw:4 nm)
Average RF: 9.90256e-006 RF StDev: 2.01995e-007 RF %RSD: 2.03982
Scaling: None LSQ Weighting: None Force Through Zero: Off
Replicate Mode: Replace
Fit Type: Linear
\[ y = 1.00886e-005x - 0.121333 \]
Goodness of fit (r^2): 0.999961

Peak 5 = ESTD - DAD: Signal A, 250 nm/Bw:4 nm
Oxypeucedanin hydrate (6)
8-((3,3-dimethyloxiran-2-yl)methyl)-7-methoxy-2H-chromen-2-one (7)

8-(3,3-dimethyloxiran-2-yl)methyl)-7-methoxy-2H-chromen-2-one (7)

7 (DAD: Signal A, 250 nm/Bw:4 nm)
Average RF: 4.66836e-005   RF StDev: 2.07068e-006   RF %RSD: 4.43556
Scaling: None   LSQ Weighting: None   Force Through Zero: Off
Replicate Mode: Replace
Fit Type: Linear
y = 4.62506e-005x - 0.174440
Goodness of fit (r^2): 0.999776
Oxypeucedanin hydrate monoacetate (8)

8 (DAD: Signal A, 250 nm/Bw:4 nm)
Average RF: 1.02565e-005   RF StDev: 1.72763e-006   RF %RSD: 16.8443
Scaling: None   LSQ Weighting: None   Force Through Zero: Off
Replicate Mode: Replace
Fit Type: Linear
\( y = 1.00862e-005x - 0.212514 \)
Goodness of fit (r^2): 0.999921

Oxypeucedanin hydrate monoacetate (8)
Chapter 1: Section B

RP-HPLC

Xanthotoxin (9)

9 (DAD: Signal A, 250 nm/Bw:4 nm)
Average RF: 3.95777e-005  RF StDev: 1.12967e-006  RF %RSD: 2.85431
Scaling: None  LSQ Weighting: None  Force Through Zero: Off
Replicate Mode: Replace  Fit Type: Linear
y = 3.95704e-005x - 0.0973815
Goodness of fit (r^2): 0.999931

Peak 9 – ESTD – DAD: Signal A, 250 nm/Bw:4 nm

Xanthotoxin (9)
4-((2-hydroxy-3-methylbut-3-en-1-yl)oxy)-7H-furo[3,2-g]chromen-7-one (10)
Chapter 1: Section B

RP-HPLC

Imperatorin (11) 18100

11 (DAD: Signal A, 250 nm/Bw:4 nm)
Average RF: 9.46914e-006  RF StDev: 1.45626e-006  RF %RSD: 15.3791
Scaling: None  LSQ Weighting: None  Force Through Zero: Off
Replicate Mode: Replace
Fit Type: Linear
y = 1.01645e-005x - 0.173190
Goodness of fit (r^2): 0.999889

Imperatorin (11)
12 (DAD: Signal A, 250 nm/Bw:4 nm)
Average RF: 2.52335e-005  RF StDev: 2.98383e-006  RF %RSD: 11.8249
Scaling: None  LSQ Weighting: None  Force Through Zero: Off
Replicate Mode: Replace
Fit Type: Linear
y = 2.61995e-005x - 0.0831525
Goodness of fit (r^2): 0.999214

Osthol (12)
Chapter 2

Studie on
Modification and Bio-Evaluation of Plant Molecules

2.1. Introduction

Natural products have been traditionally a rich source of drugs. Throughout history, many applications of natural products are seen in the field of medicine, pharmacy and biology. A number of important and new commercialized drugs have been obtained from the natural sources, and used as such or after structural modification of natural compounds, or through design of new compounds following a natural compound as model. There has been a remarkable revitalization of interest in natural product research over the last decade and is enjoying renewed attention for providing novel and interesting scaffolds. Outstanding developments have been stepped up in the area of separation science, spectroscopic techniques, and micro-plate based in vitro assays. The various hyphenated techniques, e.g., GC-MS, LC-PDA-MS, LC-MS-MS, LC-FTIR, LC-NMR, LC-NMR-MS, CE-MS, have made possible the pre-isolation analysis of crude extracts or fractions from different natural sources as well as isolation and on-line detection of natural products, chemotaxonomic studies, chemical finger printing, quality control of herbal products, de-replication of natural products and metabolomic studies.

Natural products (NP’s) continue to represent an excellent source for lead structures for drug discovery. Apart from the cases where NPs have been put to use directly as drugs, many of the drugs have come up as the structural analogues and or mimics of natural products. For a better drug or lead compound, the molecule should possess not only high potency but also contain other properties like being non-toxic and with efficient ADME (Absorption, Distribution, Metabolism, and Excretion). To address to these issues, structural modification of NPs has been undoubtedly playing critical role for the development of efficient modern medicine.

Structural modification of natural products has become an integral part of the drug discovery process. In modern methods of drug discovery processes, design and synthesis of drugs based on the biological targets is of the great interest to modern medicinal chemists. The huge structural diversity of natural compounds and their bioactive potential have meant that several products isolated from plants, and for that reason also from marine flora and microorganisms can serve as "lead" compounds for the improvement of their potential therapeutics affected by molecular modification. Additionally, semi-synthetic processes for new compounds, obtained by molecular modification of the functional groups of lead compounds, are able to generate structural analogues with greater pharmacological activity and with fewer side effects. The new structural analogues after chemical modification of a natural product give inputs to medicinal chemists to study their structure activity relationship (SAR). SAR is an important aspect of a drug to understand its medicinal properties and mechanism of action or pathway.

2.2. Objectives of Present Work

The biological studies of osthol and related molecules containing coumarin ring, carried out in last few years have provided an additional dimension to the bioactivity profile of the title compound osthol. The potential of osthol and other coumarins has not been fully exploited despite its biological importance; therefore, more efforts toward the building of diverse libraries around its chemical structure and their biological profile are in demand. Thus, based on the reported anti-cancer activity of osthol, we envisaged to carry out structural modifications for (i) improved anti-cancer and antimicrobial activity, (ii) establishment of structure activity relationship (SAR)
and (iii) understand the probable mechanism of action. Various studies towards achieving these milestones include:

1. Isolation of major constituents like osthol and other coumarins from *P. pabularia* in quantitative amounts to carry out different chemical transformations.

2. Synthesis of novel semi-synthetic analogs of the parent natural molecule for exploring its all probable reactive sites.

3. *In vitro* screening of all synthesised novel analogues on different human cancer cell lines.

4. *In vitro* screening of all synthesised analogs for antimicrobial activity.

5. Identification of lead molecules and detailed investigations related to their mechanism of action and structure activity relationship (SAR).

The first step towards the realisation of the objectives was to have the starting material osthol, and this natural product (NP) was isolated from Pet. ether extract of *Prangos pabularia* after column chromatographed on silica gel as a colourless crystalline solid, characterized by spectral analysis [details given in the experimental section].

### 2.3. Review of Literature

Since the drug discovery process is an intense, lengthy and interdisciplinary endeavor, this process has been revolutionized with the advent of genomics, proteomics, bioinformatics and efficient technologies like, combinatorial chemistry, high throughput screening (HTS), virtual screening, de novo design, in vitro, *in silico* ADMET (Absorption Distribution Metabolism Excretion Toxicity) screening and structure-based drug design. Computational tools offer the advantage of delivering new drug candidates more quickly and at a lower cost. Among these computational techniques, rational drug design with in silico approach has good application capabilities towards drug discovery. *In silico* methods can be used to analyze the target structures for possible binding/active sites, to generate candidate molecules, to check for their drug likeness, to dock these molecules to the target, to rank them according to their binding affinities, further, to optimize the molecules to improve
binding characteristics. Structural modification of a bioactive natural product for better lead molecule or candidate can be gratified with rational design of structural analogues using in silico docking methods.

Structural analogues of natural products can give inputs for the clear picture of molecular mechanistic to a medicinal chemist. Literature is full of examples wherein the modification of a natural product has led to a drug with optimized or minimized toxicities, better drug delivery or higher potency.\textsuperscript{8} Camptothecin 1 was isolated from the Chinese ornamental tree Camptotheca acuminata by Wani and Wall\textsuperscript{9} contemporaneously with the initial discovery of Taxol. As the sodium salt, camptothecin was advanced to clinical trials by NCI in the 1970s, but was dropped because of severe bladder toxicity. It was resurrected as a result of its very specific biochemical activity as an inhibitor of topoisomerase-I. The basic structure was slightly modified; leading to FDA approved anti-cancer agent irinotecan 2. And two others analogs viz 9-aminocamptothecin 3 and 9-nitrocamptothecin 4 are awaiting approval (Figure-1).

\textsuperscript{8} Liang, X-T.; Fang, W-S.(eds.) Medicinal Chemistry of Bioactive Natural Products. John Wiley & Sons, Inc. 2006.

Rhinacanthin-(M) 5 was initially used for the treatment of cancer; chemical modifications of rhinacanthin led to the development of molecules like 6 used for the treatment of malaria and were found nontoxic to normal Vero cells and show inhibition against *P. falciparum*. (Figure-1)

A study on piperine 7 analogues resulted in the identification of EPIs that led to significant increase in the potentiation of drug activity. The two most active EPIs reported, are SK-20 8 and SK-56 9 (Figure-2) exhibiting more potency than known EPIs such as reserpine 11 and verapamil 10.

---

Natural occurring epimer of Podophyllotoxin 12 which was isolated as the active anti-tumor agent from the roots of various species of the genus *Podophyllum*. Modification has led to the development of water-soluble derivative etoposide, NK-611 13, where a dimethylamino group was placed into the sugar ring, thus giving significant water solubility; the material is now in clinical trials.\textsuperscript{11}

Coumarins (and their derivatives) represent an important class of compounds reported to display numerous biological and pharmacological activities.\textsuperscript{12} For example (+)-Heraclenin, a naturally occurring furanocoumarin epoxide, is known to exhibit a

\textsuperscript{11} Wang, H.-K. *IDrugs*, 1998, 1, 92.
\textsuperscript{12} Hoult, J. R.; Payá, M. *Gen. Pharmacol.* 1996, 27, 713.
broad spectrum of biological activities, like cytotoxic,\textsuperscript{13} antiplatelet,\textsuperscript{14} anticoagulant,\textsuperscript{15} anti-inflammatory\textsuperscript{16,17} as well as mild phototoxic and photomutagenic activities.\textsuperscript{18} It has been shown to significantly induce apoptosis in Jurkat leukaemia cells.\textsuperscript{19} Spectrums of activities of several similar furanocoumarins and their glucosides have also been reported in literature.\textsuperscript{20}

The coumarin (benzopyran-2-one, or chromen-2-one) ring system, present in a large number of natural products (such as the anticoagulant Warfarin, \textsuperscript{14}) having interesting pharmacological properties,\textsuperscript{21,22} has intrigued chemists for decades to explore the natural coumarins or their synthetic derivatives for their applicability as drugs (Figure-3). Many intersecting molecules having coumarin based ring systems have been synthesized utilizing novel synthetic techniques. Some new derivatives bearing coumarin ring including the furanocoumarins (e.g., Imperatorin, \textsuperscript{15}), pyranocoumarins (e.g., Seselin, \textsuperscript{16}), and coumarin sulfamates (Coumates \textsuperscript{17}) have been found to be useful in photochemotherapy, antitumor and anti-HIV therapy and others.\textsuperscript{23,24} Among the diverse biological activities of coumarins, the notable one being their effect against breast cancer, and sulfatase and aromatase inhibitory activity.\textsuperscript{25}

\begin{thebibliography}{99}
\end{thebibliography}
Infectious diseases pose a great threat to human, animals and aqua-life. After the discovery of Penicillin in 1928 and subsequent discovery in this area coupled with the synthesis of a huge arsenal of new antibiotics, one expected control over bacterial infections and the eradication of human sufferings. Further, over a 0.1 million ton of antibiotics are manufactured commercially but irradiation/control infectious diseases hasn’t lessened. More and more strains of pathogens have become antibiotic resistance, some too many antibiotics and chemotherapeutic agents, and other pathogens to all of the commonly available anti-infectives. For example methicillin resistant *Staphylococcus aureus* (MRSA), is resistant not only to the drug methicillin but also to a host of other drugs such as aminoglycosides, macrolides, tetracyclines, chloromphenicol, lincosamides and disinfectants. MRSA can act as a major source of nosocomial infections and a community-acquired pathogen. MDR in bacteria may be generated by i) accumulation of multiple genes (occurs typically on resistance (R) plasmids), each coding for resistance to a single drug, within single cell, or by the increased expression of genes that code for multidrug efflux pumps (each of which can pump out more than one drug type). MDR efflux pumps (proteins) are said to be responsible for nearly 60% of global nosocomial infectious (WHO press release, WHO, Geneva 2000).

To solve the problem of drug resistance, development of novel class of antibacterials which offer a prolonged period of clinical effectiveness is seen one of the plausible approach or alternatively one can design syntheses of compounds which are capable to inhibit multidrug resistance proteins (efflux pumps) to combat drug resistance.

---

Efflux Pump Inhibitors (EPI)\textsuperscript{29} have been found to enhance the activity of several antibiotics identified as the substrate of these efflux pumps, by blocking their expulsion from the interior of bacterial cell.\textsuperscript{30} Efflux is an important mechanism of resistance in many clinically relevant pathogens, notably, \textit{Streptococcus pneumoniae}, \textit{Pseudomonas aeruginosa} and \textit{Neisseria gonorrhoeae}.\textsuperscript{31} \textit{Staphylococcus aureus} (\textit{S. aureus}) has shown ability to acquired resistance to several antibacterial drugs currently in the market and the pathogen is known to possess several efflux pumps, notably NorA,\textsuperscript{32} TetK,\textsuperscript{33} MsrA\textsuperscript{34} and MdeA which transports fluoroquinolones, tetracyclines, macrolides and mupirocin drugs respectively.

While simple Coumarin molecule exhibits low antibacterial activity, many of the substituted coumarin derivatives are found potent antibacterial activity e.g. ammoresinol 18 andostruthin 19 show activity against a wide spectrum of Gram +ve bacteria such as \textit{Bacillus megaterium}, \textit{Micrococcus luteus}, \textit{Micrococcus lysodeikticus}, and \textit{Staphylococcus aureus}. Anthogenol 20 (from \textit{Aegle marmelos})\textsuperscript{35} has shown activity against Enterococcus, Imperatorin 15, a furanocoumarin (from \textit{Angelica dahurica} and \textit{Angelica archangelica}, \textit{Umbelliferae}),\textsuperscript{36} has shown activity against \textit{Shigella dysenteriae}.\textsuperscript{37} Grandivittin 21, agassyllin 22, aegelinol benzoate 23 and osthole 24 (from the roots of \textit{Ferulago campestris}, \textit{Apiaceae}),\textsuperscript{38} Felamidin 25 (from \textit{Ferulago campestris}) and Aegelinol and agassyllin have shown significant antibacterial activity \textit{S.aureus} against clinically isolated Gram-positive and Gram-negative bacterial strains such as \textit{S.aureus}, \textit{Salmonella typhi}, \textit{Enterobacter cloacae}, and \textit{Enterobacter aerogenes}. Antibacterial activity was also found against \textit{Helicobacter pylori} where a dose dependent inhibition was shown between 5 and 25mg/mL. Many of the natural coumarins in existence have been isolated from higher plants; some of them have been discovered in microorganisms. The important

\begin{thebibliography}{9}
\bibitem{Lomovskaya} Lomovskaya, O.; Bostian, K. A. \textit{Biochemical pharmacology}, \textbf{2006}, 71, 910.
\bibitem{Basile} Basile, A.; Sorbo, S.; Spadaro V. \textit{Molecules}, \textbf{2009}, 14, 939.
\end{thebibliography}
coumarin members belonging to microbial sources are novobiocin, coumermycin, and chartreusin. Novobiocin 26 was isolated as fungal metabolite from *Streptomyces niveus*\(^39\) and *Streptomyces spheroides* and has exhibited broad spectrum antibacterial activity against Gram-positive organisms such as *Corinebacterium diphtheria*, *S.aureus*, *Streptomyces pneumoniae*, and *Streptomyces pyogenes* and Gram-negative organisms such as *Haemophilus influenzae*, *Neisseria meningitides*, and *Pasteurella*\(^40\) and has shown DNA gyrase inhibition activity.\(^41\) Coumermycin 27, that is, structurally similar to novobiocin is nearly 50 times more potent than novobiocin, against *Escherichia coli* and *S. aureus*, but it produces a bacteriostatic action, and the organism developed resistance gradually. Coumermycin also inhibits the super coiling of DNA catalyzed by *Escherichia coli* DNA gyrase.\(^31\)

[Diagram of coumarin members]

---

Chartreusin 28 (from *Streptomyces chartreusis*) having an uncommon structure is found predominantly active against Gram-positive bacteria, but due to its toxicity, the compound has not been tried for therapeutic application. Several of the natural products as well as synthetic compounds are reported for their efflux pump inhibitory activity. Most of them have inhibitory effect at high concentrations and possess several side effects. Therefore, it is important that EPIs may be developed which possess low MIC to be of clinical compatibility. With this objective in mind, we designed three different synthetic schemes and prepared three different series of compounds. These compounds were then studied in combination with three different drugs such as ciprofloxacin, tetracycline, erythromycin targeting the efflux pumps NorA, TetK, MsrA of *S. aureus* for their possible EPI activity. Structure--activity relationship studies, performed via the synthesis and anti-HIV activity evaluation of enantiomerically pure (+)-calanolide A 29 and its analogues, have contributed to the identification of the key structural features required for activity. 

Investigation of the ability of (+)-calanolide A and several other structurally-related pyranocoumarins revealed that (+)-calanolide A is active against a variety of *Mycobacterium tuberculosis* strains, including those resistant to the standard antitubercular drugs as shown in figure 4 and 5.

---

44 Xu, ZQ.; Barrow, WW.; Suling, WJ. *Bioorg. Med. Chem. 2004, 12*, 1199.
Cancer is the most common and fatal disease responsible for 2-3% of deaths recorded worldwide annually. About 60% of anticancer drugs used nowadays are obtained from natural sources. Cancer is presently the main cause of death in the world. The present treatment strategy includes alkylating agents, steroids, vinca alkaloids, antimetabolites, taxanes and antibiotics etc. High doses of these agents and intensive regiments are being employed aiming to cure malignancy, which result in severe side effects. Therefore, need of the hour is to develop new anticancer drugs which are safe and more effective.

As described earlier, medicinal plants can offer a promising source of novel chemotherapeutic agents including anticancer agents, vincristine and vinblastine from *Catharanthus roseus* being a good example to quote. In addition, synthesis of topotecan and irinotecan derived from campotothecin (*Camptotheca*) provided further evidence that plant derived compounds if not effective as a drug can be converted to an effective agent. The effectiveness of many anticancer drugs is limited by

---

development of drug resistance, which may be intrinsic or acquired during the course of treatment. In case of acquired drug resistance, tumors may become resistant to drug other than those initiating the resistance. Drug resistance plays a role in the initial treatment and is responsible for treatment failure in more than 90% of patients with metastatic disease.

In view of this, the chemical modification of the natural isolates is needed in order to achieve better therapeutic impact, i.e., lesser sensitivity and higher efficacy.

Coumarin based selective estrogen receptor modulators (SERMs) and coumarin estrogen conjugates have also been described as potential anti-breast cancer agents according some recently publications. Currently, tamoxifen is most commonly used adjuvant drug for estrogen receptor (ER)-positive breast cancer, which competes with estrogen and down regulates estrogenic actions in breast cancer, however, it is less effective in ER-negative breast cancer, and its safety is also controversial.

Therefore, there is a strong impetus to identify new anti-breast cancer agents with improved activity and lesser sensitivities. Osthol, 7-methoxy-8-(3-methyl-2-butenyl) coumarin is clinically ingested as an important component of medicinal plants and herbs in Tradition Chinese Medicine (TCM), and it exhibits many pharmacological and biological activities. The original study of osthol could be traced back to more than 100 years ago. It was first discovered from Peucedanum ostruthium and now found widely distributed in the plant kingdom. Cnidium monnieri (L.) Cusson, a plant reported to contain high percentage of osthol, has been used in China since several hundred years as an herbal medicine to treat male sexual dysfunction. It has been proved that osthol has some important therapeutic functions.

---

47 Kung, Sutherland, MS.; Lipps, SG.; Patnaik, N.; Gayo Fung, LM.; Khammungkune, S.; Xie, W.; Brady, HA.; Barbosa, MS.; Anderson, DW.; Stein, B. Cytokine, 2003, 23, 1.
51 Ng, T. B. Recent Prog. Med. Plants. 2006, 12, 129.
and safe profile in comparison to other natural products, which makes it a very promising lead compound in the area of drug discovery.

The studies on growth-inhibitory cytostatic activity in human cancer cell line: MCF-7 breast carcinoma cells reveals that osthol has some estrogenic activity by preventing the synthesis and action of estrogens (ER antagonists) indicating thereby that osthol has the potential to become a breast cancer treatment reagent.

2.3.1. Molecular mode of action of osthol

PI3Ks (class IA) primarily phosphorylate phosphatidylinositol-4, 5-bisphosphate (PIP2) on the plasma membrane to generate the second messenger, phosphatidylinositol-3,4,5-trisphosphate (PIP3). PI3Ks (class IA) are heterodimers that consist of a p85 regulatory and a p110 catalytic subunit. There are several isoforms of both the catalytic (p110α, p110β and p110δ) and regulatory (p50α, p55α, p85α, p85β and p55γ) subunits. PI3Ks (class IA) are most often activated by receptor tyrosine kinase (RTK) signalling, although the p110β-containing enzymes might also be activated by G protein-coupled receptors. The p85 regulatory subunit is crucial in mediating PI3Ks (class IA) activation by RTKs. The Src-homology 2 (SH2) domains of p85 bind to phosphotyrosine residues in the sequence context pYxxM (in which a ‘pY’ indicates a phosphorylated tyrosine) on activated RTKs, as in the case of platelet-derived growth factor receptors, or on adaptor molecules, such as ERBB3 or GRB2-associated binding protein 1. This binding of SH2 domain serves both to recruit the p85–p110 heterodimer to the plasma membrane, where its substrate PIP2 resides, and to relieve basal inhibition of p110 by p85. The 3'-phosphatase PTEN ephosphorylates PIP3 and therefore terminates PI3K signalling. Accumulation of PIP3 on the cell membrane leads to the colocalization of signalling proteins with pleckstrin homology (PH) domains. This leads to the activation of these proteins and propagation of downstream PI3K signalling. Akt and phosphoinositide-dependent protein kinase 1 (PDK1) directly bind to PIP3 and are thereby recruited to the plasma

membrane. The phosphorylation of Akt at T308 (which is in the activation loop of Akt) by PDK1 and at S473 (which is in a hydrophobic motif of Akt) by mTOR complex 2 (mTORC2) results in full activation of this protein kinase. In turn, Akt phosphorylates several cellular proteins, including glycogen synthase kinase 3α (GSK3α), GSK3β, forkhead box O transcription factors (FoxO), MDM2, BCL2-interacting mediator of cell death (BIM) and BCL2-associated agonist of cell death (BAD) to facilitate cell survival and cell cycle entry. In addition, Akt phosphorylates and inactivates tuberous sclerosis 2 (TSC2), a GTPase-activating protein for Ras homologue enriched in brain (RHEB).

Inactivation of TSC2 allows RHEB to accumulate in the GTP-bound state and thereby activate mTORC1. The PI3K pathway through Akt regulates the use and uptake of glucose. The therapeutic effects of perturbing cancer cell metabolism with PI3K pathway inhibitors remain largely unknown. Points of therapeutic inhibition are highlighted in the figure-6.

![Figure 6](image)

**Figure 6**

PI3K signalling: arrows indicate activating phosphorylation events, whereas perpendicular lines indicate inhibitory events.

Components in the signalling pathway that are mutated in cancers are shown in blue. AKTS1, AKT1 substrate 1(also known as PRAS40); raptor, regulatory-associated protein of mTOR; rictor, rapamycin-insensitive companion of mTOR; SIN, stress-

---

activated MAPK-interacting. Osthol can preferentially inhibit HER2-overexpressing cancer cells, elevated FASN expression which is regarded as a poor prognostic marker in breast cancer cells.\(^{60}\) It can suppress FASN expression and modulate Akt and mTOR phosphorylation in HER2-overexpressing cancer cells. These results highlight the potential of use osthol as a unique chemotherapeutic modulator in the prevention or treatment of HER2-overexpressing cancer as shown in figure-6.

However, osthol exhibits comparatively weak activity, low water solubility and limited permeability, and these properties may lower its absorption upon oral administration and bioavailability, which need improvement for better drug-likeness.\(^{46}\) According to literature analysis,\(^{61}\) 3- and 4-position of osthol might play an important role in proliferation activity. Therefore, introduction of some substituents at these positions may improve its anti-cancer activity e.g bromination and Vilsmeier–Haack reaction, to introduce bromo or a formyl group at its 3- or 4-position. However, due to the existence of double bond (reference: isopentenyl side chain), these efforts failed finally; and neither 3-position substituted nor 4-position substituted products could be successfully obtained. It was believed that the modification at 3- or 4-position could be realized after the hydrogenation of isopentenyl side chain and Platinum oxide could catalyze the hydrogenation to afford reduced osthol derivative 30 in good yield (\(>80\%\)). Bromination of the hydrogenated product 30 could be carried out under microwave irradiations selectively at 3-position to yield the important intermediate 31, which could be employed to couple with various aryl boric acid to prepare a series of novel osthol derivatives 32. After demethylation and nucleophilic substitution, a new class of osthol derivatives 33 and 34 were obtained too. The anti-cancer activity tests in vitro of the above osthol analogs were carried on human breast cancer MCF-7 and MDA-MB-231 cell lines. The structure and activity relationship studies showed the methoxy at 7-position is critical for maintaining the antitumor activity, and the modification at 3-position and the hydrogenation on the

---


double bond of isoamylene could obviously improve its antitumor activity. (Scheme 1\textsuperscript{62} and figure-7).

![Scheme 1](image)

**Reagents and conditions:** (a) PtO\textsubscript{2}, H\textsubscript{2}, 24 h, 80%; (b) NBS, NaOAc, CH\textsubscript{3}CN, MW, 2h, 60%; (c) Pd(PPh\textsubscript{3})\textsubscript{4}, phenylboronic acid, K\textsubscript{3}PO\textsubscript{4}, dioxane, 60-90%; (d) BBr\textsubscript{3}, DCM, -78°C, 40-70%; (e) bromide, DMF, NaH, 60-85%.

![Figure 7](image)

In literature, selection of osthol for its development as a hepatoprotectant and as an antipruritic agent is reported. Studies with hepatitis model mice has shown that osthol has a potential of preventing hepatitis by inhibiting the development of apoptosis, indicating the possibility of osthol to become a hepatoprotective drug candidate for various liver diseases. Osthol has been found to be a promising agent for the treatment of osteoporosis and to demonstrate the reproductive system improvement properties by activation of the central cholinergic neuronal system.

### 2.3.2. Total synthesis of osthol

While compound 41 has been obtained by heating the solution of 2, 4-dihydroxybenzaldehyde 40 and ClCH$_2$COOC$_2$H$_5$ together with Ph$_3$-CH$_2$COOC$_2$H$_5$ in ethanol, compounds 42, 43, 50 and 51 are obtained by heating the solution of 7-hydroxycoumarin and its corresponding Alkyl halide together with K$_2$CO$_3$/KI in acetone. 8-Allyl-7-hydroxycoumarin the key intermediate is easily obtained from 7-hydroxycoumarin in two steps. Treatment of 7-hydroxycoumarin 41 with 3-chloropropene in acetone in the presence of K$_2$CO$_3$/KI afforded compound 43 (90.6% yield).

---

yield), which on heating with ethylene glycol in sealed tube at 60 \( ^\circ \) C afford mainly the C-8 Claisen rearrangement product 44 together with its C-6 isomer 45 (in 4:1 ratio). Compounds 46-48 have been synthesized according to a general procedure using compound 44 and its corresponding alkyl halide together with \( \text{K}_2\text{CO}_3 \) in acetone. Methylation of compound 44 with dimethylsulfate in acetone resulted in compound 49. Following alkene metathesis procedure, and using compound 49 and 3-chloro-3-methyl-1-butylene together with Grubbs 2nd catalyst in CH\(_2\)Cl\(_2\) afforded Osthol (24) (Scheme-2).\(^{75}\)

**Scheme 2**

**Reagents and conditions:** (a) ClCH₂COOC₂H₅, Ph₃-P, CH₂COOC₂H₅, ethanol, 80 °C, 2 h, 72%. (b) 3-chloro-3-methyl-1-butylene, K₂CO₃, KI, acetone, 60 °C, 24 h, 83.6%; (c) 3-chloropropene, K₂CO₃, KI, acetone, 60 °C, 22 h, 90.6%; (d) Ethylene glycol, reflux, 6 h; (e-46), i. 3-chloropropene, K₂CO₃, KI, acetone, 60 °C, 18 h, 98.3%; (e-47) 3-chloro-2-methyl-1-propene, K₂CO₃, KI, acetone, 60 °C, 20 h, 85%; (e-48) 3-chloro-3-methyl-1-butylene, K₂CO₃, KI, acetone, 60 °C, 22 h, 60.2%; (e-49) (CH₃O)₂SO₂, K₂CO₃, KI, acetone, r.t, 5 h, 94.6% (f) (CH₃O)₂SO₂, K₂CO₃, KI, acetone, r.t, 5 h, 90.2%; (g) 3-chloro-2-methyl-1-propene, K₂CO₃, KI, acetone, 60 °C, 24 h, 87.6%; (h) 3-chloro-3-methyl-1-butylene, Grubbs 2nd catalyst, CH₂Cl₂, 45 °C, 2 h, 77.6%.
The 90 kDa heat shock protein (Hsp90) is an ATP dependent chaperone that is responsible for the folding, activation, and stabilization of more than 200 client proteins, of which approximately 50 are kinases, hormone receptors, and/or transcription factors directly associated with signaling pathways that regulate cell growth. Hsp90 exists as a homodimer in normal cells and acts as a house-keeping chaperone to maintain protein homeostasis. However, in malignant cells, Hsp90 forms a superchaperone complex that binds to cochaperones and immunophilins to modulate the function of proteins associated with all six hallmarks of cancer. These oncogenic Hsp90-dependent clients include Raf-1, Akt, CDK4, Src, hTERT, and c-Met, all of which have developed an addiction to Hsp90 for their activity in transformed cells. Not surprisingly, inhibition of the Hsp90 protein folding machinery results in simultaneous disruption of these signaling cascades and induces client protein degradation through the ubiquitin-proteasome pathway, which can eventually lead to cell death.

Design, synthesis, and evaluation of a library of 3-arylcoumarin derivatives against two breast cancer cell lines, and the initial structure–activity relationships for the phenyl appendage has been investigated, and 3-Arylcoumarin derivatives identified as novel inhibitors of the Hsp90 protein folding machinery. Compound 59a exhibited lead like activity, and western blot analysis of this compound supports binding to the Hsp90 C-terminus, as no increase in Hsp90 levels was observed. Compound 41 was first methylated with iodomethane in the presence of sodium hydride in DMF to afford compound 52, which underwent bromination with N-bromosuccinimide in the presence of catalytic sodium acetate. Subsequent demethylation of 53 with tribromoborane, followed by Mitsunobu coupling with N-methyl-4-hydroxypiperidine, generated intermediate 55. The final products were then produced by Suzuki coupling of 55 with a series of phenylboronic acids or fused hetero-

arylbronic acids to give a library of 3-arylcoumarin derivatives, 57 and 59 a, b (Scheme 2).  

\[
\begin{align*}
\text{Scheme 3}
\end{align*}
\]

Attempts made in the field of drug discovery to form a new series of compounds from osthol exist in literature. The main objective behind this invention is to provide a trans-cinnamic acid derivative by basic hydrolyses of osthol, in order to provide an anti-tumor compounds having a cytotoxic activity; where in this compound is a new trans-cinnamic acid derivatives and its pharmaceutical salts. The compound 60 and its pharmaceutical acceptable salts have activity of selectively inhibiting tumour cells and lower toxicity and can be used for preparing anti tumor drugs. The pharmaceutical acceptable salts are inorganic salt such as potassium, sodium and magnesium and organic salts such as tromethamine, diethanolamine, ammonium salt etc. (Scheme-4)  

---

Other types of compounds from osthol are also obtained: as angiogenesis inhibitors for the treatment of tumor and angioretina diseases\textsuperscript{85} (given in figure-8). Vascular endothelial cell growth inhibiting experiments showed many of these compounds (figure-8) have significant EVC-304 cell multiplication inhibitory activity being 1.4 to 4.0 times more potent than ombretastatin A-4 (CA4). The MTT results showed compounds 64 and 65 are the most active compounds having IC50 values (µg/ml) of 6.46 and 7.63 respectively.

2.4. Results and Discussion

At the very outset, two susceptible sites (I, II) of osthol were identified for the structural modification as shown in Figure-9. The idea behind the selection was to study the contribution of different functionalities towards anticancer activity, which would help in the devilment of more potent less toxic anti-cancer compounds and also possibly help in the establishment of the structure activity relationship (SAR). Thus, various chemical reactions were performed to get structurally different analogues encompassing modification at all the sites (I, II). Firstly chemical modifications carried out in the isoprenyl side chain (site-II). The modifications included:

(i) Nitration, (ii) epoxidation, (iii) oxidation of the isopropylidene ring to generate formyl functionality, followed by Wittig reaction of the formyl derivative and its subsequent hydrolysis to generate $\alpha, \beta$-unsaturated ester, (iv) reduction of the double bond, and (v) Mercuration-demercuration reaction.

After the modification at site-II, site I was taken up for modification and in this direction, ring opening of the coumarin was done following our earlier reported methodology to (i) generate O-protected cinnamic acids and (ii) generated a new library of amide molecules of these cinnamic acids by treatment via their acid chlorides with different alkyl, aryl and arylalkyl amines (Scheme-5 and Scheme-6).
Chapter 2                                                  Studies on modification and bio-evaluation of plant molecules

Scheme 5

Reagents and conditions: (a) SeO\textsubscript{2}/AcOH, (b) HNO\textsubscript{3}/Urea/AcOH, Ac\textsubscript{2}O, -10\textdegree C, (c) CH\textsubscript{2}Cl\textsubscript{2}, mCPBA, 0\textdegree C (d) aq. NaOH/DMSO, R\textsuperscript{1}I, room temp. (e) Hg (OAc)\textsubscript{2}/THF:H\textsubscript{2}O (1:1), NaBH\textsubscript{4}, room temp. (f) (C\textsubscript{2}H\textsubscript{5})\textsubscript{2}P(O)CH\textsubscript{2}COOC\textsubscript{2}H\textsubscript{5}/ NaH/ dry ether, N\textsubscript{2}-atm., 0\textdegree C, stir.

Scheme 6

Reagents and conditions: a. SOCl\textsubscript{2}/DCM, reflux, Amines.
2.5. Anti-proliferative Activity

The synthesized compounds were evaluated for their anticancer activity (in vitro study) against human cancer cell lines at four different micro-molar concentrations (7.5 µM, 15 µM, 30 µM, and 60 µM). The cell lines used in this investigation included: colon (Colo-205), lung (A549), leukemia (THP1) and breast (MCF-7). The cells were treated with compounds dissolved in DMSO at different concentrations and kept in serum media for 48 h to observe anti-proliferative activity using 3-{(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide} (MTT) assay. The results revealed efficient anti-proliferative activities for many of the compounds and inhibition of cell growth with IC$_{50}$ values in the lower micro-molar range as compared to osthol were observed. Analog 84 was found to be active against most of the cancer cell lines used (Colo-205, A549 and MCF-7) with better IC$_{50}$ values (29-30µM) than osthol (IC$_{50}$ value of >60µM for Colo-205 and MCF-7, for 46.2 µM for A549 (Table-1). Analogs 81, 85, 86, and 92 were found more potent for Colo-205 cell line (IC$_{50}$ values 24-53µM) than osthol (IC50 value of >60µM). The similar trend was observed in case of analogs 71, 79, 87, 90 and 91 against MCF-7 cell line (IC$_{50}$ values 21-48µM, osthol displayed IC$_{50}$ value of >60µM). It is noteworthy here that analog 79 emerged as the most potent molecule against MCF-7 cell line (IC50 7µM, osthol IC50 value of >60µM). Only analog 92 was found more active against leukemia cell line THP-1(IC50 5µM, osthol IC$_{50}$ 6.2µM). Also two analogs (88 and 71) were found active against A549 with IC$_{50}$ value range of 19-24µM, osthol displayed IC$_{50}$ value of 46.2µM. The percentage growth inhibition at all the tested doses against various cell lines along with IC50 values is given in Table-1.
Table 1: Percentage Growth Inhibition at 7.5 µM, 15 µM, 30 µM, 60 µM and IC$_{50}$ values of the Osthol and its analogs against Colo-205, A549, THP-1 and MCF-7 cell lines with MTT assay. BEZ-235 (0.01 µM), Paclitaxel (1 µM) and Adriamycin (1 µM) were used as positive controls.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>60</td>
<td>27</td>
<td>79</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>23</td>
<td></td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>22</td>
<td></td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>21</td>
<td></td>
<td>7.5</td>
<td>1</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>&gt;60µM</td>
<td>46.2µM</td>
<td>IC$_{50}$</td>
<td>&gt;60µM</td>
<td>6.2µM</td>
</tr>
<tr>
<td>66</td>
<td>60</td>
<td>36</td>
<td>80</td>
<td>60</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>37</td>
<td></td>
<td>30</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>23</td>
<td></td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>24</td>
<td></td>
<td>7.5</td>
<td>11</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>&gt;60µM</td>
<td>&gt;60µM</td>
<td>IC$_{50}$</td>
<td>&gt;60µM</td>
<td>6.3µM</td>
</tr>
<tr>
<td>67</td>
<td>60</td>
<td>34</td>
<td>81</td>
<td>60</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>48</td>
<td></td>
<td>30</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>27</td>
<td></td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>25</td>
<td></td>
<td>7.5</td>
<td>17</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>&gt;60µM</td>
<td>&gt;60µM</td>
<td>IC$_{50}$</td>
<td>&gt;60µM</td>
<td>6.3µM</td>
</tr>
<tr>
<td>68</td>
<td>60</td>
<td>32</td>
<td>82</td>
<td>60</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>39</td>
<td></td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>27</td>
<td></td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>25</td>
<td></td>
<td>7.5</td>
<td>8</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>&gt;60µM</td>
<td>&gt;60µM</td>
<td>IC$_{50}$</td>
<td>&gt;60µM</td>
<td>&gt;60µM</td>
</tr>
<tr>
<td>69</td>
<td>60</td>
<td>32</td>
<td>83</td>
<td>60</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>39</td>
<td></td>
<td>30</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>25</td>
<td></td>
<td>15</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>12</td>
<td></td>
<td>7.5</td>
<td>15</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>&gt;60µM</td>
<td>60µM</td>
<td>IC$_{50}$</td>
<td>&gt;60µM</td>
<td>&gt;60µM</td>
</tr>
<tr>
<td>70</td>
<td>60</td>
<td>34</td>
<td>84</td>
<td>60</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>31</td>
<td></td>
<td>30</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>22</td>
<td></td>
<td>15</td>
<td>10</td>
</tr>
</tbody>
</table>

Spanning the entire table.
Chapter 2  
Studies on modification and bio-evaluation of plant molecules

<table>
<thead>
<tr>
<th>7.5</th>
<th>12</th>
<th>11</th>
<th>15</th>
<th>19</th>
<th>7.5</th>
<th>5</th>
<th>24</th>
<th>0</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>52μM</td>
<td>&gt;60μM</td>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>29.1μM</td>
<td>29.2μM</td>
<td>51.3μM</td>
<td>30μM</td>
</tr>
<tr>
<td><strong>71</strong></td>
<td>60</td>
<td>35</td>
<td>74</td>
<td>85</td>
<td>60</td>
<td><strong>85</strong></td>
<td>60</td>
<td>71</td>
<td>24</td>
</tr>
<tr>
<td>30</td>
<td>28</td>
<td>59</td>
<td>71</td>
<td>57</td>
<td>30</td>
<td>52</td>
<td>11</td>
<td>24</td>
<td>33</td>
</tr>
<tr>
<td>15</td>
<td>25</td>
<td>38</td>
<td>66</td>
<td>40</td>
<td>15</td>
<td>46</td>
<td>3</td>
<td>11</td>
<td>30</td>
</tr>
<tr>
<td>7.5</td>
<td>22</td>
<td>34</td>
<td>56</td>
<td>37</td>
<td>7.5</td>
<td>41</td>
<td>2</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>24.5μM</td>
<td>7.2μM</td>
<td>23.4μM</td>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>25.5μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td></td>
</tr>
<tr>
<td><strong>72</strong></td>
<td>60</td>
<td>22</td>
<td>52</td>
<td>27</td>
<td>13</td>
<td><strong>86</strong></td>
<td>60</td>
<td>100</td>
<td>27</td>
</tr>
<tr>
<td>30</td>
<td>20</td>
<td>32</td>
<td>17</td>
<td>11</td>
<td>30</td>
<td>42</td>
<td>17</td>
<td>36</td>
<td>5</td>
</tr>
<tr>
<td>15</td>
<td>18</td>
<td>21</td>
<td>12</td>
<td>8</td>
<td>15</td>
<td>7</td>
<td>26</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>7.5</td>
<td>17</td>
<td>13</td>
<td>8</td>
<td>2</td>
<td>7.5</td>
<td>2</td>
<td>14</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>34.2μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
</tr>
<tr>
<td><strong>73</strong></td>
<td>60</td>
<td>21</td>
<td>41</td>
<td>22</td>
<td>70</td>
<td><strong>87</strong></td>
<td>60</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>30</td>
<td>18</td>
<td>32</td>
<td>2</td>
<td>60</td>
<td>30</td>
<td>15</td>
<td>9</td>
<td>21</td>
<td>63</td>
</tr>
<tr>
<td>15</td>
<td>16</td>
<td>30</td>
<td>1</td>
<td>46</td>
<td>15</td>
<td>7</td>
<td>6</td>
<td>19</td>
<td>41</td>
</tr>
<tr>
<td>7.5</td>
<td>16</td>
<td>27</td>
<td>1</td>
<td>26</td>
<td>7.5</td>
<td>5</td>
<td>3</td>
<td>14</td>
<td>34</td>
</tr>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>19μM</td>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
</tr>
<tr>
<td><strong>74</strong></td>
<td>60</td>
<td>3</td>
<td>27</td>
<td>52</td>
<td>70</td>
<td><strong>88</strong></td>
<td>60</td>
<td>24</td>
<td>68</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
<td>26</td>
<td>32</td>
<td>65</td>
<td>30</td>
<td>15</td>
<td>56</td>
<td>61</td>
<td>44</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>18</td>
<td>24</td>
<td>62</td>
<td>15</td>
<td>12</td>
<td>48</td>
<td>15</td>
<td>39</td>
</tr>
<tr>
<td>7.5</td>
<td>1</td>
<td>12</td>
<td>21</td>
<td>50</td>
<td>7.5</td>
<td>11</td>
<td>34</td>
<td>8</td>
<td>31</td>
</tr>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>15μM</td>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
</tr>
<tr>
<td><strong>75</strong></td>
<td>60</td>
<td>12</td>
<td>41</td>
<td>18</td>
<td>72</td>
<td><strong>89</strong></td>
<td>60</td>
<td>44</td>
<td>42</td>
</tr>
<tr>
<td>30</td>
<td>4</td>
<td>21</td>
<td>9</td>
<td>61</td>
<td>30</td>
<td>36</td>
<td>33</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>18</td>
<td>4</td>
<td>51</td>
<td>15</td>
<td>15</td>
<td>17</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>7.5</td>
<td>1</td>
<td>12</td>
<td>2</td>
<td>31</td>
<td>7.5</td>
<td>10</td>
<td>7</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>15μM</td>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
</tr>
<tr>
<td><strong>76</strong></td>
<td>60</td>
<td>34</td>
<td>29</td>
<td>32</td>
<td>45</td>
<td><strong>90</strong></td>
<td>60</td>
<td>24</td>
<td>38</td>
</tr>
<tr>
<td>30</td>
<td>13</td>
<td>24</td>
<td>16</td>
<td>41</td>
<td>30</td>
<td>15</td>
<td>29</td>
<td>17</td>
<td>44</td>
</tr>
<tr>
<td>15</td>
<td>13</td>
<td>21</td>
<td>6</td>
<td>39</td>
<td>15</td>
<td>11</td>
<td>25</td>
<td>11</td>
<td>38</td>
</tr>
<tr>
<td>7.5</td>
<td>11</td>
<td>12</td>
<td>3</td>
<td>29</td>
<td>7.5</td>
<td>9</td>
<td>13</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
</tr>
<tr>
<td><strong>77</strong></td>
<td>60</td>
<td>66</td>
<td>38</td>
<td>100</td>
<td>47</td>
<td><strong>91</strong></td>
<td>60</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>30</td>
<td>26</td>
<td>23</td>
<td>59</td>
<td>45</td>
<td>30</td>
<td>12</td>
<td>7</td>
<td>9</td>
<td>59</td>
</tr>
<tr>
<td>15</td>
<td>16</td>
<td>22</td>
<td>53</td>
<td>42</td>
<td>15</td>
<td>10</td>
<td>5</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>7.5</td>
<td>12</td>
<td>13</td>
<td>27</td>
<td>26</td>
<td>7.5</td>
<td>9</td>
<td>4</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>48μM</td>
<td>&gt;60μM</td>
<td>10.4μM</td>
<td>&gt;60μM</td>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>24μM</td>
</tr>
</tbody>
</table>
2.6. Effect of 79, 81 and 92 on Mitochondrial Membrane Potential Loss (ΔΨm) and their Cell Cycle Analysis

Mitochondrion, referred to as power house of the cell is responsible for generating cellular energy (ATP) and reducing power (NADH). Mitochondrial damage is a precursor of cell death. The induction of mitochondrial membrane potential loss (ΔΨm) by a molecule denotes its ability to induce apoptosis by intrinsic apoptotic pathway.

Molecule 92 found cytotoxic to colo-205 induced significant mitochondrial membrane potential loss (ΔΨm). (ΔΨm) is highly significant at lower concentration (1µm) and with the increase in concentration, (ΔΨm) became less pronounced. The probable reasons could be activation of extrinsic apoptotic pathway, toxicity of molecule etc.

Molecules 79 and 81 induce significant ΔΨm in respective cell lines MCF-7 and THP-1, on which they were reported to be cytotoxic. ΔΨm increases with increase in concentration in both the cases.

PI staining of cells gave us distribution of cells in various phases of cell cycle. The three cytotoxic molecules were tried for possible cell cycle arrest on their respective cell lines. However the population of apoptotic cell increases with concentration of test molecule in all the three cell lines. It could be therefore inferred that the molecules under study lead to apoptosis.
Figure 10: Effect of 79 on mitochondrial membrane potential loss ($\Delta \Psi_m$). 79 induced loss of mitochondrial membrane potential ($\Delta \Psi_m$) in breast cancer cell line (MCF-7) incubated with the compound at different concentrations (0, 1, 2, 4 and 8 μM) in 6 well plate for 48 h treatment. P2 is the percentage of loss of mitochondrial membrane potential ($\Delta \Psi_m$), which increases from 7.1% in case of negative control to 38.6% in case of 8 μM of 79. Paclitaxel (1 μM) was used as positive control. P1 and P2 represents percentage of intact and loss mitochondrial membrane potential ($\Delta \Psi_m$) respectively.

Figure 11: Effect of 81 on mitochondrial membrane potential loss ($\Delta \Psi_m$). 81 induced loss of mitochondrial membrane potential ($\Delta \Psi_m$) in colon cancer cell line (Colo-205) incubated with the compound at different concentrations (0, 1, 5, 10 and 25 μM) in 6 well plate for 48 h treatment. Figures show the representative of one of two similar experiments. P4 is the percentage of loss of mitochondrial membrane potential ($\Delta \Psi_m$), which increases from 12.4% in case of negative control to 29.9% in case of 25μM of 81. Camptothecin (1 μM) was used as positive control. P3 and P4 represents percentage of intact and loss mitochondrial membrane potential ($\Delta \Psi_m$) respectively.
Figure 12: Effect of 92 on mitochondrial membrane potential loss ($\Delta \Psi_m$). 92 induced loss of mitochondrial membrane potential ($\Delta \Psi_m$) in leukemia cell line (THP-1) incubated with the compound at different concentrations (0, 1, 2, 4 and 8 µM) in 6 well plate for 48 h treatment. P4 is the percentage of loss of mitochondrial membrane potential ($\Delta \Psi_m$), which increases from 13.3% in case of negative control to 30.5% in case of 8µM of 92. Camptothecin (1 µM) was used as positive control. P3 and P4 represents percentage of intact and loss mitochondrial membrane potential ($\Delta \Psi_m$) respectively.

Figure 13: Effect of 79 on cell cycle phase distribution of breast cancer cell line (MCF-7). Flow cytometric analysis of MCF-7 cells after propidium iodide staining. Cells were incubated for 48 h in presence of 79 at (0, 1, 2, 4 and 8 µM) concentration. Figures show the representative staining profile of one of two similar experiments. P1 (Sub-G1) is the population of apoptotic cells, which increases from 9.8% in case of negative control to 18.8% in case of 8µM of 79. The compound also induced G1 phase cell cycle arrest increasing from 43.0% and 54.4% compared to negative control of 48.1% was found. Paclitaxel (1 µM) was used as positive control. P1, P2, P3 and P4 represents Sub-G1, G1, S and G2 phases of cell cycle in the figure respectively.
Figure 14: Effect of 81 on cell cycle phase distribution of colon cancer cell line (Colo-205). Flow cytometric analysis of Colo-205 cells after propidium iodide staining. Cells were incubated for 48 h in presence of 81 at (0, 1, 5, 10 and 25 µM) concentration. Figures show the representative staining profile of one of two similar experiments. P1 (Sub-G1) is the population of apoptotic cells, which increases from 12.0% in case of negative control to 38.5% in case of 25 µM of 81. At lower concentrations of 1 and 5 µM G1 phase cell cycle arrest of 46.4% and 46.8% respectively as compared to negative control of 45.7% was found. Camptothecin (1 µM) was used as positive control. P1, P2, P3 and P4 represents Sub-G1, G1, S and G2 phases of cell cycle in the figure respectively.

Figure 15: Effect of 92 on cell cycle phase distribution of leukemic cell line (THP-1). Flow cytometric analysis of THP-1 cells after propidium iodide staining. Cells were incubated for 48 h in presence of 92 at (0, 1, 2, 4 and 8 µM) concentration. Figures show the representative staining profile of one of two similar experiments. P2 (Sub-G1) is the population of apoptotic cells, which increases from 13.8% in case of negative control to 26.9% in case of 8 µM of 92. Camptothecin (1 µM) was used as positive control. P2, P3, P4 and P5 represents Sub-G1, G1, S and G2 phases of cell cycle in the figure respectively.
Further modification at site I was carried out to prepare the triazole derivatives of osthol.

![Diagram showing the reaction process](image)

**Scheme 7**

**Reagents and conditions:** a: NaOH/DMSO, propargyl bromide, room temp. (b) RN₃, t-butanol: H₂O (1:1), CuSO₄, Sod. Ascorbate, rt.

In this direction, ring opening of lactone ring was carried out to get O-propargyl derivative which was then treated with azides in presence of ascorbic acid to generate a library of seventeen triazole derivatives (scheme-7). It may be appropriate to mention here that triazole compounds are reported to be associated with various
biological properties including anti cancer,\textsuperscript{86} immnosuppressant\textsuperscript{87} and as well as our recent findings of triazoles molecules as potent anticancer\textsuperscript{88} and immunosuppressant\textsuperscript{89} agents. The triazoles (93-109) were screened for their anticancer activity against various human cancer cell lines (colon, lung, breast, prostate, and skin) at 100 and 30 µmol concentrations. Of all the test molecules, only 93, 97, 101, and 104 were able to display anticancer activity at lower dose, with best activity in terms of %age inhibition and sensitivity of the number of cell lines observed for compound 104. The results of the above study are summarized in table-2.


Table 2: Percentage Growth Inhibition at 30 µM and 100 µM of triazoles derived from osthol against Colo-205, T47D, NCI-H322, A-549, PC-3, A-431 and Hct-116 cell lines using MTT assay.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>93</td>
<td>30</td>
<td>2</td>
<td>5</td>
<td>14</td>
<td>63</td>
<td>-</td>
<td>-</td>
<td>57</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>16</td>
<td>4</td>
<td>69</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>94</td>
<td>30</td>
<td>6</td>
<td>9</td>
<td>43</td>
<td>34</td>
<td>7</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>56</td>
<td>16</td>
<td>75</td>
<td>61</td>
<td>66</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>30</td>
<td>0</td>
<td>23</td>
<td>44</td>
<td>0</td>
<td>11</td>
<td>21</td>
<td>45</td>
</tr>
<tr>
<td>100</td>
<td>57</td>
<td>42</td>
<td>2</td>
<td>56</td>
<td>59</td>
<td>68</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>30</td>
<td>0</td>
<td>20</td>
<td>29</td>
<td>14</td>
<td>0</td>
<td>36</td>
<td>21</td>
</tr>
<tr>
<td>100</td>
<td>48</td>
<td>13</td>
<td>6</td>
<td>52</td>
<td>0</td>
<td>54</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>97</td>
<td>30</td>
<td>12</td>
<td>0</td>
<td>41</td>
<td>12</td>
<td>1</td>
<td>61</td>
<td>16</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>39</td>
<td>0</td>
<td>41</td>
<td>8</td>
<td>67</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>30</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>31</td>
<td>19</td>
<td>20</td>
<td>45</td>
<td>45</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>30</td>
<td>0</td>
<td>30</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>11</td>
<td>41</td>
<td>18</td>
<td>0</td>
<td>7</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>45</td>
<td>17</td>
<td>0</td>
<td>43</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>101</td>
<td>30</td>
<td>2</td>
<td>52</td>
<td>22</td>
<td>19</td>
<td>32</td>
<td>35</td>
<td>31</td>
</tr>
<tr>
<td>100</td>
<td>72</td>
<td>56</td>
<td>37</td>
<td>50</td>
<td>55</td>
<td>64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>30</td>
<td>3</td>
<td>40</td>
<td>27</td>
<td>0</td>
<td>28</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>100</td>
<td>97</td>
<td>46</td>
<td>14</td>
<td>27</td>
<td>30</td>
<td>28</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>103</td>
<td>30</td>
<td>0</td>
<td>31</td>
<td>36</td>
<td>7</td>
<td>33</td>
<td>35</td>
<td>19</td>
</tr>
<tr>
<td>100</td>
<td>93</td>
<td>51</td>
<td>9</td>
<td>51</td>
<td>34</td>
<td>76</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>30</td>
<td>0</td>
<td>33</td>
<td>50</td>
<td>35</td>
<td>49</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>44</td>
<td>40</td>
<td>14</td>
<td>66</td>
<td>55</td>
<td>64</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>30</td>
<td>0</td>
<td>7</td>
<td>1</td>
<td>48</td>
<td>-</td>
<td>-</td>
<td>46</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>-</td>
<td>3</td>
<td>76</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>106</td>
<td>30</td>
<td>0</td>
<td>7</td>
<td>1</td>
<td>48</td>
<td>-</td>
<td>-</td>
<td>46</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>-</td>
<td>3</td>
<td>76</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>107</td>
<td>30</td>
<td>10</td>
<td>0</td>
<td>26</td>
<td>30</td>
<td>27</td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>3</td>
<td>9</td>
<td>57</td>
<td>46</td>
<td>29</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>108</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>27</td>
<td>24</td>
<td>33</td>
<td>33</td>
<td>61</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>44</td>
<td>25</td>
<td>31</td>
<td>34</td>
<td>26</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>109</td>
<td>30</td>
<td>3</td>
<td>25</td>
<td>23</td>
<td>-</td>
<td>27</td>
<td>-</td>
<td>45</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>37</td>
<td>17</td>
<td>-</td>
<td>26</td>
<td>-</td>
<td>51</td>
<td></td>
</tr>
</tbody>
</table>

CAMPTO 1 32 54 44
CAMPTO 1 81 70 65
After the completion of the study on osthol derivatives, the study was extended to other coumarin molecules isolated from *Prangos pabularia*. The natural products chosen included 7-hydroxycoumarin, imperatorin, isoimperatorin, xanthotoxol *etc*. The strategy used for these molecules involved through ring opening, preparation of i) O-alkylated cis-cinnamic acids by using 1-1.2 equivalent alkyl halide and ii) O-alkylated cinnamic acid esters obtained by using 2-2.2 equivalent of alkyl halide [scheme-8 and 9]. All the compounds were fully characterized by detailed spectral analysis. For all the cis-cinnamic acids, the coupling constant for \(\text{a,b-unsaturated protons (CH=CH-COOH)}\) was found in the range 11-12.5 Hz and for trans compounds, coupling constant was > 15 Hz. All the synthesized compounds were screened at 60, 06, 0.6 and 0.06 µM concentration against pancreatic (MaipaCa-2), Leukemia (HL-60 and MolT-4) and colon human cancer cell lines for their possible anticancer activity. From the results obtained, pancreatic cell line was found having low sensitivity towards the synthesized compounds, and out of sixty compounds screened, only twelve compounds showed inhibitory effect at highest test dose (60 µM). Compound 129 was found the most active and displayed cytotoxicity exhibiting 100% inhibition at 60 µM conc and 59% at 0.6 µM test dose. Nearly 50% of the compounds were found active against leukemia cell line Mol with maximum inhibitory effect observed for compounds 125 and 129 displaying cytotoxic effect at all the four test doses (showed 50% inhibition at 0.06 µM). HL-60 cell line showed sensitivity towards maximum number of compounds at the highest test dose, but at lower doses, only compounds 113, 122 and 129 were found effective at 6 µM conc. displaying inhibition of 73, 58 and 97% respectively. For colo-205 cell line, sixteen compounds could exert cytotoxic effect but only at 60 µM concentration. The results of the above study are summarized in Table-3.
Chapter 2

Studies on modification and bio-evaluation of plant molecules

\[
\begin{align*}
R_1 &= H, R_2 = H, R_3 = OCH_3, R_4 = H, \\
R_1 &= H, R_2 = R_3 = \text{Ph}, R_4 = H
\end{align*}
\]

Reagents and conditions: (a): aq. NaOH/DMSO, alkyl halides, rt.

**Scheme-8**

\[
\begin{align*}
R_1 &= H, R_2 = R_3 = \text{Ph}, R_4 = H, \\
R_1 &= H, R_2 = \text{Ph}, R_3 = \text{Ph}, R_4 = H
\end{align*}
\]

Reagents and conditions: (a): aq. NaOH/DMSO, alkyl halides, rt.

**Scheme-9**
Table 3: Percentage Growth Inhibition at different concentrations of osthol analogs against MiapaCa-2, HL-60, MOLT-4, and Colo-205 cell lines with MTT assay.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Pancreatic</th>
<th>Leukemia</th>
<th>Colon</th>
<th>Tissue</th>
<th>Pancreatic</th>
<th>Leukemia</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>MiapaCa-2</td>
<td>HL-60</td>
<td>MOLT-4</td>
<td>Colo-205</td>
<td>Cell type</td>
<td>MiapaCa-2</td>
<td>HL-60</td>
</tr>
<tr>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>60</td>
<td>25</td>
<td>77</td>
<td>28</td>
<td>36</td>
<td>60</td>
<td>32</td>
</tr>
<tr>
<td><img src="image2.png" alt="Structure 1" /></td>
<td>6</td>
<td>5</td>
<td>47</td>
<td>24</td>
<td>23</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td><img src="image3.png" alt="Structure 1" /></td>
<td>0.6</td>
<td>4</td>
<td>36</td>
<td>5</td>
<td>15</td>
<td>0.6</td>
<td>7</td>
</tr>
<tr>
<td><img src="image4.png" alt="Structure 1" /></td>
<td>0.06</td>
<td>1</td>
<td>10</td>
<td>2</td>
<td>11</td>
<td>0.06</td>
<td>5</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>&gt;60μM</td>
<td>11.3</td>
<td>μM</td>
<td>&gt;60μM</td>
<td>18.1μM</td>
<td>&gt;60μM</td>
<td>M</td>
</tr>
</tbody>
</table>

... (more rows)
<p>|
| --- |
| <strong>Chapter 2</strong> | Studies on modification and bio-evaluation of plant molecules |
|
| <strong>Table:</strong> Molecules and their IC&lt;sub&gt;50&lt;/sub&gt; values. |
|</p>
<table>
<thead>
<tr>
<th>Molecule 115</th>
<th>M</th>
<th>μM</th>
<th>M</th>
<th>M</th>
<th>M</th>
<th>μM</th>
<th>M</th>
<th>M</th>
<th>M</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>57</td>
<td>92</td>
<td>83</td>
<td>19</td>
<td>60</td>
<td>40</td>
<td>16</td>
<td>44</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>25</td>
<td>38</td>
<td>18</td>
<td>6</td>
<td>6</td>
<td>12</td>
<td>23</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>5</td>
<td>24</td>
<td>37</td>
<td>8</td>
<td>0.6</td>
<td>4</td>
<td>11</td>
<td>16</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>0.06</td>
<td>2</td>
<td>10</td>
<td>33</td>
<td>2</td>
<td>0.06</td>
<td>3</td>
<td>7</td>
<td>12</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>49.3μM</td>
<td>26.9μM</td>
<td>20.5μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecule 116</td>
<td>M</td>
<td>μM</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>60</td>
<td>42</td>
<td>30</td>
<td>60</td>
<td>11</td>
<td>60</td>
<td>16</td>
<td>70</td>
<td>73</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>19</td>
<td>32</td>
<td>9</td>
<td>6</td>
<td>9</td>
<td>18</td>
<td>23</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>9</td>
<td>9</td>
<td>21</td>
<td>5</td>
<td>0.6</td>
<td>8</td>
<td>12</td>
<td>14</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>0.06</td>
<td>3</td>
<td>2</td>
<td>23</td>
<td>2</td>
<td>0.06</td>
<td>3</td>
<td>11</td>
<td>11</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>40.4μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecule 117</td>
<td>M</td>
<td>μM</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>60</td>
<td>30</td>
<td>30</td>
<td>69</td>
<td>33</td>
<td>60</td>
<td>16</td>
<td>44</td>
<td>38</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>17</td>
<td>39</td>
<td>12</td>
<td>6</td>
<td>14</td>
<td>5</td>
<td>13</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>18</td>
<td>3</td>
<td>23</td>
<td>6</td>
<td>0.6</td>
<td>13</td>
<td>4</td>
<td>11</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>0.06</td>
<td>17</td>
<td>1</td>
<td>22</td>
<td>3</td>
<td>0.06</td>
<td>11</td>
<td>2</td>
<td>4</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>25.8μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecule 118</td>
<td>M</td>
<td>μM</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>60</td>
<td>43</td>
<td>96</td>
<td>58</td>
<td>34</td>
<td>60</td>
<td>16</td>
<td>70</td>
<td>73</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>43</td>
<td>50</td>
<td>21</td>
<td>6</td>
<td>14</td>
<td>5</td>
<td>13</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>21</td>
<td>21</td>
<td>29</td>
<td>17</td>
<td>0.6</td>
<td>13</td>
<td>4</td>
<td>11</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>0.06</td>
<td>11</td>
<td>19</td>
<td>12</td>
<td>12</td>
<td>0.06</td>
<td>11</td>
<td>2</td>
<td>4</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>12.9μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecule 119</td>
<td>M</td>
<td>μM</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>60</td>
<td>25</td>
<td>55</td>
<td>68</td>
<td>32</td>
<td>60</td>
<td>16</td>
<td>70</td>
<td>73</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>23</td>
<td>52</td>
<td>41</td>
<td>27</td>
<td>6</td>
<td>7</td>
<td>12</td>
<td>44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>11</td>
<td>45</td>
<td>33</td>
<td>13</td>
<td>0.6</td>
<td>13</td>
<td>6</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.06</td>
<td>6</td>
<td>40</td>
<td>12</td>
<td>4</td>
<td>0.06</td>
<td>3</td>
<td>12</td>
<td>3</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>4.8μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecule 120</td>
<td>M</td>
<td>μM</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>60</td>
<td>58</td>
<td>91</td>
<td>21</td>
<td>37</td>
<td>60</td>
<td>40</td>
<td>16</td>
<td>44</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>44</td>
<td>85</td>
<td>13</td>
<td>23</td>
<td>6</td>
<td>6</td>
<td>19</td>
<td>31</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>15</td>
<td>37</td>
<td>11</td>
<td>12</td>
<td>0.6</td>
<td>6</td>
<td>12</td>
<td>21</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>0.06</td>
<td>11</td>
<td>12</td>
<td>6</td>
<td>11</td>
<td>0.06</td>
<td>5</td>
<td>12</td>
<td>21</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>28.9μM</td>
<td>2.1μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td>&gt;60μM</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Values:</strong></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; values in μM.</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; values in μM.</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; values in μM.</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; values in μM.</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; values in μM.</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; values in μM.</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; values in μM.</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; values in μM.</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; values in μM.</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; values in μM.</td>
</tr>
</tbody>
</table>
### Chapter 2  
Studies on modification and bio-evaluation of plant molecules

<table>
<thead>
<tr>
<th>M</th>
<th>M</th>
<th>M</th>
<th>M</th>
<th>IC(_{50})</th>
<th>M</th>
<th>μM</th>
<th>M</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>121</td>
<td>60</td>
<td>49</td>
<td>40</td>
<td>&gt;60μM M M</td>
<td>60</td>
<td>13</td>
<td>56</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>5</td>
<td>5</td>
<td>&gt;60μM M</td>
<td>60</td>
<td>13</td>
<td>56</td>
<td>53</td>
</tr>
<tr>
<td>122</td>
<td>60</td>
<td>64</td>
<td>98</td>
<td>&gt;60μM M M</td>
<td>60</td>
<td>9</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>47.5</td>
<td>4.6</td>
<td>10.9</td>
<td>&gt;60μM M</td>
<td>60</td>
<td>9</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>123</td>
<td>60</td>
<td>60</td>
<td>21</td>
<td>&gt;60μM M M</td>
<td>60</td>
<td>9</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>19</td>
<td>11</td>
<td>&gt;60μM M</td>
<td>60</td>
<td>9</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>124</td>
<td>60</td>
<td>44</td>
<td>55</td>
<td>&gt;60μM M</td>
<td>60</td>
<td>44</td>
<td>55</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>48.2</td>
<td>53.1</td>
<td>&gt;60μM M</td>
<td>60</td>
<td>44</td>
<td>55</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>60</td>
<td>21</td>
<td>21</td>
<td>&gt;60μM M M</td>
<td>60</td>
<td>100</td>
<td>67</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>12</td>
<td>12</td>
<td>&gt;60μM M</td>
<td>60</td>
<td>100</td>
<td>67</td>
<td>77</td>
</tr>
<tr>
<td>126</td>
<td>60</td>
<td>21</td>
<td>21</td>
<td>&gt;60μM M</td>
<td>60</td>
<td>21</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>21.7</td>
<td>34.8</td>
<td>&gt;60μM M</td>
<td>60</td>
<td>21</td>
<td>17</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Note: The table lists various compounds with their respective IC\(_{50}\) values in μM, indicating their bio-evaluation results.

\(\text{IC}_{50}\) typically represents the concentration at which an effect is observed in 50% of the samples.
<table>
<thead>
<tr>
<th>Chemical Structure</th>
<th>IC(_{50})</th>
<th>IC(_{50})</th>
</tr>
</thead>
<tbody>
<tr>
<td>127</td>
<td>&gt;60(\mu)M</td>
<td>&gt;60(\mu)M</td>
</tr>
<tr>
<td>128</td>
<td>&gt;60(\mu)M</td>
<td>&gt;60(\mu)M</td>
</tr>
<tr>
<td>129</td>
<td>&gt;60(\mu)M</td>
<td>&gt;60(\mu)M</td>
</tr>
<tr>
<td>130</td>
<td>&gt;60(\mu)M</td>
<td>&gt;60(\mu)M</td>
</tr>
<tr>
<td>131</td>
<td>&gt;60(\mu)M</td>
<td>&gt;60(\mu)M</td>
</tr>
<tr>
<td>132</td>
<td>&gt;60(\mu)M</td>
<td>&gt;60(\mu)M</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical Structure</th>
<th>IC(_{50})</th>
<th>IC(_{50})</th>
</tr>
</thead>
<tbody>
<tr>
<td>157</td>
<td>&gt;60(\mu)M</td>
<td>&gt;60(\mu)M</td>
</tr>
<tr>
<td>158</td>
<td>&gt;60(\mu)M</td>
<td>&gt;60(\mu)M</td>
</tr>
<tr>
<td>159</td>
<td>&gt;60(\mu)M</td>
<td>&gt;60(\mu)M</td>
</tr>
<tr>
<td>160</td>
<td>&gt;60(\mu)M</td>
<td>&gt;60(\mu)M</td>
</tr>
<tr>
<td>161</td>
<td>&gt;60(\mu)M</td>
<td>&gt;60(\mu)M</td>
</tr>
<tr>
<td>162</td>
<td>&gt;60(\mu)M</td>
<td>&gt;60(\mu)M</td>
</tr>
</tbody>
</table>
### Table 1: IC₅₀ Values for Various Molecules

<table>
<thead>
<tr>
<th>Molecule</th>
<th>IC₅₀ (μM)</th>
<th>Molecule</th>
<th>IC₅₀ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>133</td>
<td>&gt;60</td>
<td>163</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>134</td>
<td>&gt;60</td>
<td>164</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>18</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>17</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>135</td>
<td>&gt;60</td>
<td>165</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>20</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>14</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>136</td>
<td>&gt;60</td>
<td>166</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>16</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>137</td>
<td>&gt;60</td>
<td>167</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>25</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>138</td>
<td>&gt;60</td>
<td>168</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
</tbody>
</table>
Orthogonal to osthol triazole derivatives, different triazole derivatives (172-185) of 7-hydroxy coumarin (umbelliferone) were prepared by propargylation followed by click reaction using different azides (Scheme 10).

**Scheme 10**

*Reagents and conditions: (a) K₂CO₃/DMSO, propargyl bromide, room temp. (b) RN₃, t-butanol: H₂O (1:1), CuSO₄, Sod. Ascorbate, rt.*

All the triazoles were chemically characterized and bioevaluated for anticancer activity at 10 µM against seven cancer cell lines. Only two target cell lines were
found sensitivity towards the triazole dervs. Fourteen compounds showed inhibitory effect with compounds 172, 176, and 183 displaying inhibition of 70-78% followed by 177 and 178 displaying >60 % inhibition ,and rest of the compounds showed >50-59% inhibition.

The results of the study are summarized in table-4.

**Table 4:** Percentage Growth Inhibition at 100 µM of different analogs against Colo-205, T47D, NCI-H322, A-549, Hela, A-431 and Pc-3cell lines with MTT assay.

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>Conc µM</th>
<th>COLO</th>
<th>T47D</th>
<th>NCI-H322</th>
<th>A549</th>
<th>Hela</th>
<th>A431</th>
<th>PC-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>171</td>
<td>100</td>
<td>0</td>
<td>36</td>
<td>31</td>
<td>6</td>
<td>10</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>172</td>
<td>100</td>
<td>49</td>
<td>72</td>
<td>36</td>
<td>1</td>
<td>7</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td>173</td>
<td>100</td>
<td>0</td>
<td>33</td>
<td>20</td>
<td>15</td>
<td>15</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>174</td>
<td>100</td>
<td>2</td>
<td>57</td>
<td>22</td>
<td>32</td>
<td>31</td>
<td>19</td>
<td>35</td>
</tr>
<tr>
<td>175</td>
<td>100</td>
<td>34</td>
<td>51</td>
<td>12</td>
<td>33</td>
<td>12</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>176</td>
<td>100</td>
<td>43</td>
<td>78</td>
<td>42</td>
<td>35</td>
<td>39</td>
<td>35</td>
<td>45</td>
</tr>
<tr>
<td>177</td>
<td>100</td>
<td>29</td>
<td>64</td>
<td>5</td>
<td>49</td>
<td>23</td>
<td>49</td>
<td>66</td>
</tr>
<tr>
<td>178</td>
<td>100</td>
<td>71</td>
<td>60</td>
<td>0</td>
<td>32</td>
<td>38</td>
<td>32</td>
<td>41</td>
</tr>
<tr>
<td>179</td>
<td>100</td>
<td>54</td>
<td>59</td>
<td>20</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>180</td>
<td>100</td>
<td>62</td>
<td>20</td>
<td>0</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>34</td>
</tr>
<tr>
<td>181</td>
<td>100</td>
<td>73</td>
<td>55</td>
<td>0</td>
<td>38</td>
<td>43</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>182</td>
<td>100</td>
<td>3</td>
<td>52</td>
<td>4</td>
<td>8</td>
<td>23</td>
<td>23</td>
<td>29</td>
</tr>
<tr>
<td>183</td>
<td>100</td>
<td>66</td>
<td>74</td>
<td>32</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>184</td>
<td>100</td>
<td>0</td>
<td>45</td>
<td>34</td>
<td>23</td>
<td>0</td>
<td>46</td>
<td>18</td>
</tr>
<tr>
<td>185</td>
<td>100</td>
<td>0</td>
<td>39</td>
<td>42</td>
<td>37</td>
<td>23</td>
<td>34</td>
<td>45</td>
</tr>
<tr>
<td>CAMPTO</td>
<td>1</td>
<td>81</td>
<td>70</td>
<td>65</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

2.7. Structure-Activity Relationship (SAR Studies)

On analysis of the biological results in relation to chemical structure of the compounds, the following inferences could be made:

Modification of the isopentyl chain (site II) in terms of nitration, oxidation of methyl group of the isopropylidene unit or epoxidation of the olefinic bond showed deterrent effect and this resulted in the low or total loss of activity. However, hydroxylation of the isopropylidene group to get 71 (tertiary alcohol) lead to higher degree of
inhibition for leukaemia cell line than osthol (at same test concentration of both the compounds) and the product also showed 57% inhibition (at 30 µm conc.) against breast cancer cell line, osthol failed to sensitize this cell line.

Ortho-Oalkylated cinnamic acids generated by modification of site I through ring opening showed activity profiles, wherein the molecules showed activity at lower drug (substrate) concentration than osthol and were more or less specific to one cell only rather than showing inhibition across the board.

No clear inference could be drawn with respect to size of the alkyl group of O-alkylated cinnamic acid. Among various O-alkylated derivatives, OMe was less active, O-propargyl and C-5 derivatives were inactive and showed no inhibition. O-Propylated and O-allylated derivatives were effective against breast cell line showing 50% & 52% inhibition at 7.5 µm concentration respectively. O-alkylaryl derivatives were found inactive.

Among O-alkylated cinnamic acid amides, o-methoxycinnamic acid amide, toluidine and anisidine amides were found inactive while simple anilide was found active and among ortho-OMe and ortho-allyloxy amide derivatives, better potency for o-OMe than o-allyl derivative was observed. Among o-OMe and o-allyl isobutyl and disopropylamides, the latter showed higher potency. Piperidines of o-OMe and o-allyl cinnamic acids showed sensitivity but not for the same cell.

Osthol derived ortho-triazoles of cinnamic acids did not show any enhancement of activity showing thereby the determent effect with substitution of hetero atoms at the ortho position of the acid.

Biological study of the other isolated coumarin molecules (from P. pabularia) after their modification effected through the ring opening of pyranone ring (site-II) (compounds 110-169) allowed to draw some inferences which are listed below:

Except 113 found active, all O-propargyl derivatives (134, 153, 161 and 169) were found inactive. On the contrary, all O-benzyl derivatives (168, 160, 137, & 116) except 152 were found active.

In case of linear furanocoumarins (psoralen derived derivatives) 146-151, most of the ortho-substituted cis-cinnamic acid showed among all the cell lines tested, activity
mostly against one or two cell lines. But in case of angular coumarins (angelicin derived derivatives) derived orthosubstituted cis-cinnamic acids, many were found active (156, 158, 160) against all the cell lines. However, o-propargyl derivative was found inactive, which was also found true for many other propargyl derivatives (134, 153, 161 and 169).

No clear SAR could be arrived at for 7-methoxycoumarin derived ortho-Oalkylated cinnamic acids and triazoles of 7-hydroxy coumarin.

2.8. Screening of Synthesized Analogs for Antimicrobial Activity

After the bioevaluation study against cancer targets, it was thought worthwhile to screen all the synthesized molecules also for their possible antimicrobial activity.

The primary screening involved testing against Gram positive (S.aureus) and Gram-negative (E.coli, C.albicans and A.fumigatus) bacteria at 128 µg/ml concentration of the sunstrate. Only six compounds namely 66, 71, 97, 98, 105 and 136 displayed inhibitory effect, with 71 showing inhibition against all the G +ve and G -ve bacterial strains, followed by 66 which were active against three out of four targets tested. Rest of them showed inhibition against one or two strains. These six compounds were then tested at lower concentration and many of them showed MICs at 32 µg/ml or 64 µg/ml as depicted in the results summarized in table-6.
Table 5: Primary screening of synthesized molecules at 128μg/ml concentration against bacterial strains.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>126</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>66</td>
<td>Active</td>
<td>Active</td>
<td>Inactive</td>
<td>Active</td>
<td>127</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>67</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Active</td>
<td>128</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>68</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>129</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>69</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>130</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>70</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>131</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>71</td>
<td>Active</td>
<td>Active</td>
<td>Active</td>
<td>Active</td>
<td>132</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>72</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>133</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>73</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>134</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>74</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>135</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>75</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>136</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>76</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>137</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>77</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>138</td>
<td>Active</td>
<td>Inactive</td>
<td>Active</td>
<td>Active</td>
</tr>
<tr>
<td>78</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>139</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>79</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>140</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>80</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>141</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>81</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>142</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>82</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>143</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>83</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>144</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>84</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>145</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>85</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>146</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>86</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>147</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>87</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>148</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>88</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>149</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>89</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>150</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>90</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>151</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>91</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>152</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>92</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>153</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>93</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>154</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>94</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>155</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>95</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>156</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>96</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>157</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td></td>
<td>Active</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>---</td>
<td>--------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>97</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>Active</td>
<td></td>
<td></td>
<td></td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>101</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>103</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>Active</td>
<td></td>
<td></td>
<td></td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>106</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>107</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>108</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>109</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>111</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>112</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>113</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>115</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>116</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>117</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>118</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>119</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>121</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>122</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>123</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>124</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ciprofloxacin $0.125$  $0.007$

Amphotericin B $0.5$  $0.5$

Ciprofloxacin $0.125$  $0.007$

Amphotericin B $0.5$  $0.5$
### Table 6: MIC determination of the active samples

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>MIC (µg/ml)</th>
<th>S. aureus ATCC 29213</th>
<th>E. coli ATCC 25922</th>
<th>C. albicans ATCC90028</th>
<th>A. fumigatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td>64</td>
<td>-</td>
<td>128</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>71</td>
<td>32</td>
<td>64</td>
<td>128</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>97</td>
<td>128</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>64</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>32</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>138</td>
<td>64</td>
<td>&gt;256</td>
<td>128</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.125</td>
<td></td>
<td>0.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphotericin B</td>
<td></td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

2.9. Experimental

All reagents for chemical synthesis were purchased from Sigma-Aldrich and used as received. Osthol and other coumarins were isolated from *P. pabularia* in our lab and characterized by spectroscopic techniques. All the solvents used in reactions were distilled and dried before use. All reactions were monitored by TLC on 0.25 mm silica gel 60 F<sub>254</sub> plates coated on aluminum sheet (E. Merck). <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on Brucker Avance DPX-200 instrument at 200 MHz and 50 MHz respectively using CDCl<sub>3</sub> as solvent with TMS as internal standard. Chemical shift is expressed in δ (ppm) and coupling constant in Hertz. Mass spectra were recorded on Jeol MSD-300 instrument and IR spectra on Perkin Elemer FT-IR spectrometer as KBr Pellet or neat sample. Elemental analysis was performed on Elementar Vario EL-III.

2.9.1. Standard experimental procedures

2.9.1.1. General procedure for synthesis of compounds 66 and 67

Compound 66 was prepared by adding selenium dioxide (1 eq.) to a solution of osthol (1 eq.) dissolved in glacial acetic acid (7ml) and stirred for about 3 hrs. On completion of the reaction (monitored by TLC), the contents were poured into crushed ice and extracted with dichloromethane (50 ml), dried over sodium sulfate.
and concentrated on rotavapor to give crude product, which on silica gel column chromatography, using Pet. ether-ethyl acetate as the eluent, yielded pure aldehyde 66 in 60% yield.

Compound 67 was prepared by adding dry NaH (in excess) to Wittig reagent dissolved in dry ether at 0°C in an atmosphere of nitrogen, and the contents stirred for 30 minutes followed by addition of an ethereal solution of compound 66 (aldehyde, 1 eq.) and the contents stirred for 2 hrs. The reaction mixture after the completion of the reaction worked up by dilution with ethyl acetate to quench excess of NaH, followed by careful addition of water, extraction with ethyl acetate (50 ml), dried over sodium sulfate and concentrated to give crude product 3. Subsequent purification of the product by silica gel column chromatography using Pet. ether-ethyl acetate as the eluent afforded pure ester in 87% yield.

2.9.1.2. General procedure for synthesis of compounds 68 and 69

Compounds 68 and 69 were prepared by nitration using Parhan’s method. A 10 M nitrating mixture was prepared by mixing nitric acid (31.6 ml), urea (0.5 g) and diluting the solution to 50 ml with acetic acid. This nitrating mixture (6 ml) was added to a solution of osthol (2 g in acetic anhydride at -10°C) in 2-3 installments with constant stirring, keeping the temperature of the reaction mixture below 0°C. After completion of the reaction (monitored by TLC), the contents were poured in crushed ice when a yellow solid separated out. This was filtered, washed with cold water and dried. The crude solid was subjected to silica gel column chromatography using gradient of Pet. ether and ethyl acetate as the eluent. Two products (68 and 69) were obtained in 78% and 18% yields respectively, along with some un-reacted osthol.

2.9.1.3. Procedure for synthesis of compound 70

Osthol (1 eq.) was dissolved in dichloromethane and kept under chilled condition. To this, a solution of m-chloroperoxybenzoic acid (1.2 eq.) in dichloromethane was added drop wise. The reaction completed in 2 hrs (monitored by TLC). The product was extracted with ethyl acetate and purified by silica gel column chromatography.
2.9.1.4. Procedure for synthesis of compound 71

Compound 71 was prepared by Oxymecuration-demercuration process. Osthol (1 eq.) was dissolved in dichloromethane and to this added dropwise a solution of Hg (OAc)₂ in THF-Water (1:1). The reaction was monitored continuously by TLC. After completion of the reaction (5hrs), the mixture was treated with sodium borohydride (NaBH₄). After usual work up and extraction with ethyl acetate, the product was purified by silica gel column chromatography to give the title compound.

2.9.1.5. General procedure for ring opening reactions

Osthol (1 eq.) was dissolved in dimethyl sulfoxide (DMSO). Sodium hydroxide pellets (2 eq.), in 2 ml water, were added and the mixture was stirred for one hour, followed by addition of methyl iodide. The contents were worked up, following usual procedure to get the crude product, which was purified by silica gel column chromatography, using Pet. ether-ethyl acetate as the eluent to give pure compound (90%). To the purified product (1 eq.) dissolved in dry dichloromethane, was added thionyl chloride (1.2 eq.) + 2 drops of pyridine and the contents refluxed for one hour and the contents concentrated on rotavapor and re-constituted in DCM (10 ml). To this, were added appropriate amines (1eq.) as DCM solution. The contents stirred for one hour, worked up by dilution with water, extraction with DCM (30 ml). The aqueous portion extracted with DCM (30 ml) and the organic layer washed with water, dried over sodium sulfate, concentrated to give crude amide product which on subsequent purification over silica gel column resulted in the isolation of pure amides in 90-95 % yield.

2.9.1.6. General procedure for the preparation of amides

Osthol (1 eq.) and other coumarins was dissolved in dimethyl sulfoxide (DMSO). Sodium hydroxide pellets (2 eq.), in 2 ml water, were added and the mixture was stirred for one hour, followed by addition of different alkyl and aryl halides. The contents were worked up, following usual procedure to get the crude product, which was purified by silica gel column chromatography, using Pet. ether-ethyl acetate as the eluent to give pure compound (90%).
The purified product (1 eq.) dissolved in dry dichloromethane, was added thionyl chloride (1.2 eq.) + 2 drops of pyridine and the contents refluxed for one hour and the contents concentrated on rotavapor and re-constituted in DCM (10 ml). To this, were added appropriate amines (1eq.) as DCM solution. The contents stirred for one hour, worked up by dilution with water, extraction with DCM (30 ml). The aqueous portion extracted with DCM (30 ml) and the organic layer washed with water, dried over sodium sulfate, concentrated to give crude amide product which on subsequent purification over silica gel column resulted in the isolation of pure amides 17-22 in 90-95 % yield.

2.9.1.7. General procedure for the preparation of triazoles

3-(4-methoxy-3-(3-methylbut-2-enyl)-2-(prop-2-ynyloxy) phenyl) acrylic acid (3 mmol) and different freshly prepared organic azides (3 mmol) were suspended in 10 mL of a 1:1 water/tert-butanol mixture. Sodium ascorbate (0.3 mmol, 300 µL of freshly prepared 1 M solution in water) was added, followed by copper (II) sulfate pentahydrate (0.03 mmol, in 100 µL of water). The heterogeneous mixture was stirred vigorously overnight, at which point it cleared and TLC analysis indicated complete consumption of the reactants. The reaction worked up by dilution with water, extraction with DCM (30 ml). The aqueous portion extracted with DCM (30 ml) and the organic layer washed with water, dried over sodium sulfate, concentrated to give crude product which on subsequent purification over silica gel column resulted in the isolation of pure triazoles, in 90% yield.

2.9.1.8. Cell culture

Colo-205, A549 and THP-1 cells were routinely maintained in RPMI 1640 (Sigma Aldrich) while MCF-7 cells was maintained in Minimum Essential Medium MEM (Sigma Aldrich) supplemented with 10% FBS (Merck) and 1% penicillin G and streptomycin (Sigma Aldrich) at 37 °C in a humidified incubator with 5% CO₂. All stock solutions of compounds were prepared in cell culture grade DMSO and stored in -20 °C. Compounds were diluted in culture media prior to use in experiments.
2.9.1.9. Antiproliferative activity

For antiproliferative activity were dissolved in cell culture grade DMSO. We screened all the compounds and parent molecule osthol against cancer cells such as Colo-205, A549 THP-1 and MCF-7 cell lines etc. Cell viability of the compounds treated cells was measured by using MTT assay. Briefly, cells \(10^4\) cells/well were cultured in 96 well tissue culture plates and treated with different concentrations of compounds for 48 h. At the end of incubation, 20 µL of MTT (2.5 mg/mL) was added to the wells and incubated for 4 h. Absorbance was recorded at 570 nm using Eliza Plate Reader.

2.9.1.10. Cell Cycle Analysis

Cell cycle analysis was performed by propidium iodide staining (Sigma, St. Louis, MO). 2.5 lac cells were seeded in 6 well plates in 2 mL media and kept overnight in incubator for attachment. Cells were then treated with the test molecules and incubated for 24 hrs. After completion of the fixed time cells were trypsinised and washed with PBS. Cells were fixed overnight in 70% ethanol, incubated with 0.1% RNase A in PBS at 37 °C for 30 min and resuspended in PBS containing 25 µg/mL propidium iodide (PI) for 30 min at room temperature. The stained cells were analyzed for DNA content by FACS Aria (Becton Dickinson) using the FACS Diva program (Becton Dickinson).

2.9.1.11. Measurement of the Mitochondrial Membrane Potential (\(\Delta \Psi_m\))

The mitochondrial membrane potential was determined by the retention of Rh 123 dye. Rh 123 dye exhibits potential-dependent accumulation in the mitochondria indicated by a fluorescence emission shift from green (\(\sim 529\) nm) to red (\(\sim 590\) nm). Consequently, mitochondrial depolarization is indicated by a decrease in the red/green fluorescence intensity ratio. Cells \((2.5 \times 10^5)\) were seeded in 6 well plates in 2mL media and were allowed to attach overnight. Cells were then treated with the test molecules and incubated for 24 hrs. After completion of the fixed time cells were treated with Rh-123 dye (1 µM). After trypsinisation cells were washed with PBAS. Fluorescence was taken on FACS Aria (Becton Dickinson). FACS Diva program (Becton Dickinson) was used for analysis.
2.9.1.12. Antimicrobial screening

Antibacterial and antifungal activities of analogs were performed using microdilution method (1,2,3) against one gram positive strain (*Staphylococcus aureus* ATCC 29213), one gram negative strain (*Escherichia coli* ATCC 25922), one yeast strain (*Candida albicans* ATCC 90028) and one filamentous fungi (*Aspergillus fumigates*). Antibacterial testing was performed in Muller Hinton Broth (Becton-Dickenson, Cockeysville, MD, USA) whereas for antifungal testing RPMI 1640 with L-glutamine (Sigma-Aldrich, St. Louis, MO, USA) buffered to pH 7.0 supplemented with 0.165 M 3-(N-morpholino) propanesulfonic acid (MOPS) [Sigma Aldrich] was used. The stock solution of the compounds was prepared in DMSO. The MIC (Minimum Inhibitory Concentration) of the compounds was determined by serial 2 fold diluting the compounds in the above mentioned media in 100 µL volume in a 96 well U bottom microtitre plate. The final concentrations of compound ranged from 128 to 0.25 µL/ml. Amphotericin B and ciprofloxacin [16 to 0.03 µL/ml] (both from Sigma Aldrich) were used as standard antifungal and antibacterial agents respectively. The bacterial and fungal suspension of the overnight grown bacterial and fungal was prepared in sterile normal saline and the density was adjusted to 0.5 Mcfarland. The bacterial cultures were further diluted and added in 100µL volume at final inoculums of $1 \times 10^5$ CFU/ml. For fungal cultures $1 \times 10^5$CFU/ml was used. The plates were incubated at 37 °C for 24h for bacterial cultures and 48h for fungal cultures. The plates were read visually and the minimum concentration of the compound showing no turbity was recorded as MIC.
2.10. Spectra Data of Compounds

7-methoxy-8-(3-methylbut-2-enyl)-2H-chromen-2-one: 24

M.P. 83-84°C; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 1.81 (3H, s), 1.84 (3H, s), 3.42 (2H, d, \(J=7.0\)), 3.93 (3H, s), 5.26 (1H, t, \(J=7.0\) Hz), 6.18 (1H, d, \(J=9.1\) Hz), 7.10 (1H, d, \(J=8.5\) Hz), 7.38 (1H, d, \(J=8.5\) Hz), 8.14 (1H, d, \(J=9.1\) Hz). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 17.9, 21.8, 25.6, 56.1, 107.2, 112.4, 112.6, 117.5, 121.0, 126.2, 132.3, 143.4, 152.3, 159.9, 160.7. EIMS \(m/z\): 244, 229, 213, 175. IR (KBr) \(\nu_{max}\) cm\(^{-1}\): 1713, 1600, 1495, 1385, 1365.

2-Methyl-4-(7-methoxy-2-oxo-2H-chromen-8-yl)-but-2-en-1-al: 66

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) 1.95 (3H, s), 3.94 (3H, s), 3.89 (2H, d, \(J=7.69\) Hz), 6.28 (1H, d, \(J=9.46\) Hz), 6.53 (1H, t, \(J=7.34\) Hz), 7.39 (1H, d, \(J=8.62\) Hz), 6.88 (1H, d, \(J=8.6\) Hz), 7.66 (1H, d, \(J=9.5\) Hz). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \(\delta\) 195.4, 160.79, 160.30, 153.0, 150.47, 143.64, 139.81, 127.38, 114.27, 113.33, 113.10, 107.4, 50.19, 22.8, 9.27. EIMS \(m/z\): 258.2482 [M\(^+\)]. IR (KBr) \(\nu_{max}\) cm\(^{-1}\): 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

Ethyl-4-methyl-6-(7-methoxy-2-oxo-2H-chromen-8-yl)-hexa-2,4-dien-1-oate: 67

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) 1.23 (3H, t, \(J=7.19\) Hz), 1.96 (3H), 3.66 (2H, d, \(J=7.50\) Hz), 3.89 (3H, s), 4.08 (2H, q, \(J=7.11\) Hz), 5.75 (1H, d, \(J=15.33\) Hz), 5.88 (1H, t, \(J=7.48\) Hz), 6.77 (1H, d, \(J=8.6\) Hz), 7.18 (1H, d, \(J=8.19\) Hz), 7.70 (1H, d, \(J=15.86\) Hz), 7.26 (1H, d, \(J=8.7\) Hz), 6.19 (1H, d, \(J=9.46\) Hz). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \(\delta\) 210.74, 160.97, 160.49, 153.25, 143.74, 127.50, 127.50, 113.16, 113.09, 112.95, 112.05, 107.27, 64.40, 56.13, 40.91, 34.70, 31.93, 29.70, 22.69, 18.43. EIMS \(m/z\): 351.11(M\(^+\) + Na), 351, 327(M\(^+\) - 1), 283, 189, 159, 131. IR (KBr) \(\nu_{max}\) cm\(^{-1}\): 2923, 2851, 1723, 1609, 1463, 1402, 1282, 1252, 1163, 1117, 1034, 833, 774.

7-Methoxy-8-(3-methyl-4-nitro-but-2-enyl)-2H-chromen-2-one: 68

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) : 1.90 (3H, s), 3.71 & 3.51 (1H each, dd, \(J=7.2\), 7.6 Hz), 3.92 (3H, s), 5.36 (1H, t, \(J=7.2\) Hz), 5.03 (2H, d, \(J=9.2\) Hz), 6.26 (1H, d, \(J=9.2\) Hz), 6.84 (1H, d, \(J=8.8\) Hz), 7.37 (1H, d, \(J=8.4\) Hz), 7.62 (1H, d, \(J=9.6\) Hz). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\): 13.86, 24.0, 55.19, 89.70, 110.77, 111.9, 112.0, 112.24, 117.55, 127.05, 137.51, 142.68, 142.78, 152.35, 159.56. EIMS \(m/z\): (289 M\(^+\) + Na),
312, 281, 265, 212, 189, 175. IR (KBr) ν_{max} cm^{-1}: 3435, 2926, 2853, 1723, 1607, 1565, 1497, 1439, 1403, 1282, 1252, 833, 762, 697, 571, 518.

2-Methyl-4-(7-methoxy-2-oxo-2H-chromen-8-yl)-3-nitro-butan-2-yl acetate: 69

\(^1\)H NMR (CDCl\(_3\), 400 MHz) δ : 1.74 & 1.83 (3H each, s), 2.02 (3H, s), 3.23 (1H, d, J= 6.5 Hz), 3.78 (1H, d, J= 10 Hz), 3.91 (3H, s), 5.43 (1H, dd, J= 6.5 & 10.0 Hz), 6.27 (1H, d, J= 8.6 Hz), 6.84 (1H, d, J= 9.3 Hz), 7.33 (1H, d, J= 8.5 Hz), 7.61 (1H, d, J= 9.5 Hz). \(^13\)C NMR (CDCl\(_3\), 100 MHz) δ: 22.54, 23.05, 23.45, 56.20, 81.14, 91.57, 107.43, 113.03, 113.09, 113.42, 113.55, 128.14, 128.45, 143.45, 143.49, 153.09, 160.3, 160.59, 170.08. EIMS m/z: 372(M\(^+\) + Na), 261, 244, 243, 201, 189, 131.

7-Methoxy-8-\{(3,3-dimethyloxiran-2-yl)methyl\}-2H-chromen-2-one: 70

\(^1\)H NMR (CDCl\(_3\), 400 MHz): δ 1.28 (6H, s), 3.0 (2H, d, J= 5.8 Hz), 3.19 (1H, dd, J= 6.0 & 8.06 Hz), 3.93 (3H, s), 6.25 (1H, d, J= 9.2 Hz), 6.88 (1H, d, J=8.4 Hz), 7.37 (1H, d, J= 8.26 Hz), 7.65 (1H, d, J= 9.3 Hz). \(^13\)C NMR (CDCl\(_3\), 100 MHz): δ 19.14, 22.5, 24.76, 56.16, 59.29, 62.99, 107.43, 112.99, 113.15, 114.27, 127.7, 143.7, 153.45, 160.75, 161.01. EIMS m/z: 260.2741 [M\(^+\)]. IR (KBr) ν_{max} cm^{-1}: 2924, 1731, 1607, 1384, 1280, 1252, 1111, 771, 510.

8-(3-Hydroxy-3-methylbutyl)-7-methoxy-2H chromen-2-one: 71

\(^1\)H NMR (CDCl\(_3\), 400 MHz): δ 1.28 & 1.30 (6H, s), 1.69 (2H, m), 2.92 (2H, m), 3.92 (3H, s), 6.24 (1H, d, J= 9.6 Hz), 7.13 (1H, d, J= 9.2 Hz), 7.30 (1H, d, J= 8.8 Hz), 6.84 (1H, d, J= 8.4 Hz). \(^13\)C NMR (CDCl\(_3\), 100 MHz): δ 17.69, 29.08, 29.36, 42.58, 56.12, 71.05, 107.32, 112.99, 113.04, 118.8, 126.26, 143.82, 152.95, 160.20, 161.37. EIMS m/z: 285(M\(^+\) + Na), 245,189, 159,131. IR (KBr) ν_{max} cm^{-1}: 2924, 2853, 1714, 1606, 1497, 1437, 1402, 1275, 1251, 1122, 1085, 832.

3-{2,4-Dimethoxy-3-(3-methylbut-2-enyl)phenyl}prop-2-en-1-oic acid: 72

\(^1\)H NMR (CDCl\(_3\), 400 MHz) δ : 1.59 & 1.70 (3H, s), 3.27 (2H, d, J= 6.81 Hz), 3.77 & 3.89 (3H each, s), 5.19 (1H, t, J= 6.82 Hz), 5.84 (1H, d, J= 15.5 Hz), 6.57 (1H, d, J= 8.7 Hz), 7.16 (1H, d, J= 15.5 Hz), 7.55 (1H, d, J= 8.64 Hz). \(^13\)C NMR (CDCl\(_3\), 100 MHz) δ: 18.91, 21.20, 24.76, 55.10, 55.82, 106.31, 107.24, 113.0, 115.43, 122.18, 125.37, 132.25, 147.86, 159.01, 159.23, 169.98. EIMS m/z: 299.3371 [M\(^+\) + Na],
276.3276 [M⁺]. IR (KBr) ν_max (cm⁻¹): 2941, 1682, 1608, 1593, 1486, 1462, 1384, 1366, 1314, 1266, 1162, 1118, 1088, 1019, 990, 832, 807, 561.

3-(2-ethoxy-4-methoxy-3-(3-methylbut-2-enyl) phenyl) acrylic acid: 73

¹H NMR (CDCl₃, 400 MHz) δ: 1.43 – 1.39 (3H, t, J= 6.88 Hz), 1.59 & 1.70 (6H, s), 3.27 (2H, d, J= 6.81 Hz), 3.78 (3H, s), 4.35 (2H, q, J= 6.88 Hz), 5.19 (1H, t, J= 6.82 Hz), 5.84 (1H, d, J= 15.5 Hz), 6.57 (1H, d, J= 8.7 Hz), 7.16 (1H, d, J= 15.5 Hz), 7.55 (1H, d, J= 8.64 Hz).

¹³C NMR (CDCl₃, 100 MHz) δ: 15.63, 17.83, 23.08, 25.73, 55.73, 70.94, 105.93, 117.06, 121.10, 122.95, 123.17, 129.50, 131.27, 141.92, 157.09, 159.85, 171.03. EIMS m/z: 290.34 [M⁺ + Na], 267.66 [M⁺]. IR (KBr) ν_max (cm⁻¹): 2923, 2851, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615.

3-(4-methoxy-3-(3-methylbut-2-enyl)-2-propoxyphenyl) acrylic acid: 74

¹H NMR (CDCl₃, 400 MHz) δ: 0.97 – 1.01 (3H, t, J= 6.01 Hz), 1.59 & 1.70 (6H, s), 1.80-1.86 (2H, m), 3.27 (2H, d, J= 6.81 Hz), 3.78 (3H, s), 4.35 (2H, m), 5.19 (1H, t, J= 6.82 Hz), 5.84 (1H, d, J= 15.5 Hz), 6.47 (1H, d, J= 8.7 Hz), 6.97 (1H, d, J= 15.5 Hz), 7.35 (1H, d, J= 7.91 Hz).

¹³C NMR (CDCl₃, 100 MHz) δ: 10.63, 17.99, 21.37, 24.55, 24.85, 56.79, 74.62, 107.81, 120.22, 121.79, 122.55, 123.01, 129.22, 131.61, 138.85, 161.01, 162.27, 168.94. EIMS m/z: 304.45 [M⁺ + Na]. IR (KBr) ν_max (cm⁻¹): 2923, 2851, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615.

3-(2-butoxy-4-methoxy-3-(3-methylbut-2-enyl) phenyl) acrylic acid: 75

¹H NMR (CDCl₃, 400 MHz) δ: 0.97 – 1.01 (3H, t, J= 6.01 Hz), 1.59 & 1.70 (6H, s), 1.78 – 1.74 (m, 2H), 1.90-1.86 (2H, m), 3.27 (2H, d, J= 6.81 Hz), 3.78 (3H, s), 4.35 (2H, m), 5.19 (1H, t, J= 6.82 Hz), 5.84 (1H, d, J= 15.5 Hz), 6.47 (1H, d, J= 8.7 Hz), 6.97 (1H, d, J= 15.5 Hz), 7.35 (1H, d, J= 7.91 Hz).

¹³C NMR (CDCl₃, 100 MHz) δ: 13.94, 17.84, 19.26, 22.98, 25.70, 32.35, 55.65, 75.20, 105.87, 117.13, 121.10, 123.09, 129.51, 131.22, 141.69, 157.16 159.83, 170.81. EIMS m/z: 318.15 [M⁺ + Na]. IR (KBr) ν_max (cm⁻¹): 2923, 2851, 1725, 1608, 1455, 1393, 1304, 1276, 1162, 1118, 1088, 922, 830, 741, 626.
3-(4-methoxy-3-(3-methylbut-2-enyl)-2-(pentyloxy) phenyl) acrylic acid: 76

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 0.97 – 1.01 (3H, t, $J = 6.01$ Hz), 1.59 & 1.70 (6H, s), 1.78 – 1.74 (m, 2H), 1.80-1.86 (4H, m), 3.27 (2H, d, $J = 6.81$ Hz), 3.78 (3H, s), 4.35 (2H, m) 5.19 (1H, t, $J = 6.82$ Hz), 5.84 (1H, d, $J = 15.5$ Hz), 6.47 (1H, d, $J = 8.7$ Hz), 6.97 (1H, d, $J = 15.5$ Hz), 7.35 (1H, d, $J = 7.91$ Hz). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 14.63, 17.99, 22.37, 24.55, 24.85, 26.82, 31.65, 56.79, 72.62, 107.81, 120.22, 121.79, 122.55, 123.01, 129.22, 131.61, 138.85, 161.01 161.27, 168.94. EIMS $m/z$: 332.45 M$^+$ + Na. IR (KBr) $\nu_{max}$ cm$^{-1}$: 2933, 2821, 1725, 1608, 1455, 1393, 1304, 1266, 1162, 1118, 1088.

3-(2-(hexyloxy)-4-methoxy-3-(3-methylbut-2-enyl) phenyl) acrylic acid: 77

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 0.97 – 1.01 (3H, t, $J = 6.01$ Hz), 1.59 & 1.70 (6H, s), 1.78 – 1.74 (m, 2H), 1.80-1.86 (6H, m), 3.27 (2H, d, $J = 6.81$ Hz), 3.78 (3H, s), 4.35 (2H, m) 5.19 (1H, t, $J = 6.82$ Hz), 5.84 (1H, d, $J = 15.5$ Hz), 6.47 (1H, d, $J = 8.7$ Hz), 6.97 (1H, d, $J = 15.5$ Hz), 7.35 (1H, d, $J = 7.91$ Hz). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 14.63, 17.99, 22.37, 24.55, 24.85, 26.82, 31.65, 56.79, 72.62, 107.81, 120.22, 121.79, 122.55, 123.01, 129.22, 131.61, 138.85, 161.01 161.27, 168.94. EIMS $m/z$: 346.45 M$^+$ + Na. IR (KBr) $\nu_{max}$ cm$^{-1}$: 2923, 2851, 1725, 1608, 1455, 1393, 1304, 1266, 1162, 1118, 1088, 922, 830, 741, 626.

3-(4-methoxy-3-(3-methylbut-2-enyl)-2-(prop-2-ynyloxy) phenyl) acrylic acid: 78

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 1.63 (3H, s), 1.78 (3H, s), 2.52 (s, 1H), 3.38 (2H, d, $J = 6.81$ Hz), 3.86(3H, s), 4.45 (m, 2H), 5.17 (1H, t, $J = 6.82$ Hz), 5.92 (1H, d, $J = 15.5$ Hz), 6.67 (1H, d, $J = 8.7$ Hz), 7.26 (1H, d, $J = 15.5$ Hz), 7.58 (1H, d, $J = 8.64$ Hz). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 17.91, 22.69, 23.40, 55.74, 62.16, 75.55, 78.89, 106.64, 117.69, 120.99, 122.31, 129.24, 131.77, 132.15, 141.65, 155.96, 159.79, 170.89. EIMS $m/z$: 300.14 [M$^+$ + Na], 277.84 [M$^+$]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 2941, 1682, 1608, 1593, 1486, 1425, 1384, 1366, 1314, 1266, 1162, 1118, 1088.

3-{4-Methoxy-3-(3-methylbut-2-enyl)-2-(prop-2-en-1-oxy) phenyl} prop-2-en-1-oic acid: 79

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 1.58 & 1.72 (3H, s), 3.26 (2H, d, $J = 6.81$ Hz), 3.77 (3H, s), 5.16 (1H, t, $J = 6.82$ Hz), 5.82 (1H, d, $J = 15.5$ Hz), 6.57 (1H, d, $J = 8.7$ Hz),
7.16 (1H, d, \(J=15.5 \text{ Hz}\)), 7.55 (1H, d, \(J=8.64 \text{ Hz}\)). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\): 17.90, 23.06, 25.73, 55.68, 75.74, 106.09, 117.31, 121.06, 122.88, 123.22, 129.53, 131.39, 133.66, 141.76, 156.79, 159.81, 171.02. EIMS \(m/z\): 303.0748 (M\(^{+1}\)), 267.0986, 221.0808, 189.0546. IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 2936, 2850, 1675, 1608, 1590, 1481, 1466, 1422, 1385, 1366, 1310, 1259, 1160, 1117, 1080, 1019, 985, 830, 806, 565.

3-(2-(benzyloxy)-4-methoxy-3-(3-methylbut-2-enyl) phenyl) acrylic acid: 80

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\): 1.59 & 1.70 (6H, s), 3.27 (2H, d, \(J=6.81 \text{ Hz}\)), 3.80 (3H, s), 4.35 (s, 2H), 5.19 (1H, t, \(J=6.82 \text{ Hz}\)), 5.84 (1H, d, \(J=15.5 \text{ Hz}\)), 6.57 (1H, d, \(J=8.7 \text{ Hz}\)), 7.16 (1H, d, \(J=15.5 \text{ Hz}\)), 7.32 – 7.26 (m, 5H), 7.80 (1H, d, \(J=8.64 \text{ Hz}\)). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\): 17.92, 23.11, 25.74, 55.77, 64.93, 106.26, 118.17, 121.42, 123.27, 126.54, 127.12, 127.38, 129.08, 129.98, 131.41, 137.28, 140.13, 141.15, 156.55, 159.67, 169.48. EIMS \(m/z\): 352.42 [M\(^{+1}\) + Na], IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 2933, 2821, 1725, 1608, 1455, 1393, 1304, 1266, 1162, 1118, 1088.

N-Piperidyl-3-{2,4-dimethoxy-3-(3-methylbut-2-enyl) phenyl}prop-2-en-1-amide: 81

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\): 1.64-1.76 (6H, bs), 1.67 & 1.77 (6H, s), 3.36 (2H, d, \(J=7.44 \text{ Hz}\)), 3.72 (4H, bs), 3.72 & 3.84 (3H, s), 5.17 (1H, t, \(J=7.44 \text{ Hz}\)), 6.6 6(1H, d, \(J=8.64 \text{ Hz}\)), 6.90 (1H, d, \(J=15.5 \text{ Hz}\)), 7.37 (1H, d, \(J=6.8 \text{ Hz}\)), 7.79 (1H, d, \(J=15.75 \text{ Hz}\)). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\): 18.83, 21.62, 24.97, 26.01, 44.28, 56.32, 56.68, 106.01, 106.98, 113.10, 118.59, 122.98, 125.32, 133.07, 143.932, 158.63, 158.82, 166.52. EIMS \(m/z\): 344.2202, 201, 143. IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 2925, 2853, 1731, 1637, 1595, 1485, 1443, 1381, 1362, 1091, 1022, 771, 514.

N-(4-Methylphenyl)-3-{2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl}prop-2-en-1-amide: 82

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\): 1.63 & 1.78 (3H, s), 2.29 (3H, s), 3.37 (2H, d, \(J=6.8 \text{ Hz}\)), 3.79 & 3.86 (3H each, s), 5.17 (1H, t, \(J=6.8 \text{ Hz}\)), 6.53 (1H, d, \(J=15.6 \text{ Hz}\)), 6.68 (1H, d, \(J=8.8 \text{ Hz}\)), 7.15 (1H, d, \(J=8.36 \text{ Hz}\)), 7.36-7.52 (1H, m), 7.89 (1H, d, \(J=15.6 \text{ Hz}\)). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \(\delta\) 17.93, 21.96, 26.80, 56.06, 56.14, 107.38, 113.04, 113.10, 118.07, 121.16, 126.19, 126.68, 129.51, 132.63, 135.13, 143.91,
Chapter 2
Studies on modification and bio-evaluation of plant molecules

152.89, 160.26, 161.34. EIMS \( m/z \): 366.22, 329, 315, 279, 225, 149. IR (KBr) \( \nu_{max} \text{cm}^{-1} \): 2925, 1660, 1605, 1562, 1490, 1442, 1386, 1365, 1278, 1248, 1160, 1088, 1026, 830, 634, 606, 570.

N-(4-Methoxyphenyl)-3-{2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl}prop-2-en-1-amide: 83

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \( \delta \): 1.68 & 1.78 (3H, s), 3.37 (2H, d, \( J = 6.34 \text{ Hz} \)), 3.80 & 3.85 (3H, s), 5.17 (1H, t, \( J = 6.5 \text{ Hz} \)), 6.53 (1H, d, \( J = 15.6 \text{ Hz} \)), 6.67 (1H, d, \( J = 8.47 \text{ Hz} \)), 6.86 (1H, dd, \( J = 2.0, 8.9 \text{ Hz} \)), 7.39 (1H, d, \( J = 8.6 \text{ Hz} \)), 7.41-7.43 (1H, m), 7.45 (1H, d, \( J = 8.9 \text{ Hz} \)), 7.89 (1H, d, \( J = 15.58 \text{ Hz} \)), 7.79 (1H, d, \( J = 8.1 \text{ Hz} \)). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \( \delta \): 18.83, 21.46, 25.32, 55.81, 56.38, 56.79, 106.32, 107.24, 112.82, 114.31, 118.72, 122.43, 123.02, 125.37, 128.43, 133.12, 144.21, 156.73, 158.47, 158.93, 166.81. EIMS \( m/z \): 404.1821 (M\(^+\) + Na), 382.2003 (M\(^+\) + 1). IR (KBr) \( \nu_{max} \text{cm}^{-1} \): 2935, 2837, 1681, 1656, 1620, 1593, 1542, 1485, 1462, 1441, 1386, 1365, 1273, 1247, 1170, 1090, 1019, 830, 804, 610, 559.

N-Phenyl-3-{2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl}prop-2-en-1-amide: 84

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \( \delta \): 1.62 & 1.77 (3H, s), 3.36 (2H, d, \( J = 6.68 \text{ Hz} \)), 3.73 & 3.89 (3H, s), 5.17 (1H, t, \( J = 6.68 \text{ Hz} \)), 6.57 (1H, d, \( J = 15.6 \text{ Hz} \)), 6.65 (1H, d, \( J = 8.5 \text{ Hz} \)), 7.10-7.19 (1H, m), 7.26-7.39 (H, m), 7.59, d, \( J = 8.19 \text{ Hz} \)), 7.88 (1H, d, \( J = 8.0 \text{ Hz} \)), 7.91 (1H, d, \( J = 15.6 \text{ Hz} \)). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \( \delta \): 18.92, 21.81, 25.43, 56.10, 56.63, 106.72, 107.23, 112.81, 119.12, 121.34, 122, 93, 128.87, 133.04, 136.07, 144.10, 158.47, 158.82, 166.43. EIMS \( m/z \): 374.4075 [M\(^+\) + Na], 351.4074 [M\(^+\)]. IR (KBr) \( \nu_{max} \text{cm}^{-1} \): 2958, 2853, 1731, 1607, 1565, 1542, 1497, 1403, 1384, 1366, 989, 912, 831.

N,N-Bis-(1-methylethyl)-3-{2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl} prop-2-en-1-amide: 85

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \( \delta \): 1.34 (12H, bs.), 1.62 & 1.78 (3H, s), 3.36 (2H, d, \( J = 6.61 \text{ Hz} \)), 3.73 & 3.84 (3H, s), 3.83-4.25 (2H, m), 5.17 (1H, t, \( J = 6.67 \text{ Hz} \)), 6.69 (1H, d, \( J = 8.74 \text{ Hz} \)), 6.85 (1H, d, \( J = 15.5 \text{ Hz} \)), 7.36 (1H, d, \( J = 8.85 \text{ Hz} \)), 7.72 (1H, d, \( J = 15.5 \text{ Hz} \)). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \( \delta \): 18.69, 21.23, 21.97, 25.0, 43, 56.10, 56.63, 106.72, 107.23, 112.81, 119.12, 121.34, 122, 93, 128.87, 133.04, 136.07, 144.10, 158.47, 158.82, 166.43. EIMS \( m/z \): 374.4075 [M\(^+\) + Na], 351.4074 [M\(^+\)]. IR (KBr) \( \nu_{max} \text{cm}^{-1} \): 2958, 2853, 1731, 1607, 1565, 1542, 1497, 1403, 1384, 1366, 989, 912, 831.
Chapter 2  
Studies on modification and bio-evaluation of plant molecules

159.02, 161.97. EIMS m/z: 382.4810 [M+Na], 359.5133 [M++] IR (KBr) νmax cm⁻¹: 2966, 2928, 1732, 1641, 1484, 1440, 1386, 1373, 1335, 1300, 1270, 1251, 1211, 1161, 1117, 1045, 989, 925, 832, 803, 703, 634, 602, 570.

**N-(2-Methylpropyl)-3-{2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl}prop-2-en-1-amide:** 86

1H NMR (CDCl₃, 400 MHz): δ 0.95 (6H, d, J= 6.65 Hz), 1.62 & 1.77 (3H, s), 3.21 (2H, d, J= 6.35 Hz), 3.35 (2H, d, J= 6.68 Hz), 3.72 & 3.83 (3H, s), 5.16 (1H, t, J= 6.8 Hz), 6.41 (1H, d, J= 15.7 Hz), 6.65 (1H, d, J= 8.56 Hz), 7.35 (1H, d, J= 8.64 Hz), 7.27 (1H, d, J= 15.7 Hz). 13C NMR (CDCl₃, 100 MHz): δ 17.86, 20.21, 22.94, 25.76, 28.66, 47.07, 55.75, 62.06, 106.82, 119.84, 121.21, 121.26, 124.02, 126.59, 131.51, 136.19, 158.28, 159.78, 166.72. EIMS m/z: 354.2028 [M+Na], 332.2208 [-Na]. IR (KBr) νmax cm⁻¹: 2959, 2871, 1650, 1614, 1594, 1556, 1485, 1386, 1364, 1337, 1272, 1255, 1180, 1170, 1020, 990, 968, 944, 865, 805, 756.

**N-Piperidyl-3-{4-methoxy-3-(3-methylbut-2-enyl)-2-(prop-2-en-1-oxy) phenyl}prop-2-en-1-amide:** 87

1H NMR (CDCl₃, 400 MHz) δ: 1.67-1.75 (6H, s), 3.85 (3H, s), 4.30 (2H, d, J= 5.2 Hz), 5.18-5.50 (3H, m), 6.0-6.17 (1H, m), 6.68 (1H, d, J= 8.6 Hz), 6.98 (1H, d, J= 15.5 Hz), 7.30 (1H, d, J= 8.44 Hz), 7.75 (1H, d, J= 15.50 Hz). 13C NMR (CDCl₃, 100 MHz) δ: 19.11, 21.82, 25.30, 25.81, 44.72, 56.32, 72.81, 106.30, 107.28, 112.93, 116.68, 118.89, 123.20, 125.39, 132.79, 133.64, 144.16, 158.48, 158.72, 166.37. EIMS m/z: 370.2357 (M+1). IR (KBr) νmax cm⁻¹: 2934, 2855, 1643, 1593, 1485, 1441, 1374, 1273, 1217, 1173, 1137, 1089, 990, 851, 804.

**N-(4-Methylphenyl)-3-{4-methoxy-3-(3-methylbut-2-enyl)-2-(prop-2-en-1-oxy) phenyl}prop-2-en-1-amide:** 88

1H NMR (CDCl₃, 400 MHz) δ: 1.67 & 1.75 (3H, s), 2.30 (3H, s), 3.36 (2H, d, J= 6.38 Hz), 3.79 (3H, s), 4.30 (2H, d, J= 5.3 Hz), 5.16-5.40 (1H, m), 6.12-6.25 (1H), 6.58 (1H, d, J= 15.5 Hz), 5.17 (1H, t, J= 6.8 Hz), 6.66 (1H, d, J= 8.76 Hz), 7.1 (1H, d, J= 8.8 Hz), 7.36-7.52 (1H, m), 7.87 (1H, d, J= 15.6 Hz). 13C NMR (CDCl₃, 100 MHz) δ: 18.99, 21.79, 24.12, 25.71, 56.29, 72.84, 106.37, 107.32, 112.81, 116.52, 118.95,
121.72, 123.15, 125.62, 129.34, 133.01, 133.78, 144.32, 158.39, 158.81, 166.28. 
EI/MS m/z: 414.4924, 391.5026 [M+]. IR (KBr) \( \nu \) cm\(^{-1}\): 2921, 1729, 1606, 1565, 1496, 1435, 1278, 1250, 1160, 1118, 1088, 1031, 834, 606, 570, 514.

N-(4-Methoxyphenyl)-3-{4-methoxy-3-(3-methylbut-2-enyl)-2-(prop-2-en-1-oxy) phenyl}prop-2-en-1-amide: 89

\(^1\)HNMR (CDCl\(_3\), 400 MHz) \( \delta \): 1.76 & 1.84 (3H, s), 3.53 (2H, d, \( J= 6.5 \) Hz), 3.79 & 3.91 (3H, s), 4.31 (2H, d, \( J= 5.2 \) Hz), 5.17-5.31 (3H, m), 6.10-6.21 (1H, m), 6.55 (1H, d, \( J= 15.6 \) Hz), 6.83 (1H, d, \( J= 8.66 \) Hz), 7.32 (1H, d, \( J= 8.5 \) Hz), 7.86 (1H, d, \( J= 15.6 \) Hz). \(^{13}\)CNMR (CDCl\(_3\), 100 MHz) \( \delta \): 19.03, 21.71, 25.43, 56.01, 56.37, 72.82, 106.43, 107.32, 112.92, 114.50, 116.37, 118.86, 122.72, 123.15, 125.37, 128.43, 132.92, 133.71, 144.21, 156.42, 158.41, 158.78, 166.42. EI/MS m/z: 430.1969, 408.154. IR (KBr) \( \nu \) cm\(^{-1}\): 2912, 1654, 1594, 1542, 1485, 1464, 1439, 1412, 1351, 1273, 1246, 1171, 1088, 1035, 991, 830, 802, 769, 522.

N-Phenyl-3-{4-methoxy-3-(3-methylbut-2-enyl)-2-(prop-2-en-1-oxy) phenyl}prop-2-en-1-amide: 90

\(^1\)HNMR (CDCl\(_3\), 400 MHz) \( \delta \): 1.76 & 1.83 (3H, s), 3.37 (2H, d, \( J= 5.5 \) Hz), 3.91 (3H, s), 4.31 (2H, d, \( J= 5.2 \) Hz), 5.18-5.31 (3H, m), 6.10-6.21 (1H, m), 6.58 (1H, d, \( J= 15.6 \) Hz), 6.83 (1H, d, \( J= 8.56 \) Hz), 7.88 (1H, d, \( J= 15.6 \) Hz), 7.86 (1H, d, \( J= 8.4 \) Hz), 7.29-7.49 (5H, m). \(^{13}\)CNMR (CDCl\(_3\), 100 MHz) \( \delta \): 19.03, 21.71, 25.43, 56.01, 56.37, 72.82, 106.43, 107.32, 112.92, 114.50, 116.37, 118.86, 122.72, 123.15, 125.37, 128.43, 132.92, 133.71, 144.21, 156.42, 158.41, 158.78, 166.42. EI/MS m/z: 400.1865, 378.2050, 267.0989, 245.1169. IR (KBr) \( \nu \) cm\(^{-1}\): 2912, 1654, 1594, 1542, 1485, 1464, 1439, 1412, 1351, 1273, 1246, 1171, 1088, 1035, 991, 830, 802, 769, 522.

N,N-Bis-(1-methylethyl)-3-{4-methoxy-3-(3-methylbut-2-enyl)-2-(prop-2-en-1-oxy) phenyl}prop-2-en-1-amide: 91

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \( \delta \): 1.34 (12H, bs), 1.84 & 1.98 (3H, s), 3.54 (2H, d, \( J= 7.13 \) Hz), 3.89 (3H, s), 4.31 (2H, d, \( J= 5.07 \) Hz), 5.18-5.27(3H, m), 6.0-6.12(1H, m), 6.23(1H, d, \( J= 9.4 \) Hz), 6.92(1H, d, \( J= 15.49 \) Hz), 7.29 (1H, d, \( J= 8.46 \) Hz), 7.67 (1H, d, \( J= 15.45 \) Hz), 7.67 (1H, d, \( J= 8.6 \) Hz). \(^{13}\)CNMR (CDCl\(_3\), 100 MHz) \( \delta \): 18.97, 21.32, 21.71, 25.43, 45.62, 56.23, 72.82, 106.37, 107.31, 116.32, 118.85, 123.12,
Chapter 2  
Studies on modification and bio-evaluation of plant molecules

125.37, 132.86, 133.72, 144.23, 158.20, 158.86, 162.37. EIMS \( m/z \): 408.2488, 386, 267. IR (KBr) \( \nu_{\max} \text{cm}^{-1} \): 2960, 2925, 1725, 1640, 1607, 1474, 1440, 1385, 1371, 1300, 1270, 1262, 1161, 1115, 1040, 922, 830, 741, 626.

N-(2-Methylpropyl)-3-{4-methoxy-3-(3-methylbut-2-enyl)-2-(prop-2-en-1-oxy)phenyl} prop-2-en-1-amide: 92

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \( \delta \): 0.94 (6H, d, \( J=6.65 \text{ Hz} \)), 3.36 & 1.90 (3H, s), 3.20 (2H, \( d, J=6.59 \text{ Hz} \)), 3.36 (2H, \( d, J=6.45 \text{ Hz} \)), 3.84 and 3.92 (3H, s), 4.29 (2H, \( d, J=5.29 \text{ Hz} \)), 5.12 and 5.40 (3H), 6.0-6.21 (1H, m), 6.43 (1H, \( d, J=15.7 \text{ Hz} \)), 6.83 (1H, \( d, J=8.6 \text{ Hz} \)), 7.35 (1H, \( d, J=15.76 \text{ Hz} \)). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \( \delta \): 19.21, 21.62, 20.41, 25.37, 28.63, 46.92, 56.23, 72.61, 106.37, 107.28, 112.92, 116.37, 119.10, 122.93, 125.37, 133.02, 133.87, 144.01, 158.63, 158.92, 166.43. EIMS \( m/z \): 380.4537 \([M^+ + \text{Na}]\), 357.4651 \([M^+]\). IR (KBr) \( \nu_{\max} \text{cm}^{-1} \): 2923, 2851, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615.

(Z)-3-(2-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid: 93

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \( \delta \): 1.7 (s, 3H), 1.8 (s, 3H), 1.8 (s, 3H), 3.3 (d, \( J=6.5 \text{ Hz} \), 2H), 3.8 (s, 3H), 5.2 (s, 2H), 5.9 (d, \( J=12.6 \text{ Hz} \), 1H), 6.7 (d, \( J=8.6 \text{ Hz} \), 1H), 7.3 (m, 3H), 7.5 (m, 2H), 7.8 (m, 2H), 8.02 (s, 1H). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \( \delta \): 17.8, 23.0, 25.7, 29.6, 55.7, 67.5, 120.50, 120.55, 120.57, 121.6, 122.8, 123.2, 123.7, 128.9, 129.2, 129.2, 129.7, 131.3, 136.9, 138.4, 155.4, 159.2, 171.07. EIMS \( m/z \): 419.47 \([M^+ + \text{Na}]\). IR (KBr) \( \nu_{\max} \text{cm}^{-1} \): 2856, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615.

(Z)-3-(2-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid: 94

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \( \delta \): 1.7 (s, 3H), 1.8 (s, 3H), 2.2 (m, 1H), 3.4 (d, \( J=7.6 \text{ Hz} \), 2H), 3.9 (s, 6H), 5.1 (m, 3H), 5.9 (m, 1H), 7.1 (m, 2H), 7.2 (d, \( J=12.2 \text{ Hz} \), 1H), 7.6 (d, \( J=8.8 \text{ Hz} \), 2H), 7.7 (d, \( J=8.8 \text{ Hz} \), 2H), 7.97 (s, 1H). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \( \delta \): 17.9, 23.1, 25.7, 25.7, 29.6, 55.6, 55.7, 106.4, 114.7, 121.4, 121.5, 122.1, 122.6, 123.8, 123.8, 128.9, 129.4, 129.4, 130.3, 131.6, 141.3, 155.6, 159.7, 159.8, 172.01.
EIMS m/z: 449.50 [M\(^{+}\) + Na]. IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 2923, 2851, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615.

(Z)-3-(2-((1-(2-chloropyridin-3-yl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid: 95

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\): 1.3 (s, 3H), 1.7 (s, 3H), 2.2 (s, 1H), 3.3 (m, 2H), 3.9 (s, 3H), 5.1 (m, 2H), 5.9 (m, 1H), 6.6 (m, 1H), 7.2 (m, 1H), 7.4 (m, 2H), 8.0 (m, 2H), 8.5 (m, 1H). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\): 17.9, 23.1, 25.7, 33.0, 55.7, 62.1, 67.3, 106.5, 117.8, 121.2, 122.5, 123.5, 125.1, 129.5, 131.7, 131.9, 136.0, 141.5, 144.0, 144.8, 150.2, 155.9, 159.8, 171.05. EIMS m/z: 454.91 [M\(^{+}\) + Na]. IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 2953, 2791, 1725, 1608, 1437, 1393, 1304, 1276, 1196, 1032.

(Z)-3-(2-((1-(benzo[d]thiazol-2-yl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid: 96

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\): 1.5 (s, 3H), 1.8 (s, 3H), 3.4 (s, 3H), 5.2 (m, 3H), 5.9 (m, H), 6.8 (d, 1H, J=8.5 Hz), 7.3-7.6 (m, 7H), 7.8 (m, 1H), 7.9 (s, 1H). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\): 17.9, 25.7, 29.1, 31.9, 55.7, 62.1, 75.5, 78.9, 106.6, 121.0, 121.8, 122.6, 122.9, 123.3, 123.4, 125.6, 125.8, 126.1, 126.7, 129.5, 131.7, 141.3, 155.9, 159.7, 160.03, 171.06. EIMS m/z: 476.55 [M\(^{+}\) + Na]. IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 3477, 2922, 1770, 1472, 1346, 1049, 778, 688, 644, 558, 548, 490.

(Z)-3-(2-((1-(benzonitrile)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid: 97

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\): 1.7 (s, 3H), 1.8 (s, 3H), 3.3 (d, 2H, J=6.5 Hz), 3.9 (s, 3H), 5.2 (s, 2H), 6.7 (d, 1H, J=8.2 Hz), 7.3 (m, 2H), 7.4 (d,1H, J=7.9), 7.8 (d, 2H, J=8.2), 7.9 (d, 2H, J=8.2 Hz), 8.1 (s, 1H). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\): 15.7, 20.9, 23.5, 53.5, 67.2, 76.2, 105.4, 110.2, 117.6, 118.2, 118.2, 119.2, 120.2, 122.2, 127.4, 129.2, 131.7, 138.7, 139.4, 143.3, 158.4, 159.8, 170.06. EIMS m/z: 444.48 [M\(^{+}\) + Na]. IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 3732, 3314, 2918, 1764, 1681, 1585, 1461, 1299, 1170, 1126, 1050, 994, 944, 899, 845, 775, 720, 654.
(Z)-3-(2-((1-(benzylnitrile)-1H-1,2,3-triazol-4-yl) methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl) acrylic acid: 98

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 1.7 (s, 3H), 1.8 (s, 3H), 2.1 (s, 2H), 3.3 (d, 2H, $J=6.5$ Hz), 3.9 (s, 3H), 5.2 (s, 2H), 5.9 (m, 1H), 6.7 (d, 1H, $J=8.2$ Hz), 7.3 (m, 2H), 7.4 (d, 1H, $J=7.9$), 7.8 (d, 2H, $J=8.2$), 7.9 (d, 2H, $J=8.2$), 8.1 (s, 1H). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 17.9, 23.1, 25.7, 29.6, 55.5, 67.1, 75.2, 78.9, 106.6, 120.5, 120.5, 121.7, 122.6, 123.4, 127.3, 130.2, 131.1, 131.7, 140.7, 144.1, 144.5, 155.2, 155.8, 159.8, 170.07. EIMS $m/z$: 458.20 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 3480, 2932, 1764, 1700, 1601, 1478, 1298, 1208, 1167, 1124, 987, 949, 838, 733, 672, 571, 527

(Z)-3-(2-((1(3-hydroxypyrrolidin-1-yl)(phenyl)methanone)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl) acrylic acid: 99

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 1.3 (s, 3H), 1.7 (s, 3H), 1.8 (m, 2H), 2.0 (m, 1H), 3.2 (m, 2H), 3.6 (m, 2H), 3.8 (s, 3H), 5.0 (s, 2H), 5.1 (m, 1H), 5.8 (m, 2H), 6.6 (d,1H, $J=8.4$Hz), 7.1 (m, 1H), 7.5 (m, 6H), 7.9 (s, 1H). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 17.9, 23.1, 25.7, 31.0, 33.7, 54.2, 55.7, 62.0, 67.2, 69.3, 70.2, 75.5, 106.5, 121.4, 122.6, 123.4, 124.6, 124.7, 127.4, 129.3, 129.8, 130.4, 131.6, 133.2, 155.4, 159.47, 159.52, 167.08,170.01. EIMS $m/z$: 532.59 [M$^+$ + Na]. IR (KBr) $\nu_{max}$cm$^{-1}$: 3456, 2912, 2867, 1728, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615.

(Z)-3-(2-((N-phenylacetamide)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl) acrylic acid: 100

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 1.6 (s, 3H), 1.7 (s, 3H), 3.1 (m, 2H), 3.5 (s, 3H), 3.7 (s, 3H), 4.9 (m, 3H), 5.9 (m, 1H), 6.5 (d, 1H, $J=8.5$ Hz), 6.9 (m, 1H), 7.4 (m, 3H), 7.6 (d, 2H, $J=8.6$ Hz), 7.9 (s, 1H). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 17.8, 25.6, 27.1, 29.2, 55.6, 63.4, 78.9, 106.5, 118.5, 119.7, 120.7, 121.3, 121.5, 121.8, 122.6, 123.3, 129.18, 131.5, 132.4, 139.7, 144.2, 155.2, 159.5, 169.6, 171.4. EIMS $m/z$: 476.52 [M$^+$ + Na]. IR (KBr) $\nu_{max}$cm$^{-1}$: 2967, 2851, 1767, 1634, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615.
(Z)-3-((1-phenylethanol)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid: 101

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 1.7 (s, 3H), 1.8 (s, 3H), 2.5 (s, 1H), 3.4 (s, 2H), 3.9 (s, 3H), 4.5 (m, 4H), 5.1 (m, 2H), 5.9 (m, 1H), 6.5 (d, 1H, $J=8.5$ Hz), 6.9 (m, 1H), 7.4 (m, 4H), 7.6 (d, 2H, $J = 8.6$ Hz), 7.9 (s, 1H). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 18.0, 25.7, 27.2, 31.9, 55.7, 62.1, 73.5, 73.6, 78.9, 106.5, 118.5, 119.7, 120.7, 121.3, 121.5, 121.8, 122.5, 123.3, 129.1, 131.5, 132.4, 139.7, 144.2, 155.1, 159.5, 169.5. EIMS $m/z$: 463.21 [M$^+$ + Na]. IR (KBr) $\nu_{max} cm^{-1}$: 3423, 2956, 2867, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615.

(Z)-3-((1-(benzo[d][1,3]dioxol-6-yl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid: 102

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 1.7 (s, 3H), 1.8, (s, 3H), 3.3 (m, 2H), 3.9 (s, 3H), 4.5 (m, 1H), 5.1 (m, 2H), 5.3 (s, 1H), 6.1 (m, 3H), 6.7 (d, 1H, $J=8.5$ Hz), 6.9 (m, 1H), 7.4 (m, 2H), 7.6 (m, 1H), 7.9 (s, 1H). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 17.8, 23.0, 25.6, 29.6, 55.6, 67.6, 75.4, 77.3, 101.5, 102.6, 106.4, 108.7, 114.1, 121.2, 122.5, 122.6, 123.2, 129.4, 131.5, 131.6, 147.9, 148.5, 155.5, 159.6, 169.1. EIMS $m/z$: 463.48 [M$^+$ + Na]. IR (KBr) $\nu_{max} cm^{-1}$: 3467, 2988, 2851, 1726, 1645, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615.

(Z)-3-((1phenylmethanol)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid: 103

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 1.7 (s, 3H), 1.8 (s, 3H), 3.3 (m, 2H), 3.9 (s, 3H), 4.6 (m, 2H), 5.1 (m, 2H), 5.3 (s, 1H), 5.9 (m, 1H), 6.8 (m, 1H), 7.3 (m, 2H), 7.5 (m, 2H), 7.7 (m, 2H), 7.9 (s, 1H). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 17.1, 22.9, 25.3, 29.4, 55.4, 66.9, 75.2, 77.3, 106.3, 119.0, 119.8, 121.8, 122.4, 123.0, 123.2, 126.8, 128.9, 129.5, 131.3, 131.4, 136.7, 143.3, 155.0, 159.1, 169.2. EIMS $m/z$: 449.50 [M$^+$ + Na]. IR (KBr) $\nu_{max} cm^{-1}$: 3432, 2923, 2851, 1745, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615.
(Z)-3-(2-((1-(2-iodophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid: 104

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 1.7 (s, 3H), 1.8 (s, 3H), 3.3 (m, 2H), 3.8 (s, 3H), 5.1 (m, 2H), 5.3 (s, 2H), 5.9 (m, 1H), 6.8 (m, 1H), 7.3 (m, 2H), 7.5 (m, 2H), 7.7 (m, 2H), 7.9 (s, 1H). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 17.1, 29.4, 22.9, 55.4, 66.9, 75.2, 77.3, 106.3, 119.0, 119.8, 121.8, 122.4, 123.0, 123.2, 126.8, 128.9, 129.5, 131.3, 131.4, 136.7, 143.3, 155.0, 159.1, 169.2. EIMS $m/z$: 545.37 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 2923, 2851, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615.

(Z)-3-(2-((N-phenylbenzamide)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid: 105

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 1.7 (s, 3H), 1.8 (s, 3H), 3.3 (m, 2H), 3.8 (s, 3H), 5.1 (m, 2H), 5.3 (s, 1H), 5.9 (m, 1H), 6.9 (m, 2H), 7.2 (m, 4H), 7.6 (m, 4H), 7.8 (m, 2H), 8.1 (s, 1H). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 17.99, 24.57, 24.85, 56.79, 59.78, 108.33, 113.69, 119.51, 121.89, 123.01, 129.10, 131.68, 132.48, 133.12, 134.93, 136.00, 159.98, 161.02, 169.58. EIMS $m/z$: 538.39 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 3446, 2956, 2851, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615.

(Z)-3-(2-((3-(3-methoxyphenyl)-3H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid: 107

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 1.7 (s, 3H), 1.8 (s, 3H), 3.2 (m, 2H), 3.8 (s, 3H), 3.9 (s, 3H), 5.1 (m, 2H), 5.3 (m, 1H), 5.9 (m, 1H), 6.7 (m, 1H), 7.3 (m, 2H), 7.5 (m, 2H), 7.7 (m, 2H), 7.9 (s, 1H). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 17.99, 24.57, 24.85, 56.79, 59.78, 108.07, 113.21, 113.69, 118.30, 119.51, 121.89, 123.01, 129.10, 131.11, 131.61, 132.48, 133.10, 136.00, 137.84, 159.25, 161.02, 170.08. EIMS $m/z$: 449.20 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 3456, 2956, 2851, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615.

(Z)-3-(2-((3-(2-methoxyphenyl)-3H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid: 108

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 1.7 (s, 3H), 1.8 (s, 3H), 3.2 (m, 2H), 3.8 (s, 3H), 3.9 (s, 3H), 5.1 (m, 2H), 5.3 (m, 1H), 5.9 (d, 1H, $J=8.3$ Hz), 6.7 (m, 1H), 7.2 (m, 2H), 7.4
(m, 1H), 7.6 (m, 1H), 7.8 (d, 1H, J=8.2 Hz), 8.1 (s, 1H). $^{13}$C NMR (CDCl$_3$, 100 MHz) δ: 17.10, 22.97, 29.4, 55.7, 55.9, 66.2, 67.5, 73.3, 106.3, 119.0, 121.8, 122.4, 123.0, 123.2, 126.8, 128.9, 129.5, 139.2, 141.0, 143.0, 151.1, 155.7, 159.7, 166.2. EIMS m/z: 449.20 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 3476, 2920, 1763, 1598, 1457, 1298, 1039, 947, 852, 808, 750, 699, 642.

(Z)-3-((diphenylphosphonate)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid:: 109

$^1$H NMR (CDCl$_3$, 400 MHz) δ: 1.7 (s, 3H), 1.8 (s, 3H), 3.2 (m, 2H), 3.9 (s, 3H), 4.5 (m, 2H), 5.2 (m, 1H), 5.9 (d, 1H, J=8.6 Hz), 6.9 (m, 1H), 7.2-7.6 (m, 11H), 7.7 (d, 1H, J=8.6 Hz), 8.0 (s, 1H). $^{13}$C NMR (CDCl$_3$, 100 MHz) δ: 17.97, 23.41, 25.76, 55.74, 62.18, 106.65, 107.64, 112.85, 117.58, 120.23, 120.31, 122.33, 124.65, 126.15, 129.39, 129.52, 131.59, 131.94, 142.38, 155.76, 159.57, 160.64, 170.01. EIMS m/z: 575.55 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 3436, 2927, 1766, 1600, 1460, 1314, 1127, 1041, 856, 787, 710, 666, 637, 564.

(Z)-3-(7-(3-methylbut-2-enyloxy)-6-methoxybenzofuran-5-yl)acrylic acid :110

$^1$H NMR (CDCl$_3$, 200 MHz) δ: 1.82 (6H, s), 3.80 (s, 3H), 4.96 (2H, d, J=7.2 Hz), 5.62 (1H, t, J= 7.2 Hz), 5.98 (1H, d, J=12.4 Hz), 6.74 (1H, d, J=2.14 Hz), 6.9 (1H, s), 7.25 (1H, d, J=12.4 Hz), 7.5 (1H, d, J=2.14 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) δ: 14.39, 18.0, 25.6, 62.26, 69.9, 106.48, 117.48, 120.25, 121.53, 122.94, 137.99, 140.85, 144.74, 156.26, 157.09, 167.73. EIMS m/z: 302.32 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 1718, 1595, 1497, 1383, 1360.

(Z)-3-(7-(3-methylbut-2-enyloxy)-6-ethoxybenzofuran-5-yl)acrylic acid: 111

$^1$H NMR (CDCl$_3$, 200 MHz) δ: 1.2 (3H, t, J=7.1 Hz), 1.82 (6H, s), 4.1 (2H, q, J=6.9 Hz), 4.96 (2H, d, J=7.2 Hz), 5.62 (1H, t, J= 7.2 Hz), 5.98 (1H, d, J=12.4 Hz), 6.74 (1H, d, J=2.14 Hz), 6.9 (1H, s), 7.25 (1H, d, J=12.4 Hz), 7.5 (1H, d, J=2.14 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) δ: 14.39, 15.68, 25.6, 65.44, 69.26, 106.84, 113.24, 117.19, 119.41, 120.24, 122.94, 124.11, 137.99, 140.13, 144.46, 152.19, 157.92, 167.57. EIMS m/z: 316.35 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 1718, 1595, 1497, 1383, 1360.
(Z)-3-(7-(3-methylbut-2-enyloxy)-6-(allyloxy)benzofuran-5-yl)acrylic acid: 112

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 1.82 (6H, s), 4.51 (2H, d, $J$=6.47 Hz), 4.96 (2H, d, $J$=7.2 Hz), 5.34 (2H, m), 5.62 (1H, t, $J$= 7.2 Hz), 5.88 (1H, m), 5.98 (1H, d, $J$=12.4 Hz), 6.74 (1H, d, $J$=2.14 Hz), 6.9 (1H, s), 7.25 (1H, d, $J$=12.4 Hz), 7.5 (1H, d, $J$=2.14 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 15.68, 25.6, 69.26, 74.32, 106.84, 113.24, 117.19, 119.41, 120.24, 122.94, 124.11, 133.40, 137.99, 140.13, 144.46, 152.19, 155.92, 167.60. EIMS m/z: 328.36 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 1718, 1595, 1497, 1383, 1360.

(Z)-3-(7-(3-methylbut-2-enyloxy)-6-(prop-2-ynyloxy)benzofuran-5-yl)acrylic acid: 113

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 1.79 (6H, s), 2.48 (1H, s), 4.76 (2H, d, $J$=2.4 Hz), 4.96 (2H, d, $J$=7.2 Hz), 5.98 (1H, d, $J$=12.4 Hz), 6.74 (1H, d, $J$=2.14 Hz), 6.9 (1H, s), 7.25 (1H, d, $J$=12.4 Hz), 7.5 (1H, d, $J$=2.14 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 17.68, 25.6, 59.70, 69.26, 78.06, 79.75, 106.84, 113.24, 117.19, 120.24, 122.94, 124.11, 133.40, 137.99, 140.13, 144.46, 152.19, 155.92, 167.60. EIMS m/z: 326.34 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 1718, 1595, 1497, 1383, 1360.

(Z)-3-(7-(3-methylbut-2-enyloxy)-6-butoxybenzofuran-5-yl)acrylic acid: 114

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 0.92 (3H, t, $J$=7.3 Hz), 1.26 (2H, m), 1.82 (6H, s), 1.8-1.92 (2H, m), 4.12 (2H, q, $J$=6.9 Hz), 4.96 (2H, d, $J$=7.2 Hz), 5.62 (1H, t, $J$= 7.2 Hz), 5.98 (1H, d, $J$=12.4 Hz), 6.74 (1H, d, $J$=2.14 Hz), 6.9 (1H, s), 7.25 (1H, d, $J$=12.4 Hz), 7.5 (1H, d, $J$=2.14 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 17.47, 22.87, 29.29, 33.47, 35.89, 57.32, 77.75, 99.99, 110.88, 116.56, 117.22, 123.73, 128.07, 129.20, 143.32, 149.19, 150.76, 171.06. EIMS m/z: 344.4 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 1728, 1593, 1497, 1373, 1365.

(Z)-3-(7-(3-methylbut-2-enyloxy)-6-(hexyloxy)benzofuran-5-yl)acrylic acid: 115

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 0.92 (3H, t, $J$=7.3 Hz), 1.26-1.30 (4H, m), 1.82 (6H, s), 1.8-1.92 (4H, m), 4.12 (2H, q, $J$=6.9 Hz), 4.96 (2H, d, $J$=7.2 Hz), 5.62 (1H, t, $J$= 7.2 Hz), 5.98 (1H, d, $J$=12.4 Hz), 6.74 (1H, d, $J$=2.14 Hz), 6.9 (1H, s), 7.25 (1H, d, $J$=12.4 Hz), 7.5 (1H, d, $J$=2.14 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 17.39, 22.87, 29.29, 33.47, 35.89, 57.32, 77.75, 99.99, 110.88, 116.56, 117.22, 123.73, 128.07, 129.20, 143.32, 149.19, 150.76, 171.06. EIMS m/z: 332.4 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 1728, 1593, 1497, 1373, 1365.
120.24, 122.94, 124.11,137.99, 140.13, 144.46, 152.19, 154.92, 167.57. EIMS m/z: 372.47 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 1712, 1595, 1498, 1376, 1312.

(Z)-3-(7-(3-methylbut-2-enyloxy)-6-(benzyloxy)benzofuran-5-yl)acrylic acid: 116

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 1.82 (6H, s), 4.96 (2H, d, $J=7.2$ Hz), 5.2 (2H, s), 5.62 (1H, t, $J= 7.2$ Hz), 5.98 (1H, d, $J=12.4$ Hz), 6.74 (1H, d, $J=2.14$ Hz), 6.9 (1H, s), 7.12 (5H, m), 7.25 (1H, d, $J=12.4$ Hz), 7.5 (1H, d, $J=2.14$ Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 17.99, 25.6, 69.9, 70.59, 106.48, 113.24, 117.48, 120.25, 120.83, 121.53, 122.94, 127.14, 128.53, 137.99, 140.85, 144.74, 156.26, 157.09, 167.73. EIMS m/z: 378.42 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 1725, 1589, 1497, 1383, 1355.

(Z)-3-(2-ethoxy-4-methoxyphenyl)acrylic acid: 118

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 1.43 (6H, t, $J=6.69$ Hz), 3.78(3H, s), 4.07(2H, q, $J=7.01$Hz), 6.25 (1H, d, $J=12.61$ Hz), 6.45-6.50 (2H, m), 7.32 (1H, d, $J=12.59$ Hz), 7.78 (1H, d, $J=8.3$& 2.34 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 13.39, 56.03, 64.52, 102.56, 107.48, 114.24, 116.25, 130.13, 133.25, 159.49, 162.01, 167.73. EIMS m/z: 222.24 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 1732, 1610, 1501, 1383, 1356.

(Z)-3-(2-(allyloxy)-4-methoxyphenyl)acrylic acid: 119

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 3.78(3H, s), 4.5 (2H, m), 5.32 (2H, m), 6.03 (1H, m), 6.25 (1H, d, $J=12.61$ Hz), 6.45-6.50 (2H, m), 7.32 (1H, d, $J=12.59$ Hz), 7.76 (1H, d, $J=8.34$& 2.34 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 56.07, 69.32, 99.52, 105.37, 112.98, 116.48, 117.75, 132.14, 133.96, 138.36, 157.72, 160.68, 169.07. EIMS m/z: 234.09 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 1726, 1595, 1497, 1382, 1365.

(Z)-3-(4-methoxy-2-(prop-2-ynyloxy)phenyl)acrylic acid: 120

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 2.61 (1H, s), 3.72(3H, s), 4.70 (2H, s), 6.25 (1H, d, $J=12.58$ Hz), 6.45-6.50 (2H, m), 7.32 (1H, d, $J=14.15$ Hz), 7.60 (1H, d, $J=8.34$& 2.34 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 56.22, 59.28, 77.84, 78.24, 101.92, 108.07, 112.98, 117.20, 129.07, 132.24, 155.68, 161.10, 168.07. EIMS m/z: 232.23 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 1718, 1595, 1497, 1383, 1360.
(Z)-3-(2-butoxy-4-methoxyphenyl)acrylic acid: 121

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 1.05 (3H, t, $J=7.26$ Hz), 1.54 (4H, m), 3.78(3H, s), 4.01 (2H, m), 5.80 (1H, d, $J=12.64$ Hz), 6.45 (2H, m), 7.21 (1H, d, $J=12.63$ Hz), 7.79 (1H, d, $J=8.34$ Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 14.13, 20.05, 30.70, 56.22, 59.28, 99.52, 105.37, 112.98, 117.48, 129.07, 133.96, 157.72, 160.68, 169.07. EIMS $m/z$: 250.29 [M$^+$ + Na]. IR (KBr) $\nu$ max cm$^{-1}$: 1724, 1598, 1492, 1381, 1360.

(Z)-3-(2-(hexyloxy)-4-methoxyphenyl)acrylic acid: 122

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 0.93 (3H, t, $J=6.50$ Hz), 1.33 (6H, m), 1.77(2H, m), 3.78(3H, s), 3.97 (2H, m), 5.80 (1H, d, $J=12.64$ Hz), 6.45 (2H, m), 7.21 (1H, d, $J=12.63$ Hz), 7.74 (1H, d, $J=8.44$ Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 14.02, 22.57, 22.59, 25.70, 40.50, 56.22, 68.21, 99.03, 103.19, 113.04, 117.48, 129.07, 132.26, 158.55, 161.67, 170.37. EIMS $m/z$: 278.34 [M$^+$ + Na]. IR (KBr) $\nu$ max cm$^{-1}$: 1723, 1596, 1497, 1383, 1360.

(Z)-3-(2-(benzyloxy)-4-methoxyphenyl)acrylic acid: 123

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 3.78(3H, s), 5.13 (2H, s), 6.28 (1H, d, $J=12.64$ Hz), 6.93 (2H, m), 7.21 (1H, d, $J=12.63$ Hz), 7.43 (5H, m), 7.66 (1H, d, $J=8.44$ Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 56.22, 70.52, 99.03, 101.93, 112.74, 113.23, 117.48, 127.51, 128.38, 128.80, 135.78, 143.36, 155.82, 161.14, 161.88, 167.94.EIMS $m/z$: 360.40 [M$^+$ + Na]. IR (KBr) $\nu$ max cm$^{-1}$: 1728, 1588, 1490, 1383, 1372.

(Z)-3-(2,4-dimethoxyphenyl)acrylic acid: 124

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 3.85 (s, 6H), 6.25 (1H, d, $J=12.61$ Hz), 6.45-6.50 (2H, m), 7.32 (1H, d, $J=12.59$ Hz), 7.78 (1H, d, $J=8.3& 2.34$ Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 56.04, 56.79, 98.56, 106.48, 114.24, 118.25, 130.13, 133.25, 159.49, 162.01, 167.73. EIMS $m/z$: 208.076 [M$^+$ + Na]. IR (KBr) $\nu$ max cm$^{-1}$: 1712, 1585, 1480, 1383, 1360, 1280.

(Z)-3-(2,4-diethoxyphenyl)acrylic acid: 125

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 1.43 (m, 6H), 4.07(m, 4H), 6.25 (1H, d, $J=12.61$ Hz), 6.45-6.50 (2H, m), 7.32 (1H, d, $J=12.59$ Hz), 7.78 (1H, d, $J=8.3& 2.34$ Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 13.39, 14.02, 63.26, 64.9, 102.56, 107.48, 114.24, 116.25,
130.13, 133.25, 159.49, 162.01, 167.73. EIMS \textit{m/z}: 236.25 [M$^+$ + Na]. IR (KBr) $v_{\text{max}}$ cm$^{-1}$: 1720, 1595, 1497, 1383, 1360.

**(Z)-3-(2,4-bis(allyloxy)phenyl)acrylic acid: 126**

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 4.5 (4H, m), 5.32 (4H, m), 6.03 (2H, m), 6.25 (1H, d, $J$=12.61 Hz), 6.45-6.50 (2H, m), 7.32 (1H, d, $J$=12.59 Hz), 7.76 (1H, d, $J$=8.34 & 2.34 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 68.03, 69.32, 99.52, 105.37, 112.98, 117.48, 117.75, 117.88, 118.32, 132.14, 133.96, 138.36, 157.72, 160.68, 169.07 EIMS \textit{m/z}: 260.19 [M$^+$ + Na]. IR (KBr) $v_{\text{max}}$ cm$^{-1}$: 1732, 1582, 1490, 1383, 1346.

**(Z)-3-(2,4-bis(prop-2-ynyloxy)phenyl)acrylic acid: 127**

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 2.61 (2H, m), 4.70-4.77 (4H, m), 6.25 (1H, d, $J$=12.58 Hz), 6.45-6.50 (2H, m), 7.32 (1H, d, $J$=14.15 Hz), 7.60 (1H, d, $J$=8.34 & 2.34 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 56.22, 59.28, 77.84, 78.24, 101.92, 108.07, 112.98, 117.20, 129.07, 132.24, 143.61, 144.18, 155.68, 161.10, 168.07. EIMS \textit{m/z}: 256.08 [M$^+$ + Na]. IR (KBr) $v_{\text{max}}$ cm$^{-1}$: 1718, 1620, 1595, 1497, 1383, 1360.

**(Z)-3-(2,4-dibutoxyphenyl)acrylic acid: 128**

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 1.05 (6H, t, $J$=7.26 Hz), 1.54 (4H, m), 1.73(4H, m), 4.01 (4H, m), 5.80 (1H, d, $J$=12.64 Hz), 6.45 (2H, m), 7.21 (1H, d, $J$=12.63 Hz), 7.79 (1H, d, $J$=8.34 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 13.80, 19.19, 19.36, 30.74, 67.78, 68.09, 99.12, 104.66, 116.47, 117.00, 132.12, 138.66, 158.38, 161.42, 169.25. EIMS \textit{m/z}: 292.17 [M$^+$ + Na]. IR (KBr) $v_{\text{max}}$ cm$^{-1}$: 1728, 1625, 1595, 1497, 1383, 1340.

**(Z)-3-(2,4-bis(hexyloxy)phenyl)acrylic acid: 129**

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 0.93 (6H, t, $J$=6.50 Hz), 1.33 (12H, m), 1.77(4H, m), 3.97 (4H, m), 5.80 (1H, d, $J$=12.64 Hz), 6.45 (2H, m), 7.21 (1H, d, $J$=12.63 Hz), 7.74 (1H, d, $J$=8.44 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 14.02, 22.57, 22.59, 25.70, 25.74, 40.50, 68.07, 68.21, 99.03, 103.19, 113.04, 132.26, 140.28, 158.55, 161.67, 170.37. EIMS \textit{m/z}: 348.48 [M$^+$ + Na]. IR (KBr) $v_{\text{max}}$ cm$^{-1}$: 1726, 1620, 1595, 1497, 1383, 1360.
(Z)-3-(2,4-bis(benzyloxy)phenyl)acrylic acid: 130

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 5.13 (2H, s), 5.30 (2H, s), 6.28 (1H, d, $J$=12.64 Hz), 6.93 (2H, m), 7.21 (1H, d, $J$=12.63 Hz), 7.43 (10H, m), 7.66 (1H, d, $J$=8.44 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 70.52, 101.93, 112.74, 113.23, 127.51, 128.38, 128.80, 135.78, 143.36, 155.82, 161.14, 161.88, 167.94. EIMS $m/z$: 360.40 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 1721, 1624, 1595, 1497, 1383, 1360.

(Z)-3-(6,7-dimethoxybenzofuran-5-yl)acrylic acid: 131

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 3.69 (3H, s), 3.77 (3H, s), 6.05 (1H, d, $J$=12.51 Hz), 6.72 (1H, d, $J$=2.17 Hz), 7.19 (1H, s), 7.29 (1H, d, $J$=12.94 Hz), 7.59 (1H, d, $J$=2.15 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 56.6, 56.7, 106.20, 112.24, 117.26, 122.81, 125.06, 133.00, 133.60, 147.10, 148.65, 167.60. EIMS $m/z$: 248.23 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 1716, 1618, 1595, 1497, 1383, 1360.

(Z)-3-(6,7-diethoxybenzofuran-5-yl)acrylic acid: 132

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 1.01 (6H, m), 4.0-4.28 (4H, m), 6.03 (1H, d, $J$=12.56 Hz), 6.71 (1H, d, $J$=2.16 Hz), 7.19 (1H, s), 7.29 (1H, d, $J$=13.04 Hz), 7.60 (1H, d, $J$=2.17 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 13.62, 14.02, 59.61, 60.15, 107.20, 112.24, 117.26, 122.81, 124.06, 133.00, 133.60, 143.16 147.10, 150.65, 168.60. EIMS $m/z$: 276.08 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 1718, 1600, 1497, 1383, 1360.

(Z)-3-(6,7-bis(allyloxy)benzofuran-5-yl)acrylic acid: 133

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 4.77 (4H, m), 5.30 (4H, m), 6.03 (1H, d, $J$=12.56 Hz), 6.06 (2H, m), 6.71 (1H, d, $J$=2.14 Hz), 7.21 (1H, s), 7.29 (1H, d, $J$=13.04 Hz), 7.60 (1H, d, $J$=2.17 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 68.03, 69.32, 76.03, 76.35, 78.79, 106.18, 117.48, 117.75, 117.88, 118.32, 122.81, 124.06, 132.14, 133.96, 143.16, 147.10, 150.65, 167.94. EIMS $m/z$: 300.11 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 1722, 1605, 1497, 1383, 1360.

(Z)-3-(6,7-bis(prop-2-ynyloxy)benzofuran-5-yl)acrylic acid: 134

$^1$H NMR (CDCl$_3$, 200 MHz) $\delta$: 2.31 (2H, m), 4.16 (4H, m), 6.10 (1H, d, $J$=13.64 Hz), 6.83 (1H, d, $J$=1.80 Hz), 7.24 (1H, s), 7.30 (1H, d, $J$=12.45 Hz), 7.62 (1H, d, $J$=1.87 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 61.11, 67.93, 76.03, 76.35, 78.79, 106.18,
112.47, 119.20, 123.49, 126.90, 129.19, 139.56, 140.55, 144.98, 146.78, 172.24.
EI/MS m/z: 296.27 [M⁺ + Na]. IR (KBr) νmax cm⁻¹: 1725, 1600, 1505, 1383, 1360.

(Z)-3-(6,7-dibutoxybenzofuran-5-yl)acrylic acid: 135

¹H NMR (CDCl₃, 200 MHz) δ: 0.96(6H, m), 1.51(4H, m), 1.79(4H, m), 4.00 (2H, t, J=6.54 Hz), 4.33 (2H, t, J=6.56), 6.03(1H, d, J=12.56 Hz), 6.70 (1H, d, J=2.20 Hz), 7.26 (1H, s), 7.36(1H, d, J=12.63 Hz), 7.56 (1H, d, J=2.27 Hz). ¹³C NMR (CDCl₃, 100MHz) δ: 13.91, 14.05, 20.11, 22.99, 30.05, 31.44, 74.73, 77.25, 106.92, 116.93, 118.66, 125.29, 137.52, 140.25, 145.74, 148.09, 170.81. EI/MS m/z: 332.16 [M⁺ + Na]. IR (KBr) νmax cm⁻¹: 1728, 1600, 1497, 1383, 1362.

(Z)-3-(6,7-bis(hexyloxy)benzofuran-5-yl)acrylic acid: 136

¹H NMR (CDCl₃, 200 MHz) δ: 0.81(6H, m), 1.45-1.75(16H, m), 3.92 (2H, t, J=6.62 Hz), 4.22 (2H, t, J=6.54), 5.90(1H, d, J=12.59 Hz), 6.61 (1H, d, J=2.15 Hz), 6.87 (1H, s), 7.47(1H, d, J=12.43 Hz), 7.54(1H, d, J=2.09 Hz). ¹³C NMR (CDCl₃, 100MHz) δ: 14.04, 14.11, 20.58, 22.30, 22.42, 29.70, 31.19, 33.82, 71.22, 75.06, 107.10, 114.08, 116.19, 124.51, 125.24, 137.51, 139.28, 142.15, 145.14, 146.99, 148.10, 171.19. EI/MS m/z: 332.16 [M⁺ + Na]. IR (KBr) νmax cm⁻¹: 1718, 1595, 1490, 1380, 1360.

(Z)-3-(6,7-bis(benzyloxy)benzofuran-5-yl)acrylic acid: 137

¹H NMR (CDCl₃, 200 MHz) δ: 5.04(2H, s), 5.39(2H, s), 5.97(1H, d, J=12.51 Hz), 6.71 (1H, d, J=2.06 Hz), 7.15 (1H, s), 7.31(10H, m), 7.50(1H, d, J=12.67 Hz), 7.61(1H, d, J=2.04 Hz). ¹³C NMR (CDCl₃, 100MHz) δ: 75.13, 76.45, 107.19, 116.62, 117.05, 118.88, 126.62, 127.08, 127.91, 128.07, 128.31, 128.43, 128.47, 128.87, 137.08, 137.23, 137.26, 139.75, 141.95, 145.41, 147.94, 170.29. EI/MS m/z: 400.42 [M⁺ + Na]. IR (KBr) νmax cm⁻¹: 1718, 1605, 1492, 1383, 1354.

(E)-ethyl 3-(2-ethoxyphenyl)acrylate: 138

¹H NMR (CDCl₃, 500 MHz) δ: 1.42-1.36 (6H, t, J= 6.9Hz), 1.60- 3.98 (2H, q, J= 6.8Hz), 4.31- 4.19 (2H, q, J=7.1Hz), 6.58 (1H, d, J=16.1 Hz), 6.9 (1H, d, J=7.5 Hz), 6.95 (1H, t, J=7.5 Hz), 7.34 (1H, m), 7.52 (1H, d, J=7.5 Hz), 8.1 (1H, d, J=16.1 Hz). ¹³C NMR (CDCl₃, 100 MHz) δ: 14.08, 14.53, 60.51, 63.99, 112.08, 118.20, 120.52, 123.15, 128.89, 131.57, 140.60, 141.00, 157.72, 169.91. EI/MS m/z: 220.26 [M⁺ + Na]. IR (KBr) νmax cm⁻¹: 3065, 2834, 1724, 1607, 1565, 1542, 1497, 1403, 1375, 989.
(E)-allyl 3-(2-(allyloxy)phenyl)acrylate: 139

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 4.6 (2H, d, $J=3.8$Hz), 4.7 (2H, d, $J=5.9$Hz), 5.5 (4H, m), 6.1 (2H, m), 6.58 (1H, d, $J=16.1$ Hz), 6.9 (1H, d, $J=7.5$ Hz), 6.95 (1H, t, $J=7.5$ Hz), 7.34 (1H, m), 7.52 (1H, d, $J=7.5$ Hz), 8.1 (1H, d, $J=16.1$ Hz). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 66.06, 70.12, 113.06, 117.81, 118.60, 120.61, 121.17, 125.68, 131.73, 131.74, 133.60, 134.77, 140.71, 157.62, 167.68. EIMS $m/z$: 244.11 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 3060, 2853, 1731, 1607, 1565, 1542, 1497, 1375, 989, 912, 831.

(E)-butyl 3-(2-butoxyphenyl)acrylate: 140

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 0.98 (m, 6H), 1.6 (m, 2H), 1.6 (m, 2H), 1.7 (m, 2H), 1.9 (2H, m), 4.0 (2H, t, $J=2.5$ Hz), 4.2 (2H, t, $J=2.5$ Hz), 6.60 (1H, d, $J=16.1$ Hz), 6.92 (1H, d, $J=7.5$ Hz), 6.96 (1H, t, $J=7.5$ Hz), 7.34 (1H, m), 7.52 (1H, d, $J=7.5$ Hz), 8.1 (1H, d, $J=16.1$ Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 13.70, 13.87, 19.13, 19.31, 30.57, 31.30, 64.00, 67.97, 111.26, 119.66, 124.33, 130.29, 130.69, 131.79, 139.18, 156.76, 166.53. EIMS $m/z$: 276.17 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 2960, 2925, 1725, 1640, 1607, 1474, 1440, 1385, 1371, 1300, 1270, 1262, 1161, 1115, 1040, 922, 830, 741, 626.

(E)-isopropyl 3-(2-isopropoxyphenyl)acrylate: 141

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 1.3 (6H, d, $J=6.2$ Hz), 1.4 (6H, d, $J=6.4$ Hz), 4.6 (1H, m), 5.1 (1H, m), 6.60 (1H, d, $J=16.1$ Hz), 6.92 (1H, d, $J=7.5$ Hz), 6.96 (1H, t, $J=7.5$ Hz), 7.34 (1H, m), 7.52 (1H, d, $J=7.5$ Hz), 8.1 (1H, d, $J=16.1$ Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 21.65, 21.99, 22.44, 22.65, 69.97, 74.14, 116.01, 118.76, 120.81, 126.93, 128.79, 130.69, 140.55, 156.87, 167.52. EIMS $m/z$: 248.14 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 2935, 2837, 1681, 1656, 1620, 1593, 1542, 1511, 1485, 1462, 1441, 1386, 1365, 1273, 1247, 1170, 1090, 1019, 830, 804, 610, 559.

(E)-prop-2-ynyl 3-(2-(prop-2-ynyloxy)phenyl)acrylate: 142

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 2.48 (1H, s), 2.52 (1H, s), 4.76 (2H, d, $J=2.4$ Hz), 4.8 (2H, d, $J=2.3$ Hz), 6.10 (1H, d, $J=16.1$ Hz), 7.0 (1H, d, $J=7.5$ Hz), 6.96 (1H, t, $J=7.5$ Hz), 7.34 (1H, m), 7.29 (1H, m), 8.1 (1H, d, $J=16.1$ Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 51.63, 56.09, 74.92, 76.01, 111.94, 117.64, 120.88, 124.55, 129.09, 131.50, 140.39,
156.26, 166.68. EIMS m/z: 240.08 [M⁺ + Na]. IR (KBr) ν<sub>max</sub> cm<sup>-1</sup>: 2941, 1682, 1608, 1593, 1486, 1425, 1384, 1366, 1314, 1266, 1162, 1118, 1088.

**(E)-propyl 3-(2-propanoxyphenyl)acrylate: 143**

**1H NMR** (CDCl<sub>3</sub>, 500 MHz) δ: 0.9 (3H, t, J=7.4 Hz), 1.1 (3H, t, J=7.4 Hz), 1.7 (2H, m), 1.88 (2H, m), 3.9 (2H, m), 4.1 (2H, m), 6.01 (1H, d, J=16.2 Hz), 6.9 (2H, m), 7.4 (1H, m), 7.52 (1H, d, J=1.4 Hz), 8.0 (1H, d, J=16.2 Hz). 13C NMR (CDCl<sub>3</sub>, 100MHz) δ: 10.39, 10.60, 22.03, 22.60, 65.76, 69.91, 111.35, 119.70, 124.36, 130.28, 130.72, 139.12, 156.77, 166.53. EIMS m/z: 248.14 [M⁺ + Na]. IR (KBr) ν<sub>max</sub> cm<sup>-1</sup>: 2960, 2925, 1725, 1640, 1607, 1474, 1440, 1385, 1371, 1305, 1270, 1262, 1161, 1115, 1040, 922, 830, 741, 626.

**(E)-isobutyl 3-(2-isobutoxyphenyl)acrylate: 144**

**1H NMR** (CDCl<sub>3</sub>, 500 MHz) δ: 0.9 (1H, d, J=6.1 Hz), 1.1(6H, d, J=6.6 Hz), 2.0 (1H, m), 2.2 (1H, m), 3.8 (2H, d, J=6.1 Hz), 4.0 (2H, d, J=6.6 Hz), 6.6 (1H, d, J=16.1 Hz), 6.9 (2H, m), 7.3 (1H, m), 7.4 (1H, m), 7.6 (1H, d, J=9.1 Hz), 8.1 (1H, d, J=16.1 Hz). 13C NMR (CDCl<sub>3</sub>, 100MHz) δ: 19.12, 19.32, 27.62, 28.30, 70.42, 74.57, 111.24, 116.87, 119.68, 124.48, 130.77, 139.36, 143.56, 156.82, 166.63. EIMS m/z: 276.37 [M⁺ + Na]. IR (KBr) ν<sub>max</sub> cm<sup>-1</sup>: 3065, 2853, 1731, 1607, 1565, 1542, 1497, 1403, 1375, 989, 912, 831.

**(E)-benzyl 3-(2-(benzyloxy)phenyl)acrylate: 145**

**1H NMR** (CDCl<sub>3</sub>, 500 MHz) δ: 5.2 (4H, s), 6.68 (1H, d, J=16.1 Hz), 6.9 (2H, m), 7.4 (11 H, m), 7.64 (1H, d, J=6.9 Hz), 8.20 (1H, d, J=16.1 Hz). 13C NMR (CDCl<sub>3</sub>, 100MHz) δ: 67.57, 71.43, 116.69, 117.56, 118.63, 120.94, 127.11, 127.73, 128.65, 129.76, 140.63, 143.63, 157.43, 167.21. EIMS m/z: 344.14 [M⁺ + Na]. IR (KBr) ν<sub>max</sub> cm<sup>-1</sup>: 3065, 1725, 1616, 1565, 1542, 1497, 1403, 1305, 1103, 989, 912, 831.

**(E)-ethyl 3-(6-ethoxybenzofuran-5-yl)acrylate: 146**

**1H NMR** (CDCl<sub>3</sub>, 500 MHz) δ: 1.2 (3H, t, J=7.1 Hz), 1.46 (3H, t, J=6.9 Hz), 4.13 (2H, q, J=6.9 Hz), 4.20 (2H, q, J=7.03Hz), 6.49 (1H, d, J=16.45 Hz), 6.74 (1H, d, J=2.14 Hz), 7.46 (1H, s), 7.52 (1H, d, J=2.14 Hz), 7.85 (1H, s), 8.15 (1H, d, J=16.11 Hz). 13C NMR (CDCl<sub>3</sub>, 100MHz) δ: 14.39, 14.67, 60.26, 64.49, 94.80, 106.48, 117.48, 120.25, 120.53, 120.94, 140.85, 144.74, 156.26, 157.09, 167.73. EIMS m/z:
260.29 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}} \text{cm}^{-1}$ 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

(E)-allyl 3-(6-(allyloxy)benzofuran-5-yl)acrylate: 147

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 4.51 (2H, d, $J$=6.47 Hz), 4.73 (2H, d, $J$=6.69 Hz), 5.34 (4H, m), 5.88 (2H, m), 6.49 (1H, d, $J$=16.17 Hz), 6.74 (1H, d, $J$=2.14 Hz), 7.00 (1H, s), 7.52 (1H, d, $J$=2.12 Hz), 7.74 (1H, s), 8.12 (1H, d, $J$=16.06 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 64.81, 69.63, 95.86, 106.43, 117.09, 117.55, 117.88, 120.68, 120.79, 132.60, 132.64, 140.50, 141.01, 144.87, 155.69, 156.93, 167.14. EIMS $m/z$: 284.31

(E)-butyl 3-(6-butoxybenzofuran-5-yl)acrylate: 148

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 0.92 (3H, t, $J$=7.3 Hz), 1.04 (3H, t, $J$=7.4 Hz), 1.26 (2H, m), 1.5 (6H, m), 1.8-1.92 (4H, m), 4.01 (2H, m), 4.20 (2H, m), 6.51 (1H, d, $J$=16.10 Hz), 6.74 (1H, d, $J$=2.22 Hz), 7.12 (1H, s), 7.57 (1H, d, $J$=2.12 Hz), 7.74 (1H, s), 8.20 (1H, d, $J$=16.12 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 13.78, 13.86, 14.13, 14.37, 31.13, 31.16, 60.23, 68.68, 95.44, 106.70, 117.47, 120.51, 120.74, 122.98, 140.66, 144.73, 156.39, 157.11, 167.74. EIMS $m/z$: 316.39 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}} \text{cm}^{-1}$ 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

(E)-propyl 3-(6-propoxybenzofuran-5-yl)acrylate: 149

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 0.92 (3H, t, $J$=7.3 Hz), 1.04 (3H, t, $J$=7.4 Hz), 1.26 (2H, m), 1.5 (4H, m), 4.01 (2H, m), 4.20 (2H, m), 6.51 (1H, d, $J$=16.10 Hz), 6.74 (1H, d, $J$=2.22 Hz), 7.12 (1H, s), 7.57 (1H, d, $J$=2.12 Hz), 7.74 (1H, s), 8.20 (1H, d, $J$=16.12 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 13.78, 14.13, 31.13, 32.16, 60.23, 68.68, 95.44, 106.70, 117.47, 120.51, 120.74, 122.98, 140.66, 144.73, 156.39, 157.11, 167.74. EIMS $m/z$: 288.34 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}} \text{cm}^{-1}$ 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

(E)-isobutyl 3-(6-isobutoxybenzofuran-5-yl)acrylate: 150

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 0.91 (1H, d, $J$=6.1 Hz), 1.1(6H, d, $J$=6.6 Hz), 2.0 (1H, m), 2.2 (1H, m), 3.8 (2H, d, $J$=6.1 Hz), 4.0 (2H, d, $J$=6.6 Hz), 6.51 (1H, d, $J$=16.09
Hz), 6.74 (1H, d, J=2.22 Hz), 7.12 (1H, s), 7.57 (1H, d, J=2.12 Hz), 7.74 (1H, s), 8.20 (1H, d, J=16.13 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 19.51, 19.83, 26.88, 27.62, 60.01, 75.34, 95.44, 106.46, 117.41, 120.57, 120.74, 122.98, 140.66, 144.73, 156.39, 157.11, 167.67. EIMS m/z: 316.39 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$ 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

(E)-isopropyl 3-(6-isopropoxybenzofuran-5-yl)acrylate: 151

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 1.3 (6H, d, J=6.2 Hz), 1.4 (6H, d, J= 6.4 Hz), 4.6 (1H, m), 5.1 (1H, m), 6.60 (1H, d, J=16.32 Hz), 6.74 (1H, d, J=2.22 Hz), 7.12 (1H, s), 7.57 (1H, d, J=2.12 Hz), 7.74 (1H, s), 8.20 (1H, d, J=16.06 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 21.65, 21.99, 22.44, 22.65, 69.97, 74.14, 95.44, 106.46, 117.41, 120.57, 120.74, 122.98, 140.66, 144.73, 156.39, 157.11, 167.67. EIMS m/z: 288.34 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$ 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

(E)-benzyl 3-(6-(benzyloxy)benzofuran-5-yl)acrylate: 152

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 5.2 (4H, s), 6.68 (1H, d, J=16.32 Hz), 6.90 (1H, d, J=2.22 Hz), 7.12 (11H, m), 7.57 (1H, d, J=2.12 Hz), 7.74 (1H, s), 8.20 (1H, d, J=16.13 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 60.25, 70.77, 96.25, 106.47, 117.69, 120.56, 120.67, 120.83, 127.14, 128.07, 128.53, 136.39, 140.47, 144.92, 155.70, 156.87, 167.53. EIMS m/z: 384.42 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$ 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

(E)-prop-2-ynyl 3-(6-(prop-2-yloxy)benzofuran-5-yl)acrylate: 153

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 2.48 (1H, s), 2.52 (1H, s), 4.76 (2H, d, J= 2.4 Hz), 4.8 (2H, d, J=2.3 Hz), 6.60 (1H, d, J=16.10 Hz), 6.74 (1H, d, J=2.22 Hz), 7.12 (1H, s), 7.57 (1H, d, J=2.12 Hz), 7.74 (1H, s), 8.20 (1H, d, J=16.06 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 56.52, 59.70, 78.06, 78.79, 79.75, 80.00, 95.44, 106.46, 117.41, 120.57, 120.74, 122.98, 140.66, 144.73, 156.39, 157.11, 167.67. EIMS m/z: 280.27 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$ 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.
**(E)-ethyl 3-(7-ethoxybenzofuran-6-y1)acrylate: 154**

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 1.66 (3H, t, $J=7.10$ Hz), 1.75 (3H, t, $J=7.07$ Hz), 4.2 (2H, q, $J=7.1$ Hz), 4.3 (2H, q, $J=7.0$ Hz), 6.5 (1H, d, $J=16.1$ Hz), 6.9 (1H, d, $J=2.2$ Hz), 7.4 (1H, d, $J=8.6$ Hz), 7.67 (1H, d, $J=2.2$ Hz), 8.18 (1H, d, $J=8.1$ Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 14.38, 15.68, 53.44, 69.26, 105.83, 106.84, 117.19, 119.41, 120.24, 124.11, 140.13, 144.46, 152.19, 157.92, 167.57. EIMS m/z: 260.21 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

**(E)-allyl 3-(7-(allyloxy)benzofuran-6-y1)acrylate: 155**

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 4.7 (2H, m), 4.8 (2H, m), 5.3 (2H, m), 5.4 (2H, m), 6.1 (1H, m), 6.2 (1H, m), 6.5 (1H, d, $J=16.1$ Hz), 6.9 (1H, d, $J=2.2$ Hz), 7.4 (1H, d, $J=8.6$ Hz), 7.67 (1H, d, $J=2.2$ Hz), 8.18 (1H, d, $J=16.1$ Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 60.27, 64.99, 105.10, 107.21, 117.93, 118.20, 118.67, 119.54, 120.38, 124.04, 133.23, 133.65, 140.42, 144.63, 151.96, 158.57, 167.06. EIMS m/z: 284.10 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

**(E)-butyl 3-(7-butoxybenzofuran-6-y1)acrylate: 156**

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 1.0 (6H, m), 1.49 (2H, m), 1.56 (2H, m), 1.8 (2H, m), 1.92 (2H, m), 4.21(2H, t, $J=6.66$ Hz), 4.26 (2H, t, $J=6.47$ Hz), 6.48 (1H, d, $J=16.2$ Hz), 6.92 (1H, d, $J=2.2$ Hz), 7.21 (1H, d, $J=8.7$ Hz), 7.48 (1H, d, $J=8.7$ Hz), 7.6 (1H, d, $J=2.2$ Hz), 8.18 (1H, d, $J=16.2$ Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 13.78, 13.87, 19.27, 30.86, 32.24, 64.19, 73.47, 105.27, 106.76, 117.16, 119.26, 120.11, 123.99, 139.96, 144.39, 152.41, 157.97, 167.62. EIMS m/z: 316.39 [M$^+$ + Na]. IR (KBr) $\nu_{max}$ cm$^{-1}$: 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

**(E)-propyl 3-(7-propoxybenzofuran-6-y1)acrylate: 157**

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 1.0 (6H, m), 1.8 (2H, m), 1.92 (2H, m), 4.21(2H, t, $J=6.66$ Hz), 4.26 (2H, t, $J=6.47$ Hz), 6.48 (1H, d, $J=16.2$ Hz), 6.92 (1H, d, $J=2.2$ Hz), 7.21 (1H, d, $J=8.7$ Hz), 7.48 (1H, d, $J=8.7$ Hz), 7.6 (1H, d, $J=2.2$ Hz), 8.18 (1H, d, $J=16.2$ Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 13.78, 13.87, 30.86, 32.24, 64.19, 73.47.
Chapter 2

Studies on modification and bio-evaluation of plant molecules

105.27, 106.76, 117.16, 119.26, 120.11, 123.99, 139.96, 144.39, 152.41, 157.97, 167.62. EIMS m/z: 288.35 [M^+ + Na]. IR (KBr) νmax cm⁻¹ 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

(E)-isobutyl 3-(7-isobutoxybenzofuran-6-yl)acrylate: 158

$^1$H NMR (CDCl$_3$, 500 MHz) δ: 1.04 (6H, d, J=6.5 Hz), 1.20 (6H, d, J=6.3 Hz), 2.02 (1H, m), 2.20 (1H, m), 4.0 (2H, d, J=6.5 Hz), 4.1 (2H, d, J=6.3 Hz), 6.3 (1H, d, J=16.1 Hz), 6.9 (1H, d, J=2.2 Hz), 7.22 (1H, d, J=8.6 Hz), 7.52 (1H, d, J=8.6 Hz), 7.6 (1H, d, J=2.2 Hz), 8.2 (1H, d, J=16.1 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) δ: 19.18, 19.25, 27.88, 28.25, 70.44, 79.98, 105.32, 106.67, 117.04, 119.05, 119.88, 123.85, 126.60, 139.85, 144.34, 152.43, 157.99, 167.56. EIMS m/z: 316.39 [M^+ + Na]. IR (KBr) νmax cm⁻¹ 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

(E)-isopropyl 3-(7-isopropoxybenzofuran-6-yl)acrylate: 159

$^1$H NMR (CDCl$_3$, 500 MHz) δ: 1.3 (6H, d, J=4.5 Hz), 1.4 (6H, d, J=5.1 Hz), 4.6 (1H, m), 5.2 (1H, m), 6.4 (1H, d, J=16.2 Hz), 6.8 (1H, d, J=2.2 Hz), 7.4 (1H, d, J=8.6 Hz), 7.66 (1H, d, J=2.2 Hz), 8.18 (1H, d, J=16.2 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) δ: 21.75, 22.00, 67.50, 76.60, 104.84, 106.64, 117.06, 120.46, 122.54, 123.57, 140.05, 144.55, 151.24, 157.66, 166.13. EIMS m/z: 288.34 [M^+ + Na]. IR (KBr) νmax cm⁻¹ 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

(E)-benzyl 3-(7-(benzyloxy)benzofuran-6-yl)acrylate: 160

$^1$H NMR (CDCl$_3$, 500 MHz) δ: 5.2 (4H, s), 6.3 (1H, d, J=16.1 Hz), 6.9 (1H, d, J=2.2 Hz), 7.22 (1H, d, J=8.6 Hz), 7.35 (10H, m), 7.52 (1H, d, J=8.6 Hz), 7.6 (1H, d, J=2.2 Hz), 8.2 (1H, d, J=16.1 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) δ: 66.56, 73.89, 106.98, 118.41, 120.69, 126.29, 127.93, 127.93, 129.74, 137.28, 140.46, 142.86, 144.03, 146.66, 168.04. EIMS m/z: 384.14 [M^+ + Na]. IR (KBr) νmax cm⁻¹ 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

(E)-prop-2-ynyl 3-(7-(prop-2-ynyloxy)benzofuran-6-yl)acrylate: 161

$^1$H NMR (CDCl$_3$, 500 MHz) δ: 2.48 (1H, s), 2.52 (1H, s), 4.76 (2H, d, J=2.4 Hz), 4.8 (2H, d, J=2.3 Hz), 6.3 (1H, d, J=16.1 Hz), 6.9 (1H, d, J=2.2 Hz), 7.22 (1H, d, J=8.6 Hz), 7.52 (1H, d, J=8.6 Hz), 7.6 (1H, d, J=2.2 Hz), 8.2 (1H, d, J=16.1 Hz). $^{13}$C NMR
(CDCl₃, 100MHz) δ: 56.52, 59.70, 78.06, 78.79, 79.75, 80.00, 105.10, 107.21, 117.93, 118.20, 118.67, 119.54, 120.38, 124.04, 133.23, 133.65, 140.42, 144.63, 151.96, 158.57, 167.06. EIMS m/z: 280.27 [M⁺ + Na]. IR (KBr) νmax cm⁻¹: 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

(E)-ethyl 3-(6-ethoxy-4-methoxybenzofuran-5-yl)acrylate: 162

¹H NMR (CDCl₃, 500 MHz) δ: 1.2 (3H, t, J=7.1 Hz), 1.46 (3H, t, J=6.9 Hz), 3.88 (s, 3H), 4.13 (2H, q, J=6.9 Hz), 4.20 (2H, q, J=7.03 Hz), 6.49 (1H, d, J=16.45 Hz), 6.74 (1H, d, J=2.14 Hz), 7.52 (1H, d, J=2.14 Hz), 7.78 (1H, s), 8.15 (1H, d, J=16.11 Hz).

¹³C NMR (CDCl₃, 100MHz) δ: 14.39, 14.67, 59.12, 60.26, 64.49, 94.80, 106.48, 117.48, 120.25, 120.94, 140.85, 144.74, 152.46, 156.26, 157.09, 167.73. EIMS m/z: 290.31 [M⁺ + Na]. IR (KBr) νmax cm⁻¹: 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

(E)-allyl 3-(6-(allyloxy)-4-methoxybenzofuran-5-yl)acrylate: 163

¹H NMR (CDCl₃, 500 MHz) δ: 3.83 (s, 3H), 4.51 (2H, d, J=6.47 Hz), 4.73 (2H, d, J=6.69 Hz), 5.34 (4H, m), 5.88 (2H, m), 6.49 (1H, d, J=16.17 Hz), 6.74 (1H, d, J=2.14 Hz), 7.52 (1H, d, J=2.12 Hz), 7.74 (1H, s), 8.12 (1H, d, J=16.06 Hz).

¹³C NMR (CDCl₃, 100MHz) δ: 60.12, 64.81, 69.63, 95.86, 106.43, 114.09, 117.55, 117.88, 120.79, 132.60, 132.64, 140.50, 141.01, 144.87, 152.46, 155.69, 156.93, 167.14. EIMS m/z: 314.12 [M⁺ + Na]. IR (KBr) νmax cm⁻¹: 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

(E)-butyl 3-(6-butoxy-4-methoxybenzofuran-5-yl)acrylate: 164

¹H NMR (CDCl₃, 500 MHz) δ: 0.92 (3H, t, J=7.3 Hz), 1.04 (3H, t, J=7.4 Hz), 1.26 (2H, m), 1.5 (6H, m), 1.8-1.92 (4H, m), 3.83 (s, 3H), 4.01 (2H, m), 4.20 (2H, m), 6.51 (1H, d, J=16.10 Hz), 6.74 (1H, d, J=2.22 Hz), 7.57 (1H, d, J=2.12 Hz), 7.74 (1H, s), 8.20 (1H, d, J=16.12 Hz).

¹³C NMR (CDCl₃, 100MHz) δ: 13.78, 13.86, 14.13, 14.37, 31.13, 31.16, 59.65, 60.23, 68.68, 95.44, 106.70, 117.47, 120.51, 120.74, 140.66, 144.73, 152.20, 156.39, 157.11, 167.74. EIMS m/z: 346.18 [M⁺ + Na]. IR (KBr) νmax cm⁻¹: 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.
\((E)-\)propyl 3-(4-methoxy-6-propoxybenzofuran-5-yl)acrylate: 165

\(^{1}\)H NMR (CDCl\(_3\), 500 MHz) \(\delta\): 0.92 (3H, t, \(J=7.3\) Hz), 1.04 (3H, t, \(J=7.4\) Hz), 1.26 (2H, m), 1.5 (4H, m), 3.78 (s, 3H), 4.01 (2H, m), 4.40 (2H, m), 6.51 (1H, d, \(J=16.10\) Hz), 6.74 (1H, d, \(J=2.22\) Hz), 7.57 (1H, d, \(J=2.12\) Hz), 7.74 (1H, s), 8.12 (1H, d, \(J=16.12\) Hz). \(^{13}\)C NMR (CDCl\(_3\), 100MHz) \(\delta\): 13.78, 14.13, 31.13, 32.16, 59.65, 60.23, 68.68, 95.44, 106.70, 117.47, 120.51, 120.74, 140.66, 144.73, 152.20, 156.39, 157.11, 167.74. EIMS \(m/z\): 318.15 [M\(^{+}\) + Na]. IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 2923, 1728, 1685, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

\((E)-\)isobutyl 3-(6-isobutoxy-4-methoxybenzofuran-5-yl)acrylate: 166

\(^{1}\)H NMR (CDCl\(_3\), 500 MHz) \(\delta\): 0.91 (1H, d, \(J=6.1\) Hz), 1.1 (6H, d, \(J=6.6\) Hz), 2.0 (1H, m), 2.2 (1H, m), 3.74 (s, 3H), 3.8 (2H, d, \(J=6.1\) Hz), 4.0 (2H, d, \(J=6.6\) Hz), 6.51 (1H, d, \(J=16.09\) Hz), 6.74 (1H, d, \(J=2.22\) Hz), 7.57 (1H, d, \(J=2.12\) Hz), 7.74 (1H, s), 8.20 (1H, d, \(J=16.13\) Hz). \(^{13}\)C NMR (CDCl\(_3\), 100MHz) \(\delta\): 19.51, 19.83, 26.88, 27.62, 58.65, 60.01, 75.34, 95.44, 106.46, 117.41, 120.57, 122.98, 140.66, 144.73, 151.78, 156.39, 157.11, 167.67. EIMS \(m/z\): 346.18 [M\(^{+}\) + Na]. IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

\((E)-\)isopropyl 3-(6-isopropoxy-4-methoxybenzofuran-5-yl)acrylate: 167

\(^{1}\)H NMR (CDCl\(_3\), 500 MHz) \(\delta\): 1.3 (6H, d, \(J=4.5\) Hz), 1.4 (6H, d, \(J=5.1\) Hz), 3.80 (s, 3H), 4.6 (1H, m), 5.2 (1H, m), 6.4 (1H, d, \(J=16.2\) Hz), 6.8 (1H, d, \(J=2.2\) Hz), 7.4 (1H, d, \(J=8.6\) Hz), 7.66 (1H, d, \(J=2.2\) Hz), 8.18 (1H, d, \(J=16.2\) Hz). \(^{13}\)C NMR (CDCl\(_3\), 100MHz) \(\delta\): 21.75, 22.00, 58.65, 67.50, 76.60, 104.84, 106.64, 117.06, 120.46, 122.54, 123.57, 140.05, 144.55, 151.24, 157.66, 166.13. EIMS \(m/z\): 318.15 [M\(^{+}\) + Na]. IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

\((E)-\)benzyl 3-(6-(benzyloxy)-4-methoxybenzofuran-5-yl)acrylate: 168

\(^{1}\)H NMR (CDCl\(_3\), 500 MHz) \(\delta\): 3.80 (s, 3H), 5.2 (4H, s), 6.68 (1H, d, \(J=16.32\) Hz), 6.90 (1H, d, \(J=2.22\) Hz), 6.93-7.12 (10H, m), 7.57 (1H, d, \(J=2.12\) Hz), 7.74 (1H, s), 8.20 (1H, d, \(J=16.13\) Hz). \(^{13}\)C NMR (CDCl\(_3\), 100MHz) \(\delta\): 59.65, 60.25, 70.77, 96.25, 106.47, 117.69, 120.56, 120.67, 120.83, 127.14, 128.07, 132.53, 136.39, 140.47,
144.92, 151.70, 158.87, 168.53. EIMS m/z: 414.15 [M⁺ + Na]. IR (KBr) νmax cm⁻¹: 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

*(E)*-prop-2-ynyl 3-(4-methoxy-6-(prop-2-ynyloxy)benzofuran-5-yl)acrylate : 169

1H NMR (CDCl₃, 500 MHz) δ: 2.48 (1H, s), 2.52 (1H, s), 3.74 (s, 3H), 4.76 (2H, d, J= 2.4 Hz), 4.8 (2H, d, J=2.3 Hz), 6.60 (1H, d, J=16.10 Hz), 6.74 (1H, d, J=2.22 Hz), 7.57 (1H, d, J=2.12 Hz), 7.74 (1H, s), 8.20 (1H, d, J=16.06 Hz). IR (KBr) νmax cm⁻¹: 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510.

7-(prop-2-ynyloxy)-2H-chromen-2-one: 171

1H NMR (CDCl₃, 400 MHz) δ: 2.59(1H, s), 5.20(2H, s), 6.28 (1H, d, J=9.60 Hz), 6.90- 6.92 (2H, m), 7.32(1H, d, J=8 Hz), 7.67(1H, d, J=9.3 Hz). 13C NMR (CDCl₃, 100MHz) δ: 56.20, 76.55, 77.33, 102.11, 113.16, 113.47, 128.87, 155.87, 160.61, 161.41. EIMS m/z: 200.19 [M⁺ + Na]. IR (KBr) νmax cm⁻¹: 1718, 1595, 1497, 1383, 1360.

7-((1-(2-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one: 172

1H NMR (CDCl₃, 400 MHz) δ: 3.9(3H, s), 5.30(2H, s), 6.17 (1H, d, J=9.50 Hz), 7.06 (2H, m), 7.19 (2H, m), 7.27 (2H, m), 7.33(1H, d, J=8 Hz), 7.81(1H, d, J=9.3 Hz), 8.29 (2H, m). 13C NMR (CDCl₃, 100MHz) δ: 55.91, 62.20, 102.02, 112.23, 112.79, 112.87, 113.23, 121.13, 125.26, 125.93, 128.83, 130.25, 143.28, 150.96, 155.59, 160.97, 161.31. EIMS m/z: 349.34 [M⁺ + Na]. IR (KBr) νmax cm⁻¹: 1718, 1595, 1497, 1383, 1360.

7-((1-(2-chloropyridin-3-yl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one: 173

1H NMR (CDCl₃, 400 MHz) δ: 5.38(2H, s), 6.30 (1H, d, J=9.50 Hz), 6.98 (2H, m), 7.41 (1H, m), 7.43 (1H, m), 7.66(1H, d, J=9.5 Hz), 8.08 (1H, d, J=6.19 Hz), 8.24 (1H, s), 8.59 (1H, d, J=3.08 Hz). 13C NMR (CDCl₃, 100MHz) δ: 62.16, 102.20, 112.79, 112.79, 113.20, 113.71, 123.42, 124.80, 128.96, 131.92, 135.95, 143.18, 143.45,
144.92, 150.45, 155.79, 160.93, 161.17. EIMS m/z: 354.75 [M^+ + Na]. IR (KBr) νmax cm⁻¹: 1718, 1595, 1497, 1383, 1360.

4-(4-((2-oxo-2H-chromen-7-yloxy)methyl)-1H-1,2,3-triazol-1-yl)benzonitrile: 174

^1^H NMR (CDCl₃, 500 MHz) δ: 5.38(2H, s), 6.31 (1H, d, J=9.40 Hz), 7.03 (2H, m), 7.51 (1H, m), 7.80(1H, d, J=9.48 Hz), 7.92 (2H, d, J=8.85 Hz), 8.06 (2H, d, J=8.56 Hz), 8.56 (1H, s). ^13^C NMR (CDCl₃, 100MHz) δ: 61.23, 101.43, 111.87, 112.37, 112.64, 112.81, 117.10, 120.27, 121.63, 128.81, 133.53, 139.32, 143.81, 143.89, 155.11, 160.96, 161.63. EIMS m/z: 344.32 [M^+ + Na]. IR (KBr) νmax cm⁻¹: 1718, 1595, 1497, 1383, 1360.

7-((1-(benzo[d][1,3]dioxol-6-yl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one: 175

^1^H NMR (CDCl₃, 500 MHz) δ: 5.32(2H, s), 5.38(2H, s), 6.13 (1H, d, J=9.48 Hz), 6.68 (2H, m), 7.02 (3H, m), 7.32 (1H, m), 7.59(1H, d, J=9.50 Hz), 8.62 (1H, s). ^13^C NMR (CDCl₃, 100MHz) δ: 61.23, 102.12, 103.97, 107.73, 109.31, 112.90, 113.46, 114.01, 118.90, 119.90, 128.95, 134.99, 143.07, 146.62, 147.86, 149.33, 155.70, 160.96, 161.63. EIMS m/z: 363.32 [M^+ + Na]. IR (KBr) νmax cm⁻¹: 1718, 1595, 1497, 1383, 1360.

7-((1-(benzo[d][1,3]dioxol-6-yl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one: 176

^1^H NMR (CDCl₃, 500 MHz) δ: 5.38(2H, s), 6.39(2H, s), 6.13 (1H, d, J=9.50 Hz), 7.10 (2H, m), 7.32 (2H, m), 7.58 (1H, m), 7.59(1H, d, J=9.70 Hz), 8.33 (1H, d, J=8.33 Hz), 8.41 (1H, d, J=8.05 Hz), 8.61 (1H, s). ^13^C NMR (CDCl₃, 100MHz) δ: 61.23, 103.97, 112.90, 113.46, 114.01, 117.08, 119.52, 122.47, 122.64, 127.18, 128.95, 134.56, 141.33, 143.07, 150.60, 151.97, 155.70, 160.96, 161.63. EIMS m/z: 376.39 [M^+ + Na]. IR (KBr) νmax cm⁻¹: 1718, 1595, 1497, 1383, 1360.

N-(4-(4-((2-oxo-2H-chromen-7-yloxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)benzamide: 177

^1^H NMR (CDCl₃, 400 MHz) δ: 5.36(2H, s), 6.27 (1H, d, J=9.49 Hz), 7.01 (2H, m), 7.55 (2H, d, J=8.24 Hz), 7.57 (2H, d, J=8.30 Hz), 7.66(1H, d, J=9.48 Hz), 7.76 (1H, d, J=8.93 Hz), 8.0 (2H, d, J=8.39 Hz), 8.03 (1H, m), 8.05 (2H, d, J=8.94 Hz), 8.33
1H, s). $^{13}$C NMR (CDCl$_3$, 125MHz) $\delta$: 62.21, 102.07, 112.90, 113.46, 114.01, 119.90, 122.14, 124.36, 128.33, 128.58, 128.95, 131.75, 133.17, 134.97, 136.18, 143.07, 147.86, 155.70, 161.83, 162.32, 166.56. EIMS $m/z$: 438.43 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 3477, 2922, 1770, 1472, 1346, 1049, 778, 688, 644, 558, 548, 490.

**N-(4-(4-((2-oxo-2H-chromen-7-yloxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)acetamide:** 178

1H NMR (CDCl$_3$, 400 MHz) $\delta$: 2.19(3H, s), 5.34(2H, s), 6.31 (1H, d, $J=9.50$ Hz), 6.90 (2H, m), 7.32(1H, m), 7.44 (2H, d, $J=8.87$ Hz), 7.68(1H, d, $J=11.41$ Hz), 7.76 (1H, d, $J=8.65$ Hz), 8.13 (1H, m). $^{13}$C NMR (CDCl$_3$, 125MHz) $\delta$: 23.80, 62.21, 103.97, 112.90, 113.46, 114.01, 119.90, 122.14, 123.12, 128.95, 135.57, 136.70, 143.07, 147.86, 155.70, 161.83, 162.32, 170.43. EIMS $m/z$: 376.37 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 1718, 1595, 1497, 1383, 1360.

**7-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one:** 179

1H NMR (CDCl$_3$, 400 MHz) $\delta$: 5.36(2H, s), 6.20 (1H, d, $J=8.10$ Hz), 6.98 (3H, m), 7.40 (1H, d, $J=8.10$ Hz), 7.55 (2H, d, $J=8.10$ Hz), 7.66(1H, d, $J=9.48$ Hz), 7.76(2H, d, $J=8.2$ Hz), 8.10 (1H, s). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 62.31, 102.20, 103.97, 113.14, 113.60, 120.67, 121.23, 128.98, 129.85, 136.86, 143.29, 143.74, 155.76, 161.06, 161.24. EIMS $m/z$: 319.32 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 3720, 2921, 1595, 1499, 1301, 1228, 1168, 1045, 947, 851, 766, 681, 489, 450.

**7-((1-(3-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one:** 180

1H NMR (CDCl$_3$, 400 MHz) $\delta$: 3.88(3H, s), 5.33(2H, s), 6.28 (1H, d, $J=10$ Hz), 6.99 (3H, m), 7.27 (1H, m), 7.35 (1H, m), 7.40 (2H, m), 7.65(1H, d, $J=10$ Hz), 8.12 (1H, s). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 55.61, 62.18, 102.07, 103.26, 106.44, 112.26, 112.41, 112.71, 113.05, 114.82, 121.36, 128.94, 129.0, 130.56, 137.76, 143.31, 143.71, 155.65, 160.59, 161.18. EIMS $m/z$: 349.34 [M$^+$ + Na]. IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 1718, 1595, 1497, 1383, 1360.

**7-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one:** 181

1H NMR (CDCl$_3$, 400 MHz) $\delta$: 3.87(3H, s), 5.33(2H, s), 6.29 (1H, d, $J=10$ Hz), 6.94-6.99 (3H, m), 7.03 (2H, d, $J=8.1$ Hz), 7.41 (2H, d, $J=8.12$ Hz), 7.64(1H, d, $J=10$ Hz), 8.03 (1H, s). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 55.42, 61.93, 102.66, 111.51, 112.74,
113.01, 113.12, 113.34, 114.67, 121.62, 122.10, 128.75, 128.90, 129.97, 143.55, 159.90, 160.47, 161.38. EIMS \textit{m/z}: 349.34 \textit{[M$^+ + \text{Na}$]}. IR (KBr) $\nu_{\text{max}} \text{cm}^{-1}$: 1718, 1595, 1497, 1383, 1360.

7-((1-(2-iodophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one: 183

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 5.39 (2H, s), 6.27 (1H, d, $J=10$ Hz), 6.98 (2H, m), 7.24 (1H, m), 7.39-7.52 (3H, m), 7.63 (1H, d, $J=10$ Hz), 7.94 (1H, s), 8.02 (1H, d, $J=4-5$ Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 61.23, 93.36, 103.97, 112.90, 113.46, 114.01, 120.91, 121.65, 128.81, 134.66, 143.07, 144.35, 147.65, 155.70, 160.96, 161.83. EIMS $m/z$: 445.21 \textit{[M$^+ + \text{Na}$]}. IR (KBr) $\nu_{\text{max}} \text{cm}^{-1}$: 1718, 1595, 1497, 1383, 1360.

7-((1-phenylmethanol)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one: 184

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 4.75 (2H, s), 5.36 (2H, s), 6.30 (1H, d, $J=10$ Hz), 7.01 (2H, m), 7.45-7.53 (3H, m), 7.64-7.66 (2H, m), 7.68 (1H, d, $J=10.01$ Hz), 8.13 (1H, s). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 61.07, 62.31, 103.97, 112.90, 113.46, 114.01, 119.83, 123.05, 123.65, 128.68, 128.95, 136.40, 136.81, 143.07, 147.86, 155.70, 161.83, 162.32. EIMS $m/z$: 349.34 \textit{[M$^+ + \text{Na}$]}. IR (KBr) $\nu_{\text{max}} \text{cm}^{-1}$: 3720, 2921,1596,1499,1301,1228,1168,1045, 947, 851, 766, 681, 489, 450.

2-(4-(4-(2-oxo-2H-chromen-7-yloxy)methyl)-1H-1,2,3-triazol-1-yl)phenylacetonitrile: 185

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 3.35 (2H, s), 5.38 (2H, s), 6.31 (1H, d, $J=9.40$ Hz), 7.03 (2H, m), 7.51 (1H, m), 7.78 (1H, d, $J=9.48$ Hz), 7.91 (2H, d, $J=8.85$ Hz), 8.0 (2H, d, $J=8.56$ Hz), 8.56 (1H, s). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$: 23.41, 61.23, 101.43, 111.87, 112.37, 112.64, 112.81, 117.10, 120.27, 121.63, 128.81, 133.53, 139.32, 143.81, 143.89, 155.11, 160.96, 161.63. EIMS $m/z$: 358.35 \textit{[M$^+ + \text{Na}$]}. IR (KBr) $\nu_{\text{max}} \text{cm}^{-1}$: 1718, 1595, 1497, 1383, 1360.
Chapter 2

2.11. Spectra of Some Compounds

\[ \text{\textsuperscript{1}H and \textsuperscript{13}C NMR spectra of compound (66)} \]
$^1$H & $^{13}$C NMR spectra of compound (67)
Studies on modification and bio-evaluation of plant molecules

$^1$H$^{13}$C NMR spectra of compound (70)
$^1$H and $^{13}$C NMR spectra of compound (71)
Chapter 2

Studies on modification and bio-evaluation of plant molecules

$^1$H$^13$C NMR spectra of compound (72)
Chapter 2                                                  Studies on modification and bio-evaluation of plant molecules

$^{1}H$&$^{13}C$ NMR spectra of compound (73)
$^1$H & $^{13}$C NMR spectra of compound (75)
$^1\text{H}$ and $^{13}\text{C}$ NMR spectra of compound (77)
$^1$H and $^{13}$C NMR spectra of compound (78)
$^1$H$^{13}$C NMR spectra of compound (79)
**Chapter 2**

Studies on modification and bio-evaluation of plant molecules

\[ ^1H \& ^13C \text{NMR spectra of compound (82)} \]
Chapter 2

Studies on modification and bio-evaluation of plant molecules

$^1$H and $^{13}$C NMR spectra of compound (92)
Chapter 2

Studies on modification and bio-evaluation of plant molecules

$^1$H and $^{13}$C NMR spectra of compound (95)
$^1\text{H}$ and $^{13}\text{C}$ NMR spectra of compound (138)
$^1$H & $^{13}$C NMR spectra of compound (148)
Studies on modification and bio-evaluation of plant molecules

$^1$H&$^{13}$C NMR spectra of compound (155)
Chapter 3: Section A

CuCN Catalyzed One Pot Synthesis of γ-Keto Diesters: Domino Double Michael Addition Followed by Nef Reaction

3.1. Introduction

Chemists have achieved great success in the field of chemistry taking into consideration its importance in the current lifestyle. However, due to the increasing concern about the environment on account of production of large amount of waste products during preparation of a large number of fine chemicals, there are environmental concerns involved which could negatively influence the ecological balance. Today, the issues concern more about how to synthesize compounds in an eco friendly environment rather than what to synthesize. In the synthesis of complex molecules that require large number of steps, the involvement of different conditions, reagents, solvents, catalysts and production of large number of waste products at each step is a serious concern for the environment. Environmental and economic pressures are now forcing the chemical community to search for more efficient ways of performing chemical transformations.¹

The main aim in the development of synthesis is the formation of new eco-friendly reaction conditions taking into the consideration the aspects of green chemistry.² In contemporary organic synthesis, catalysis by non-hazardous and environmentally benign organic molecules is a major breakthrough, and rapid construction of structurally complex and functionally dense molecules from simple and readily available precursors in one operation, referred to as a domino reaction, is an exciting aspect.³

---

3.2. Objectives of the Present Work

Synthesis of such biologically significant cyclic compounds rely on the elaboration of polycarbonyl compounds among which 1,4-dicarboxyls have proven particularly useful. A few approaches towards the synthesis of such functional arrangements involve multicomponent coupling of siloxycyclopropane and carbon monoxide, carbonylative dimerisation of siloxycyclopropane, carbonylative arylation of siloxycyclopropane, conjugate addition of nitroalkanes to enones followed by reduction of nitro group to carbonyl group. Most of the previously reported methods suffer from drawbacks such as requirement of a stoichiometric amount of the Lewis acid, elongated reaction time, hazardous carbon monoxide as source for carbonyl group, and above all expensive and highly toxic catalysts. Surprisingly, apart from these valuable strategies for the generation of \( \gamma \)-keto diesters, there exists no general one pot method available for the synthesis of the said class. Hence, more efficient and practical alternative methods using inexpensive and easily available reagents are warranted. Herein, we unveil one pot synthesis of \( \gamma \)-keto diesters in presence of \( \text{Cs}_2\text{CO}_3 \) through \( \text{CuCN} \) catalyzed domino double Michael reaction followed by Nef reaction to afford the target products, achieved in few minutes, with moderate to good yields.

Despite these valuable strategies for the generation of \( \gamma \)-keto diesters, there is no general one pot process available for their synthesis. Hence, a more efficient and practical alternative using an inexpensive and easily available reagent is warranted.

---


In the work presented here, a successful attempt has been made to achieve one-pot synthesis for the preparation of \( \gamma \)-keto diesters by \( \text{Cs}_2\text{CO}_3 \) mediated and \( \text{CuCN} \) catalysed domino double Michael reaction followed by Nef reaction to afford the target products achieved in few minutes, with moderate to good yields.

### 3.3. Review of Literature

Domino reactions are defined as formation of two or more bond forming transformations under widely identical conditions in which the subsequent reactions take place at the functionalities obtained in the former transformation. The development and use of domino reactions are an appropriate answer to the need for minimizing work up processes, the use of energy, reactives, and relative reduction of solvents, inorganic salts and purification steps. Among these aspects, the minimum use of the solvents the generation of less hazardous substances and the development of new less expensive processes are the most important. Moreover, the one-pot process can drive the equilibria to the desired direction. Thus, these reactions would allow an ecologically and economically favorable production. A one-pot process can be planned by the right choice of different parameters such as (i) reaction conditions, (ii) catalysts, (iii) addition sequence of reactants, or (iv) use of starting materials with a high chemical versatility, e.g., nitroalkanes. These features are found in domino reactions. Large number of activating groups that helps in the formation of carbon-carbon bonds often need to be replaced by some other functional group in synthetic chemistry.

High degree of atom economy, increased efficiencies and low production of environmental hazards are the main characteristics of these reactions, particularly those involving C-C bond formation\(^9\)\(^{10}\)\(^{11}\) such as Mannich reaction\(^12\), Michael

---


reaction\textsuperscript{13} and Baylis-Hillman reaction,\textsuperscript{14} Robinsons annulation reaction,\textsuperscript{15} Knoevenagel condensation,\textsuperscript{16} and Diels-Alder cycloaddition reaction.\textsuperscript{17}

These reactions represent crucial aspects in organic synthesis. Activating groups that promote C-C bond formation need to be replaced by other functional entities after their assistance in the targeted synthetic strategy. Interconversion of functional groups and the accessibility of a reliable number of such transformations for a particular group can therefore be conceived as strong tool in synthetic chemistry. In this respect, nitroalkanes 1 are significantly important as staring materials due to their easy conversion into corresponding nucleophilic nitronate 2 anion because of the high electron-withdrawing power of the nitro group that provides an outstanding enhancement of the hydrogen acidity at the \( \alpha \)-position.

\[ \begin{align*}
\text{1} & \quad \text{2} \quad \text{3} \quad \text{4} \quad \text{5} \quad \text{6}
\end{align*} \]

Scheme 1

Nitronate salts can therefore act as carbon nucleophiles with a range of electrophiles including haloalkanes,\textsuperscript{18} aldehydes\textsuperscript{19, 20} and Michael acceptors,\textsuperscript{21} leading to carbon–carbon bond formation. (Scheme-1)

\textsuperscript{17} Li, X.; Xu, J. *J. Org. Chem.* 2013, 78, 347.
Once these adducts have been formed, the nitro group can be retained in the molecular framework if this is useful for a further nucleophilic addition or it can be transformed into other functionalities following a defined synthetic strategy. It is easy to synthesize and it is even easier to exploit its potential for the synthesis of complex target molecules. These act as suitable Michael donors leading to the formation of C-C bond.

Reduction of the nitro group in compound 7 allows the preparation of a primary amine 8, in which a simple modification of the oxidation state of the nitrogen atom is carried out. Alternatively, the nitro group can be removed from the molecule by replacing it with hydrogen giving the corresponding denitrated product 9 or by elimination as nitrous acid, introducing a double bond in the molecular structure 10. A further option consists of the conversion of the nitro group into a carbonyl group 11 (Scheme-2).

Scheme 2


This process is probably the most exploited transformation of the nitro group, since it definitively reverses the polarity of the neighbouring carbon atom from nucleophilic to electrophilic.

As discussed above the nitro compounds can be easily converted into carbonyls. The main focus of our work currently will be on the conversion of aliphatic nitro compounds followed by Michael reaction into the corresponding carbonyl compounds by using Nef reaction.

### 3.3.1. Nef reaction

The original procedure for the nitro to carbonyl transformation as described by Nef in 1894 was the hydrolysis of a nitronate salt produced (by the basic treatment of a nitroalkane) in strongly acidic conditions (Scheme 3).

![Scheme 3: Mechanism for the original Nef reaction](image)

Hydrolysis occurs on a protonated form of the corresponding nitronic acid, giving as intermediate that, by loss of water and hyponitrous acid give the carbonyl derivative. In this reaction the formation and distribution of the product is strongly affected by the acidity level of the system. When pH of the system is greater than one, oximes as well as other hydroxyl nitroso compounds can be formed in significant amounts. For this reason, a rapid acidification of the nitronate salt is required and it is very often operationally desirable to add the nitronate salt to the acid solution. The harsh conditions in which this conversion is usually carried out (pH 1) have spurred the development of alternative methods that can be performed in oxidative, reductive, as well as almost neutral, conditions. It is interesting to note that a common factor in all of the oxidative procedures is the formation of the corresponding nitronate anion as the reactive species; the subsequent cleavage occurs on the carbon–nitrogen double bond to give the carbonyl derivative. Conversely,

---

reductive methods can be carried out both on the nitronate anion or directly on the nitroalkane, even in acidic conditions. An important aspect of this transformation concerns the nature of the nitroalkane used as the substrate. Indeed, secondary nitro compounds are conveniently transformed into ketones, but primary nitro derivatives can be converted into aldehydes or carboxylic acids, depending on the reaction conditions. Particular care need to be taken in this reaction, especially when oxidative procedures are chosen to transform primary nitroalkanes into aldehydes. Nitroalkenes that are powerful Michael acceptors can also be used as substrates for the Nef reaction. In addition; the conjugate addition of nucleophilic reagents to nitro olefins provides the formation of a nitronate anion as an intermediate that can usually be transformed by a tandem process into the corresponding carbonyl derivative.

The most common methods on Nef reaction which are reproduced in detail include. Dimethylidoxirane (DMD) is a strong oxidising agent readily prepared by the reaction of oxone with acetone and, among its various applications includes the regeneration of the carbonyl group from acetals, hydrazones and other derivatives. DMD attacks nitronate anions obtained from nitro compounds such as 18, giving the corresponding carbonyl derivative 19 in good yields (Scheme 4).

\[
\begin{align*}
18 & \xrightarrow{1. \text{t-BuOK, THF}} 19 \\
& \xrightarrow{2. \text{rt, 73\%}} \\
\end{align*}
\]

**Scheme 4:** Nef reaction using dimethylidoxirane

KMnO₄ is certainly the most widely used oxidant for this purpose and, in controlled conditions; it is able to convert primary nitro compounds into aldehydes. Buffered permanganate solutions (pH¼11) can oxidise primary nitroalkanes such as 20 into

---

29 Perekalin, V. V.; Lipina, E. S.; Berestovitskaya, V. M.; Efremov, D. A. *Nitroalkenes conjugated nitro compounds; Wiley: Chichester,* 1994.
alkanoic acids 21 without affecting other functions such as esters, amides, primary alcohols and acetals33 (Scheme 5).

\[ R \text{CH}_2\text{NO}_2 \xrightarrow{\text{KMnO}_4, \text{KOH, K}_2\text{HPO}_4, \text{t-BuOH, rt}} R \text{CH}_2\text{CO}_2\text{H} \]

(R = 3,4-Cl$_2$C$_6$H$_3$CH$_2$, R$_1$ = CO$_2$Et (99%); 
R = EtO$_2$CCH$_2$, R$_1$ = CO$_2$Et (96%); 
R = HO(CH$_2$)$_8$, R$_1$ = H (99%); 
R = THO(CH$_2$)$_8$, R$_1$ = H (90%))

**Scheme 5**: Permanganate oxidation of primary nitroalkanes to carboxylic acids

Molecular oxygen is cheap, readily available and environmental friendly reagent provides an interesting system to perform oxidations. There are some nitro compounds such as 22 that can be converted into the corresponding carbonyl derivatives 23 when exposed to air in the presence of copper salts as catalyst34 (Scheme 6).

\[ \text{NO}_2 \xrightarrow{\text{Cu(OAc)}_2 \cdot \text{H}_2\text{O}, \text{10%mol, air}} \text{CO}_2\text{H} \]


Reaction of nitromethane with D-xylose 24 produces an epimeric pair of nitrosugars. This compound can be directly transformed into D-idose 25 under classical Nef conditions in 68% yield35 (Scheme 7).
The ability of DBU as a base to promote a conjugate addition of nitroalkanes 1 to enones as well as a Nef reaction on secondary nitroalkanes can be used in a tandem process that permits the direct synthesis of $\gamma$-diketones and $\gamma$-keto esters 26\(^\text{36}\) (Scheme 8).

The syntheses are achieved by the conjugate addition of the cyclic nitro ketones 27 with the appropriate enone acrolein (R = H), methyl vinyl ketone (R = Me), or methyl acrylate (R = OMe). The polyfunctionalized nitronate formed, 28 can be directly treated with KMnO\(_4\)/MgSO\(_4\) and the 1,4-dicarbonyl derivatives 29 formed are accordingly synthesized in one-pot, in moderate to high overall yields (50–92\%)\(^\text{37}\) (Scheme 9).

---

**References**


3.3.2. Synthesis of 1, 4-dicarbonyls using a conjugate addition–Nef reaction

As previously stated, conjugate addition of nitroalkanes to enones and enoates provides a rapid entry to the corresponding γ-nitro carbonyl derivatives that can undergo a Nef reaction giving 1,4-dicarbonyl compounds. Preparation of 1,4-dicarbonyls requires reversing the natural polarity of the carbonyl group, so is more tricky. This procedure represents one of the most exploited methods to prepare such difunctionalized derivatives that find a number of applications in the synthesis of important target molecules. 1,4-Difunctionalized derivatives are valuable intermediates in organic synthesis, since they can be transformed into a plethora of valuable compounds such as cyclopentenones and several heterocyclic systems. In particular, 1,4-dicarbonyl is the key building blocks in the preparation of several important target molecules. Functionalized 1,4-diketones and g-oxoaldehydes are both valuable classes of compounds, because of their importance in the synthesis of cyclopentenones and heterocyclic systems such as furans, pyrroles, thiophenes, and pyridazines, while γ-oxoesters are highly useful intermediates for the preparation of lactones, lactam antibiotics, isoquinolines, and lactonic sex pheromones. The

---

\[ \text{Scheme 9} \]


utilization of nitro compounds for the synthesis of useful building blocks or in multistep procedures devoted to the preparation of complex molecules is a field that is experiencing a rapid growth. In many of these synthetic pathways, the nitro to carbonyl conversion plays a central role, so that the array procedures to carry out the Nef reaction represent a formidable tool for every organic chemist. In this part of the report, the application of the Nef reaction to the preparation of carbonyl derivatives or in multistep syntheses is presented. Application and importance of the Nef reaction for the preparation of the large number of natural products and bioactive compounds may be emphasized in the following lines:

Cyclopentenones are important moieties present in many natural products such as allylrethrone, which is an important component of insecticidal pyrethroids and an important intermediate for the synthesis of allethrolone and pyrethrins. Commercial 5-nitro-1-pentene 30 has been chosen for an improved synthesis of allylrethrone. Its preparation can be realized in three distinct steps, starting from the nitroalkene and methyl vinyl ketone. The obtained Michael adduct 31 is converted into the diketone 32 by a hydrolytic Nef reaction and is then cyclised to allylrethrone 33 under basic conditions\(^\text{41}\) (Scheme 10).

\[
\begin{align*}
\text{NO}_2 \quad \text{O} \quad \text{NO}_2 \quad \text{O} \\
\text{Al}_2\text{O}_3, \text{7h} \quad \text{MeONa/MeOH} \\
78\% \quad \text{H}^+, -35^\circ\text{C}, 90\%
\end{align*}
\]

\[
\begin{align*}
\text{MeOH, 0.5N NaOH} \\
\text{reflux, 8h} \quad \text{93}\%
\end{align*}
\]

Scheme 10

In recent years, chemistry of medium and large size ring lactones has attracted considerable attention because many of the molecules belonging to this group

have revealed diverse and significant biological activities.\textsuperscript{42,43,44} Patulolide A shows significant antifungal, antibacterial and anti-inflammatory activities.\textsuperscript{45,46,47} Recent reports indicate that the R-antipode of Patulolide A inhibits IgE induced release of histamine for human leucocytes better than the degeneration inhibitor, theophylin.\textsuperscript{48} The optically active nitro-acetate 34 can be readily prepared by enzymatic acylation from the corresponding racemic nitro-alcohol and reacts efficiently with methyl propiolate giving the corresponding adduct 35. The nitro group is then converted into the keto-ester 36 using the McMurry method,\textsuperscript{49} and after ester hydrolysis the resulting hydroxy acid is lactonized to the macrolide (R) - patulolide A 37\textsuperscript{50} (Scheme 11).

A related strategy is also effective for the total synthesis of optically active (R, R)-(-)-pyrenophorin which is used as antifungal metabolite and is one of the representatives

\textsuperscript{44} Omura, O.; Nakagawa, A. \textit{J. Antibiotics} \textbf{1975}, 28, 401.
\textsuperscript{50} Kalita, D.; Khan, A. T.; Barua, N. C.; Bez, G. \textit{Tetrahedron} \textbf{1999}, 55, 5177
of naturally occurring diolides.\textsuperscript{51} The synthesis of this molecule (R, R)-(-)-pyrenophorin 41 starts from optically active nitro-acetate 38 through Nef reaction\textsuperscript{52} (Scheme 12).

![Scheme 12](image)

Nitroalkanes can be profitably employed as carbanionic precursors for the assembly of dihydroxy ketone frameworks, suitable for the preparation of spiroketals. Nef reaction of the resulting carbon-nitrogen bond and its conversion to carbonyl group results in the generation of required electrophilic function to obtain the spiroketal framework.\textsuperscript{53} The spiroketal moiety is a key motif present in the plethora of natural compounds present in plant, fungi, insect secretions, shellfish toxin and other living organisms with promising biological activities like antibiotics.\textsuperscript{54} A double Michael addition of nitromethane using two different α,β-unsaturated carbonyl derivatives can be used as a key strategy for the preparation of racemic 2-ethyl-1,6-dioxaspiro[4.4]nonane, known as “Chalcogran” which is the main component in the aggregation pheromone of the bark beetle Pityogenes chalcogrobus (L).\textsuperscript{55} Addition of nitromethane to 1-penten-3-one in the presence of basic alumina under solvent-free conditions affords the corresponding 4-nitro-ketone 42 that is suitable for a subsequent conjugate addition to acrolein under the same conditions, leading to the nitro-diketone 43.

Reduction of the carbonyl groups with NaBH₄ generates the nitro-diol 44, which upon nitro- to carbonyl- conversion (Nef reaction) spontaneously produces the spiroketal system of chalcogran as a mixture of stereoisomers 45a and 45b in 20% overall yield₅⁶ (Scheme 13).

![Scheme 13](image)

The rubromycins are a class of antibiotics isolated from cultures of Streptomyces₅⁷ that exhibit activity against Gram positive bacteria. In figure-1, β-Rubromycin 47 and γ-rubromycin 48a exhibit potent inhibition of human telomerase,₅₈ with IC₅₀ values of 3µM, and are active against the reverse transcriptase of human immunodeficiency virus-1.₅₉ The fact that α-rubromycin 46, which lacks this aryl spiroketal moiety, exhibits substantially decreased inhibitory potency toward telomerase (IC₅₀ > 200 µM), suggests that this spiroketal system plays an essential role in the observed inhibition of telomerase.

Structurally related to the rubromycins are purpuromycin,₆₀ 48b a potential topical agent for vaginal infections,₆¹ heliquino- mycin,₆² 48c and an inhibitor of DNA
helicase. All of these compounds can act as bioreductive alkylating agents as postulated by Moore.  

![Chemical structures](image)

\( \text{a-rubromycin} \)
\( \text{b-rubromycin} \)
\( \text{g-rubromycin} \)

\( \text{purpuromycin} \)
\( \text{heliquinomycin} \)

Figure 1

Since oximes are known intermediates in the reductive conversion of a nitro group to a carbonyl function, it is sometimes preferable to realise this Nef reaction in two distinct steps, namely nitro-to-oxime conversion, followed by oxime hydrolysis. A related procedure can be applied for the total synthesis of Calphostin D, a potent inhibitor of protein kinase C, an enzyme that controls cell division and differentiation. Because of this activity it has been potentially used as anti cancer and anti-HIV agents. The nitroalkene 49 is directly transformed into the ketone 50 using CrCl₂, which avoids the formation of the intermediate oxime (Scheme 14).

65 Nishizuka, Y. Cancer, 1989, 63, 1892.
A retro-Henry reaction on the nitrocyclohexanol 52 affords the open-chain nitroketone 53 that undergoes a tandem Michael addition-Nef reaction upon reaction with NaOMe, followed by acidification with H$_2$SO$_4$ to give the ketoester 54.$^{68}$ This derivative is a key intermediate in the synthesis of optically active (R)-a-lipoic acid (Scheme 15).$^{21}$

A family of sesquiterpenes sharing the hypothetical cuparane skeleton has been isolated from several natural sources.$^{69}$ Most of them show diverse biological activities.$^{70,71,72}$ Their syntheses have attracted the attention of numerous organic synthetic chemists, due to the difficulty associated with the construction of the vicinal quaternary centers on the five-membered ring. Although several basic approaches

---


---
have been reported for the construction of the cuparane skeleton.\textsuperscript{73} A related procedure provides a rapid synthesis of a functionalized cyclopentenone derivative, an advanced intermediate for the preparation of the sesquiterpene \textit{cuparene}\textsuperscript{74} (Scheme-16).

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\textbf{Scheme 16}};
\end{tikzpicture}
\end{center}

Diastereoselective conjugate addition of nitroalkanes \textbf{1} to the chiral (Z) - enoate \textbf{61} leads to the synthesis of compounds with predominance of the syn stereoisomer \textbf{62}\textsuperscript{75-76} (Scheme 17). Reduction of the nitro group and ring closure affords pyrrolidinones \textbf{63} that can be used as synthetic intermediates in the preparation of several interesting compounds.

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\textbf{Scheme 17}};
\end{tikzpicture}
\end{center}

The allylic acetates \textbf{64} obtained from the Baylis–Hillman procedure, react with nitroalkanes through a conjugate addition–elimination process that leads to the


\textsuperscript{76} Ballini, R.; Petrini, M. Issue 5th Eurasian Conference on Heterocyclic Chemistry Arkivoc \textbf{2009} (ix) 195.
formation of the unsaturated esters 65. An hydrolytic nitro to carbonyl conversion on these compounds efficiently affords the (E)-alkylidene-1,4-diketones 66 (Scheme 18).

![Scheme 18](image)

An unusual behaviour can be observed upon reaction of the nitroalkanes 1 with dimethyl citraconate in 67 in the presence of DBU. As observed by NMR analysis, in the presence of DBU, there is an equilibrium between its regioisomer that is probably more reactive towards Michael addition with nitroalkanes. The adducts 68 formed by the usual conjugate addition are therefore subsequently transformed into the keto diesters 69 by a Nef reaction (Scheme 19).

![Scheme 19](image)

---

Amberlyst A21 is a macroreticular resin that is particularly efficient in promoting Conjugate addition of nitro derivatives to acrylate esters.\(^{79}\) The resulting adducts \(^{71}\) can be easily converted into the 1,4-dicarbonyl compounds \(^{72}\) (Scheme 20).

\[
\begin{align*}
\text{NO}_2 R + \text{CH} = \text{CHOMe} & \xrightarrow{\text{Amberlyst A21, rt, 51-85\%}} \text{NO}_2 \text{RCH(OMe)CH}_2\text{OMe} \\
\text{1} & \quad \text{70} & \quad \text{71} \\
1. \text{CH}_3\text{OLi, CH}_3\text{OH, 0°C} & \quad 2. \text{Na}_2\text{B}_4\text{O}_7, \text{H}_2\text{O}, \text{rt} & \quad 3. \text{KMnO}_4, \text{H}_2\text{O}, \text{rt, 60-85\%} \\
\text{72} \\
\end{align*}
\]

### 3.4. Results and Discussion

During our initial studies, the reaction of ethyl acrylate \(^{73}\) (1 eq.) and nitro methane \(^1\) (1 eq.) in DMSO in presence of K\(_2\)CO\(_3\) (1.1 eq) as a base and CuCN as the catalyst at 20-25°C afforded two products (\(^{74a}\) and \(^{74b}\)) Scheme 21 in disproportionate ratio (Table 1), which were isolated in good yields and the products identified by spectral analysis (\(^{1}H\) NMR, \(^{13}C\) NMR, IR and MS) and derivatization (reduction of \(^{74a}\) to amino ester and of \(^{74b}\) to hydroxy ester).

Prolonged reaction time though known to influence the ratio of the products\(^{84,80}\) did not result in any incremental increase of \(^{3b}\) content even after extending the reaction up to eight days.

#### 3.4.1. Reaction of ethyl acrylate and nitromethane

\[
\begin{align*}
\text{CH} = \text{CHOMe} + \text{CH}_3\text{NO}_2 & \xrightarrow{\text{K}_2\text{CO}_3, \text{CuCN, DMSO, rt,}} \text{O}_2\text{NCH} = \text{CHCOOMe} \\
\text{73} & \quad \text{1} & \quad \text{74a} \quad \text{74b} \\
\end{align*}
\]


Encouraged by the initial success with regard to the formation of 74b, a comprehensive optimization study was performed with the objective of time, space and yields minimizing the temperature range, shrunken reaction times, and exploration study towards the use of other solvents and bases for better yields of keto diester 74b over 74a due to the biological and synthetic importance of the latter as described earlier. In the first set of optimization experiments, with CuCN as the catalyst, DMSO as the reaction solvent, replacement of K$_2$CO$_3$ by other bases selected from a list of organic bases such as DBU, DABCO, Et$_3$N, Pyridine, Pyrrolidine, Piperidine and cesium carbonate was carried out in a temperature range of 30-32°C. In presence of piperidine, pyridine, and pyrrolidine as the base, compound 74a was obtained as the sole product. A disproportionate separable mixture of 74a and 74b with lower content of 74b than achieved with K$_2$CO$_3$ was obtained with other organic bases in % overall yields. With cesium carbonate as the base, the reaction was complete in 4hrs compared to other bases that involved longer times (6hrs), to give a mixture of 3a:3b in the same ratio as obtained with K$_2$CO$_3$ as the base. The reaction when carried out at lower temperature of 0-5°C did not disturb the ratio of 74a:74b to any measurable extent than observed at evaluated temperature of 30-35 °C (table-1). The change of solvent over DMSO to ACN, DMF and DCM had profound effect in terms of time saving as the reaction was complete in 0.5hr only. With DCM, the desired keto diester compound was obtained without disturbing the ratio of 74a:74b or the overall isolated yields of the products which was not so with DMF and ACN as can be seen from the results summarized in table-1.

In the absence of CuCN, only Michael reaction product 74a was obtained. Further, the reaction when carried out on the nitro intermediate in the absence of the catalyst, no keto product was formed. However, the same reaction in the presence of CuCN afforded the desired keto product 74b. In a bid to switch over the reaction more predominantly towards the production of 74b due to its biological and synthetic importance as described earlier, the reaction conditions were optimized in terms of solvent, base and temperature. The results of these experiments are summarized in Table-1.
**Table 1:** Reaction of ethyl acrylate and nitro methane with CuCN as the catalyst at different temperature in presence of different bases in DMSO and DCM solvents.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Solvents</th>
<th>Base</th>
<th>t (°C)</th>
<th>Time (h)</th>
<th>Ratio of 74a:74b and (combined isolated % yield)</th>
<th>DMSO</th>
<th>DCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMSO</td>
<td>DCM</td>
<td>K₂CO₃</td>
<td>0-5°C</td>
<td>6</td>
<td>38:68</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>DMSO</td>
<td>DCM</td>
<td>DBU</td>
<td>0-5°C</td>
<td>6</td>
<td>47:53</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>DMSO</td>
<td>DCM</td>
<td>DABCO</td>
<td>0-5°C</td>
<td>6</td>
<td>42:58</td>
<td>81</td>
</tr>
<tr>
<td>4</td>
<td>DMSO</td>
<td>DCM</td>
<td>Et₃N</td>
<td>0-5°C</td>
<td>6</td>
<td>49:51</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>DMSO</td>
<td>DCM</td>
<td>Cs₂CO₃</td>
<td>0-5°C</td>
<td>6</td>
<td>32:68</td>
<td>87</td>
</tr>
<tr>
<td>6</td>
<td>DMSO</td>
<td>DCM</td>
<td>Pyridine</td>
<td>0-5°C</td>
<td>6</td>
<td>80:00</td>
<td>80-83</td>
</tr>
<tr>
<td>7</td>
<td>DMSO</td>
<td>DCM</td>
<td>Piperidine</td>
<td>0-5°C</td>
<td>6</td>
<td>85:00</td>
<td>82-85</td>
</tr>
<tr>
<td>8</td>
<td>DMSO</td>
<td>DCM</td>
<td>Pyrrolidine</td>
<td>0-5°C</td>
<td>6</td>
<td>80:00</td>
<td>82-85</td>
</tr>
<tr>
<td>9</td>
<td>DMSO</td>
<td>DCM</td>
<td>K₂CO₃</td>
<td>20-25°C</td>
<td>2</td>
<td>33:67</td>
<td>87</td>
</tr>
<tr>
<td>10</td>
<td>DMSO</td>
<td>DCM</td>
<td>DBU</td>
<td>20-25°C</td>
<td>2</td>
<td>45:55</td>
<td>85</td>
</tr>
<tr>
<td>11</td>
<td>DMSO</td>
<td>DCM</td>
<td>DABCO</td>
<td>20-25°C</td>
<td>2</td>
<td>40:60</td>
<td>85</td>
</tr>
<tr>
<td>12</td>
<td>DMSO</td>
<td>DCM</td>
<td>Et₃N</td>
<td>20-25°C</td>
<td>2</td>
<td>45:55</td>
<td>85</td>
</tr>
<tr>
<td>13</td>
<td>DMSO</td>
<td>DCM</td>
<td>Cs₂CO₃</td>
<td>20-25°C</td>
<td>2</td>
<td>30:70</td>
<td>89</td>
</tr>
<tr>
<td>14</td>
<td>DMSO</td>
<td>DCM</td>
<td>Pyridine</td>
<td>20-25°C</td>
<td>2</td>
<td>80:00</td>
<td>80-83</td>
</tr>
<tr>
<td>15</td>
<td>DMSO</td>
<td>DCM</td>
<td>Piperidine</td>
<td>20-25°C</td>
<td>2</td>
<td>85:00</td>
<td>82-85</td>
</tr>
<tr>
<td>16</td>
<td>DMSO</td>
<td>DCM</td>
<td>Pyrrolidine</td>
<td>20-25°C</td>
<td>2</td>
<td>80:00</td>
<td>82-85</td>
</tr>
<tr>
<td>17</td>
<td>DMSO</td>
<td>DCM</td>
<td>K₂CO₃</td>
<td>30-32°C</td>
<td>0.5</td>
<td>27:73</td>
<td>87</td>
</tr>
<tr>
<td>18</td>
<td>DMSO</td>
<td>DCM</td>
<td>DBU</td>
<td>30-32°C</td>
<td>0.5</td>
<td>39:61</td>
<td>83</td>
</tr>
<tr>
<td>19</td>
<td>DMSO</td>
<td>DCM</td>
<td>DABCO</td>
<td>30-32°C</td>
<td>0.5</td>
<td>34:66</td>
<td>83</td>
</tr>
<tr>
<td>20</td>
<td>DMSO</td>
<td>DCM</td>
<td>Et₃N</td>
<td>30-32°C</td>
<td>0.5</td>
<td>38:62</td>
<td>85</td>
</tr>
<tr>
<td>21</td>
<td>DMSO</td>
<td>DCM</td>
<td>Cs₂CO₃</td>
<td>30-32°C</td>
<td>0.5</td>
<td>22:78</td>
<td>88</td>
</tr>
<tr>
<td>22</td>
<td>DMSO</td>
<td>DCM</td>
<td>Pyridine</td>
<td>30-32°C</td>
<td>0.5</td>
<td>80:00</td>
<td>80-83</td>
</tr>
<tr>
<td>23</td>
<td>DMSO</td>
<td>DCM</td>
<td>Piperidine</td>
<td>30-32°C</td>
<td>0.5</td>
<td>85:00</td>
<td>82-85</td>
</tr>
<tr>
<td>24</td>
<td>DMSO</td>
<td>DCM</td>
<td>Pyrrolidine</td>
<td>30-32°C</td>
<td>0.5</td>
<td>80:00</td>
<td>82-85</td>
</tr>
</tbody>
</table>
Table 2: Reaction of ethyl acrylate and nitro methane in different solvents with Cs$_2$CO$_3$ as the base, and CuCN as a catalyst.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Solvent</th>
<th>t ($^\circ$C)</th>
<th>Time (h)</th>
<th>Ratio of 74a:74b</th>
<th>Isolated Yields</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMSO</td>
<td>30-32</td>
<td>0.5</td>
<td>22:78</td>
<td>88</td>
</tr>
<tr>
<td>2</td>
<td>DCM</td>
<td>30-32</td>
<td>0.5</td>
<td>16:84</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>DMF</td>
<td>30-32</td>
<td>0.5</td>
<td>34:66</td>
<td>86</td>
</tr>
<tr>
<td>4</td>
<td>CH$_3$CN</td>
<td>30-32</td>
<td>0.5</td>
<td>35:65</td>
<td>86</td>
</tr>
<tr>
<td>5</td>
<td>THF</td>
<td>30-32</td>
<td>0.5</td>
<td>39:61</td>
<td>85</td>
</tr>
<tr>
<td>6</td>
<td>CH$_3$OH</td>
<td>30-32</td>
<td>0.5</td>
<td>No reaction</td>
<td>_</td>
</tr>
<tr>
<td>7</td>
<td>H$_2$O</td>
<td>30-32</td>
<td>0.5</td>
<td>No reaction</td>
<td>_</td>
</tr>
<tr>
<td>8</td>
<td>Ethylene glycol</td>
<td>30-32</td>
<td>0.5</td>
<td>No reaction</td>
<td>_</td>
</tr>
</tbody>
</table>

Ratio of products calculated based on isolated yields. Combined yield of pure isolated products.

Best results were obtained at 30-32 $^\circ$C with Cs$_2$CO$_3$/K$_2$CO$_3$ as base and DCM as solvent of choice in high yields (85-90%). With organic bases, 3a was obtained as the sole product. Reaction at elevated temperature (> 40 $^\circ$C) failed to furnish 3b. Since the reaction at higher temperature failed to afford 3b, microwave and sonication method were tried. Microwave assisted reaction of methyl acrylate and nitro methane adsorbed on silica gel (mesh 60-120) at 55 $^\circ$C for 2 minutes with Cs$_2$CO$_3$ as base and CuCN as catalyst failed to furnish product 74b. However, under ultrasonication at 30 $^\circ$C with reaction time of 30 minutes, 74b was isolated as the major product (ratio of 74a:74b/1:3.5, combined isolated yields 90 %). From the above results, it may be concluded that the temperature affects not only the ratio of the products, their yields, but also the reaction time. With protic solvents like H$_2$O, CH$_3$OH, the reaction did not take place while in aprotic solvents such as DMSO, ACN, DMF, THF and DCM; best results were obtained in DCM (reaction temp. 30-32 $^\circ$C) to achieve a ratio of >5:1 for 74b:74a (Table-2).

Further optimization study was carried out using different copper salt and the best results were obtained with CuCN catalyst (Table-3).
Table 3: Study of different Cu-salts on time, space and yield of the reaction products of ethyl acrylate and nitro methane.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Catalyst</th>
<th>Ratio of 74a:74b in DCM, with base</th>
<th>Ratio of 74a:74b in DMSO, with base</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>K₂CO₃</td>
<td>Cs₂CO₃</td>
</tr>
<tr>
<td>1</td>
<td>CuCN</td>
<td>25:75</td>
<td>16:84</td>
</tr>
<tr>
<td>2</td>
<td>CuCl</td>
<td>31:69</td>
<td>26:74</td>
</tr>
<tr>
<td>3</td>
<td>CuI</td>
<td>28:72</td>
<td>24:76</td>
</tr>
<tr>
<td>4</td>
<td>Cu(CN)₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>CuCl₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>CuI₂</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reaction time: 0.5 h, Temp: 30-32 °C

Further reactions were carried out to validate the feasibility of the methodology using six different acrylates and subjecting them to react with nitro methane (Scheme-22) and nitro ethane (Scheme-23) under the optimized reaction conditions of temperature (30-32 °C), base (Cs₂CO₃), solvent (DCM) using CuCN as the catalyst.

3.4.2. Reaction of different alkyl acrylates and nitromethane

![Scheme 22](image-url)
3.4.3. Reaction of different alkyl acrylates and nitroethane

In the sets of experiments involving nitromethane and nitroethane, the reactions were carried out in DCM and DMSO as the solvents and a marginal difference in the ratio of the keto diester and nitro diester products was observed for all the reactions. The reactions proceeded smoothly with good to excellent overall yields as can be seen from the results summarized in the table-3 and table -4.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Substrate</th>
<th>Reaction with CH$_3$NO$_2$</th>
<th>Reaction with C$_2$H$_5$NO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>74a:74b</td>
<td>76a:76b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ratio</td>
<td>Combined yield (%)</td>
</tr>
<tr>
<td>1</td>
<td>Ethyl acrylate</td>
<td>16:84</td>
<td>83-90</td>
</tr>
<tr>
<td>2</td>
<td>Methyl acrylate</td>
<td>19:79</td>
<td>80-85</td>
</tr>
<tr>
<td>3</td>
<td>Butyl acrylate</td>
<td>28:72</td>
<td>70 -72</td>
</tr>
<tr>
<td>4</td>
<td>Methyl methacrylate</td>
<td>27:73</td>
<td>69-70</td>
</tr>
<tr>
<td>5</td>
<td>Ethyl methacrylate</td>
<td>29:71</td>
<td>65-70</td>
</tr>
<tr>
<td>6</td>
<td>Butyl methacrylate</td>
<td>30:70</td>
<td>66-70</td>
</tr>
</tbody>
</table>

Reaction time: 0.5 h, Temp: 30-32 ºC

Next, we tried to expand the scope of the reaction on other α, β-unsaturated systems such as citral, acrolein, carvone, and cinnamaldehyde. However, all these substrates failed to furnish the desired products. These results lead to imply that the ester group of the acrylates apparently seems to contribute for the reaction to click.
Chapter 3: Section A

The reactions proceeded smoothly with good to excellent overall yields and with substituted acrylates (Table-4), the desired product was obtained as single entity (diastereoisomeric ratio: >99%, confirmed by spectral analysis, entries 74b- III-V and 76a- III-V). The proton spectrum of compound 74b IV on addition of CSA [S(+)-1-(9-anthryl)-2,2,2-trifluoro-ethanol] showed fine splitting of methyl and methine proton signals (Figure-2) leading thereby to conclude that the diastereoisomer is apparently a racemic mixture rather than a meso compound. The splitting pattern of proton signals was also observed for 76a- IV.

Figure 2: $^1$H NMR spectra of 74b- IV in the absence and presence of CSA. 1a: Spectra in absence of CSA and 1c and 1e its expanded spectra. 1b: Spectra in presence of CSA and 1d and 1f its expanded spectra.
From mechanistic point of view, account for the formation of the keto diester, a plausible mechanism has been proposed as shown in Scheme 24. The mechanism involves the attack of the carbanion (:CH$_2$NO$_2$) on the ethyl acrylate under basic conditions to give Michael product “x” (a carbanion) which attacks the second ethyl acrylate molecule to give 4-nitro diester $74a$. The nitro group of the latter forms a complex with CuCN which undergoes deprotonation of C-4 to form “y”, followed by attack of water molecule to form intermediate “z” and subsequent expulsion of HNO group to result in the formation of keto diester $74b$.

**Plausible mechanism of the reaction**
3.5. Conclusions

We have developed a copper catalyzed one pot synthesis of γ-keto diesters using simple available substrates in desirable yields. The procedure described is simple and involves relatively mild reaction conditions.

3.6. Standard Experimental Procedure

NMR spectra were recorded on Bruker 200, 400 and 500 MHz spectrometers with TMS as the internal standard. Chemical shifts were expressed in parts per million (δ ppm). Reagents and solvents used were mostly LR grade. Silica gel coated aluminum plates from M/s Merck were used for TLC. MS were recorded on High Resolution Mass Spectrometer MS Q-TOF LC/MS, Agilent Technologies 6540. Optical rotations were measured on Perkin-Elmer 241 polarimeter at 25°C using sodium D light. Melting points were determined on Buchi B-542 apparatus by open capillary method and are uncorrected. Chemicals were purchased from M/s Aldrich Chemicals, Mumbai. All anhydrous reactions were carried out under nitrogen atmosphere using freshly dried solvents. The organic extracts were dried over anhydrous Na₂SO₄.

3.6.1. General Procedure for synthesis of Diesters

To DCM solution (10 mL) of nitro methane/nitro ethane (1 eq.) was added Cs₂CO₃ (1 eq.) and the contents stirred for five minutes, followed by the addition of a mixture of appropriate acrylate (1 eq., in 3 mL DCM) and catalytic amount of CuCN (pinch) and the reaction mixture stirred at 30-32 °C. The progress of reaction was monitored by TLC. On completion of reaction, the contents were diluted with water, the organic layer separated and the aqueous portion extracted with DCM (3×50 mL). The combined organic layer washed with water (2×10 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure at <40°C. The crude reaction product was purified by column chromatography over silica gel (mesh 60-120) using hexane and ethyl acetate (19:1) as eluent to obtain nitro and keto diester products.
3.7. Spectral Data of Some Selected Compounds

**Diethyl 4-nitroheptanedioate: (74a).** Viscous liquid (157 mg);
\(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta 4.61-4.70\) (m, 1H), 4.15 (q, \(J = 7.15\) Hz, 4H), 2.10-2.42 (m 8H), 1.26 (t, \(J = 7.15\) Hz, 6H); \(^{13}\)C NMR (50 MHz, CDCl\(_3\)): \(\delta 171.7, 86.6, 60.9, 30.1, 28.6, 14.1\); IR (neat) \(\nu_{\text{max}}\) 1773, 1660, 1554, 1444, 1376 cm\(^{-1}\); MS at \(m/z\) 284 (M\(^+\)+Na).

**Diethyl 4-oxoheptanedioate: (74b).** Viscous liquid (915 mg);
\(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta 4.12\) (q, \(J = 7.1\) Hz, 4H), 2.78 (t, \(J = 6.5\) Hz, 4H), 2.60 (t, \(J = 6.5\) Hz, 4H), 1.25 (t, \(J = 7.1\) Hz, 6H);
\(^{13}\)C NMR (50 MHz, CDCl\(_3\)): \(\delta 207.0, 172.7, 60.6, 37.1, 28.0, 14.1\); IR (neat) \(\nu_{\text{max}}\) 2983, 1735, 1660, 1554, 1444, 1376, 1183, 1030 cm\(^{-1}\); MS at \(m/z\) 253 (M\(^+\)+Na).

**Dimethyl 4-oxoheptanedioate: (74b-I).** Viscous liquid (185 mg);
\(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta 3.67\) (s, 6H), 2.78 (t, \(J = 6.4\) Hz, 4H), 2.63 (t, \(J = 6.4\) Hz, 4H); \(^{13}\)C NMR (50 MHz, CDCl\(_3\)): \(\delta 206.9, 173.1, 51.8, 37.1, 27.7\); IR (neat) \(\nu_{\text{max}}\) 2922, 1744, 1715, 1705, 1637, 1617, 1440, 1333, 1205, 1104, 991 cm\(^{-1}\); MS at \(m/z\) 225 (M\(^+\)+Na).

**Dibutyl 4-oxoheptanedioate: (74b-II).** Viscous liquid (216 mg);
\(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta 4.07\) (t, \(J = 6.5\) Hz, 4H), 2.77 (t, \(J = 6.5\) Hz, 2H), 2.60 (t, \(J = 6.4\) Hz, 2H), 2.28-2.36 (m, 4H), 1.50-1.63 (m, 4H), 1.27-1.45 (m, 4H), 0.92 (t, \(J = 7.0\) Hz, 6H); \(^{13}\)C NMR (50 MHz, CDCl\(_3\)): \(\delta 206.9, 172.4, 64.6, 37.1, 30.3, 28.0, 19.1, 13.7\). IR (neat) \(\nu_{\text{max}}\) 1718, 1635, 1551, 1465, 1437, 1205, 1163 cm\(^{-1}\); MS at \(m/z\) 286 (M\(^+\)).

**Diethyl 2,6-dimethyl-4-oxoheptanedioate: (74b-III).** Viscous liquid (357 mg); \(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta 4.16\) (q, \(J = 7.13\) Hz, 4H), 2.96-2.82 (m, 4H), 2.53-2.39 (m, 2H), 1.24 (t, \(J = 7.12\) Hz, 6H), 1.14 (d, \(J = 6.9\) Hz, 6H); \(^{13}\)C NMR (50Hz, CDCl\(_3\)): \(\delta 206.9, 175.7, 60.8, 45.8, 36.2, 16.5, 14.1\); IR (neat) \(\nu_{\text{max}}\) 1732,

1685, 1555, 1462, 1433, 1198, 1167 cm\(^{-1}\); MS at \(m/z\) 281 (M\(^{+}\)+Na).

**Dimethyl 2,6-dimethyl-4-oxoheptanedioate:** (74b-IV). Colorless semisolid (181 mg); \(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta\) 3.66 (s, 6H), 2.97-2.86 (m, 4H), 2.52-2.40 (m, 2H), 1.12 (d, \(J = 7.1\) Hz, 6H); \(^{13}\)C NMR (50 MHz, CDCl\(_3\)): \(\delta\) 206.6, 176.0, 51.8, 45.9, 34.5, 17.0; IR (neat) \(\nu_{\text{max}}\) 2953, 1736, 1462, 1205, 1107 cm\(^{-1}\); MS at \(m/z\) 253 (M\(^{+}\)+Na).

**Dibutyl 2,6-dimethyl-4-oxoheptanedioate:** (74b-V). Liquid (326 mg); \(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta\) 4.17-4.0 (m, 4 H), 2.84-2.48 (m, 4H), 2.50-2.43 (m, 2H), 1.17 (d, \(J = 6.77\) Hz, 3H), 1.14 (d, \(J = 6.80\) Hz, 3H), 0.92 (t, \(J = 7.1\) Hz, 6H); \(^{13}\)C NMR (50 MHz, CDCl\(_3\)): \(\delta\) 206.5, 172.9, 64.5, 50.9, 45.8, 34.7, 30.6, 19.1, 17.0, 13.7; IR (neat) \(\nu_{\text{max}}\) 1732, 1551, 1465, 1437, 1205, 1163, 1651 cm\(^{-1}\); MS at \(m/z\) 237 (M\(^{+}\)+Na).

**Ethyl 4-oxopentanoate:** (76b). Viscous liquid (267 mg); \(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta\) 4.1 (q, \(J = 7.0\) Hz, 2H), 2.75 (t, \(J = 6.4\) Hz, 2H), 2.57 (t, \(J = 6.4\) Hz, 2H), 2.19 (s, 3H), 1.2 (t, \(J = 7.0\) Hz, 3H); \(^{13}\)C NMR (50 MHz, CDCl\(_3\)): \(\delta\) 206.81, 172.8, 60.6, 37.9, 29.9, 28.0, 14.1; IR (neat) \(\nu_{\text{max}}\) 1735, 1655, 1437, 1205 cm\(^{-1}\); MS at \(m/z\) 167 (M\(^{+}\)+Na).

**Butyl 3-methyl-4-oxopentanoate:** (76b-V). Colorless liquid (352 mg); \(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta\) 4.12 (t, \(J = 6.63\) Hz, 2H), 2.89 (m, 2H), 2.50 (m, 1H), 2.16(3H, s), 1.54 (m, 2H), 1.32 (m, 2H), 1.20 (d, \(J = 7.92\) Hz, 3H), 0.89 (t, \(J = 7.25\) Hz, 3H); \(^{13}\)C NMR (50 MHz, CDCl\(_3\)): \(\delta\) 206.6, 175.8, 64.5, 46.6, 34.8, 30.6, 30.0, 19.4, 17.4, 13.5. IR (neat) \(\nu_{\text{max}}\) 1744, 1717, 1705, 1637, 1617, 1440, 1333, 1215, 1107 cm\(^{-1}\); MS at \(m/z\) 209 (M\(^{+}\)+Na).
3.7.1. Spectra’s of some representative compounds

$^1\text{H} \& ^{13}\text{C}$ NMR spectra of compound (74a)
$^1$H & $^{13}$C NMR spectra of compound (74b)
$^1$H & $^{13}$C NMR spectra of compound (74b-I)
$^1$H & $^{13}$C NMR spectra of compound (74b-II)
$^1$H & $^{13}$C NMR spectra of compound (74b-III)
\(^1\text{H} \& ^{13}\text{C} \text{NMR spectra of compound (74b-IV)}\)
$^1$H & $^{13}$C NMR spectra of compound (74b-V)
\(^1\text{H} \& ^{13}\text{C} \text{NMR spectra of compound (76b)}\)
Chapter 3: Section A

Nef Reaction

$^1$H & $^{13}$C NMR spectra of compound (74b-V)
\[ ^1H \text{ NMR spectra of compound (74b-IV)} \]
$^1$H NMR spectra of compound (74b-IV)
\[^1H\text{ NMR spectra of compound (74b-IV)}\]
3.8. Introduction

Nature has been a source of therapeutic agents for thousands of years, and an impressive number of modern drugs have been derived from natural sources, many based on their use in traditional medicine. Over the last century, a number of top selling drugs have been developed from natural products (artemisinin from *Artemesia annua*, vincristine from *Vinca rosea*, morphine from *Papaver somniferum*, taxol from *T. brevifolia*, etc.). In recent years, a significant revival of interest in natural products as a potential source for new medicines has been observed among academia as well as pharmaceutical companies. Several modern drugs (~40% of the modern drugs in use) have been developed from natural products. The reason for this success in drug discovery can probably be explained by their high chemical diversity, the effects of evolutionary pressure to create biologically active molecules, and/or the structural similarity of protein targets across many species. Structural modification of natural products has become an integral part of the drug discovery process. Many potential drugs and lead compounds derived by structural modification of natural compounds, or by the synthesis of new compounds, have been designed following a natural compound as a model.\(^1\) In modern methods of drug discovery processes, design and synthesis of drugs based on the biological targets is of the great interest to modern medicinal chemists. Additionally, semi-synthetic processes for new compounds, obtained by molecular modification of the functional groups of lead compounds, are able to generate structural analogues with greater pharmacological activity and with

---

fewer side effects. A constant enrichment in the science of organic synthesis through improvement of the synthetic methodologies is observed, driven by the needs to improve the capability to synthesize molecules in more facile, efficient and in economical ways. The paradigms of organic synthesis have shifted from the traditional concept of efficiency in terms of chemical yield to one that also considers economic and ecological values. The efficiency of a chemical reaction generally means the ability to assemble the target molecule from readily available building blocks in relatively few operations with only minimal resource requirement (raw material, energy, labour etc.) and least waste generation. While selectivity and atom economy issues were considered the sole criteria that judge the efficiency of a chemical synthesis in the past, efficiency criteria regarding the reaction processing are being equally emphasized now. The significance of a particular reaction can be judged on its capability to form products with dimensions of high yield, chemo-, regio-, stereo- or enantioselectivity. Further requirements may include generality over a wide range of starting materials as well as productivity aiming at more diverse and complex products using a rather low number and complexity of starting materials. An “ideal” organic reaction would fulfill at least one of these criteria.

3.9. Objectives of the Present Work

Cancer is presently the main cause of death in the world. The present treatment strategy includes alkylating agents, steroids, vinea alkaloids, anti-metabolites, taxanes and antibiotics etc. High doses of these agents and intensive regiments are being employed, aiming to cure malignancy, which result in severe side effects. There is a need of anticancer drugs which are safe and more effective. Besides exploitation of natural resources (plants, microbes and fungi) for the discovery of new anticancer drugs or drug candidates, preparation of synthetic new chemical entities with potent therapeutic profiles can be equally explored. In this direction, Biginelli reaction catalysed by heteropolyacids can be one of the sources for the synthesis of large number of new molecules. Heteropolyacid (HPA) catalysts are widely exploited for the production of fine organic chemicals, health care products, pharmaceuticals and

---

agrochemical products.\textsuperscript{5} HPAs are more reactive catalysts than conventional inorganic and organic acids for reactions in solution.\textsuperscript{6} Heteropolyacids have been used as catalysts for many of the organic transformations, such as synthesis of acylals,\textsuperscript{7} tetrahydroxylation of phenols,\textsuperscript{8} thioacetalization and transacetalization reactions.\textsuperscript{9} They are also used as industrial catalysts for several liquid-phase reactions,\textsuperscript{10} including alcohol dehydration,\textsuperscript{11} alkylation\textsuperscript{12} and esterification.\textsuperscript{13}

The work embodied in the present section of the chapter relates to the synthesis of dihydropyrimidinone analogues by Biginelli reaction and there \textit{in vitro} screening against different cancer cell lines. The methodology involves the multicomponent reaction catalyzed by “HPA-Montmorillonite-KSF” as a reusable and heterogeneous catalyst.

\textbf{3.10. Review of Literature}

Most of the organic reactions in the chemistry comprise of one or two starting materials. Reactions that use more than two different starting materials are called multi-component reactions (MCRs). An “ideal” MCR should not only comprise of more than two starting materials of different nature but should also incorporate all or most of the atoms of those starting materials into the final product for better chemical efficiency and generation of variety of molecular libraries. In last one decade, there has been an incredible increase on the accessibility of MCRs, and much still remains to be accomplished.\textsuperscript{14} Some of these transformations are important cornerstones in the diversity-oriented construction of molecular complexity due to their ability to


incorporate, in a fast and efficient manner, three or more components into a single product. Reactions that build up carbon-carbon bonds and at the same time introduce nitrogen-containing functionalities into the structural framework are especially attractive for the rapid construction of organic molecules.

Multicomponent reactions (MCR) have attracted considerable interest owing to their extraordinary synthetic efficiency.\(^\text{15}\) The utmost attribute of MCRs is the inherent bond forming efficiency (BFE),\(^\text{16}\) i.e. the number of bonds that are formed in one process is an important measure to determine the quality of a multicomponent reaction, without isolating the intermediates, changing the reaction conditions, or adding further reagents, unlike the usual stepwise formation of individual bonds in the target molecule. It is obvious that the adoption of such strategies would allow minimization of both waste production and expenditure of human labor. As MCRs are one-pot processes with simpler experimental conditions that do not require the isolation of intermediates, they are perfect candidates for combinatorial, and generation of drug like molecules.

The Strecker reaction\(^\text{17}\) has been viewed as the earliest recognized multicomponent reaction. This involves a three-component coupling of an amine and an aldehyde with hydrogen cyanide to give α-aminonitriles which represent important precursors for α-amino acids. Over the years, safer, milder, and even asymmetric\(^\text{18}\) reaction conditions have been developed and inspired new interest in this reaction. In Mannich reaction,\(^\text{19}\) the α-aminoalkylation of ketones has proven an extremely valuable transformation for the synthesis of large number of natural product. Robinson’s synthesis of tropinone in 1917 is generally recognized as the first multicomponent synthesis of a natural product.\(^\text{20}\) Other historically significant MCRs that are based on the reactivity of


\(^{17}\) Strecker, A.; Liebigs Ann. Chem. 1850, 75, 27.


carbonyl and amine functionalities include the Hantzsch pyrrole synthesis,\(^{21}\) Biginelli’s synthesis of dihydropyrimidines,\(^{22}\) and the four-component Bucherer-Bergs reaction,\(^{23}\) an extension of the Strecker reaction for the synthesis of amino acids and hydantoin. In the Ugi-4-component reaction, the four reactants viz aldehyde, amine, isonitrile, and carboxylic acid combine to give a peptidoic \(\alpha\)-acylamino carboxamide.\(^{24}\) These historically significant MCRs discussed above are summarized in figure-1.

![Figure 1](image_url)

The discovery and development of novel MCRs goes unabated for research and industrial chemistry groups and poses a new challenge for organic chemists besides understanding of organic chemistry itself. Among all the mentioned MCRs, a prominent one that produces an interesting class of nitrogen heterocycles is the venerable Biginelli’s dihydropyrimidine synthesis.

Nitrogen containing compounds have tremendous potential in the field of chemistry and possible enormous biological properties. Functionalized nitrogen-heterocycles play a prominent role in medicinal chemistry and therefore they have been intensively used as scaffolds in the field of drug development. In this context pyrimidine derivatives are of particular interest because of their pharmacological profile. Dihydropyrimidin-2(1H)-ones (DHPM) of type also known as Biginelli compounds are easily accessible via a multicomponent condensation process, first reported more than a century ago.

Historically, Italian chemist Pietro Biginelli (University of Florence) reported this reaction (a three component reaction) for the first time involving acid-catalyzed condensation of ethyl acetoacetate, benzaldehyde, and urea in ethanol in refluxing conditions to obtain (on cooling) a solid crystalline product 3,4-dihydropyrimidin-2(1H)-one (Scheme-1).
3.10.1. Mechanism

Several studies related to mechanistic aspects of the reaction have been carried out. After forty years of Biginelli’s report, Folkers and Johnson first conducted its mechanistic studies. Different possible ways, by which these three reaction components can combine, were examined for the preparation of dihydropyrimidine as shown in figure-2.

\[ \text{Figure 2} \]

---

(I) the instantaneous reaction between benzaldehyde, ethyl acetoacetate, and urea, (II) the combination of ethyl acetoacetate and benzal-bisurea, (III) the reaction of benzaldehyde and ethyl β-carbamidocrotonate, and (IV) the reaction of ethyl α-benzalacetoacetate and urea. They proposed that the simultaneous combination of the three reaction components in A was improbable.

D was ruled out on the basis of the low reaction yields (2%). In contrast, B and C gave high yields of 6 (80%). The authors note that B may undergo fragmentation of the benzal-bisurea, regenerating the three reaction components, which may then form the product by another pathway. Further, the authors posit that the β-carbamidocrotonate in C hydrolyzes to the original three reaction components. Therefore, they conclude that 6 is likely formed from cyclization of 27 [figure-2], which can be generated from either B or C.

Sweet and Fissekis proposed the second mechanistic approach after Folkers’ revolutionary work.29 In this proposal there is an aldol condensation between benzaldehyde and ethyl acetoacetate to form a stabilized carbenium ion 28. Trapping with N-methylurea gives 29, which can cyclize to form 30 (Figure-2).

Kappe further explored the mechanism of the Biginelli reaction using NMR spectroscopy and trapping experiments.30 He proposed the formation of N-acyliminium 32 from benzaldehyde and urea via an unobservable (1H NMR) hemiaminal 31 (Figure-2). Interception of 32 with the enol tautomer of ethyl acetoacetate gives 33, the precursor to dihydropyrimidine 26. Kappe suggests that the first step, formation of 31, is rate limiting, thus preventing the observation of intermediates 32 and 33 by NMR.

The synthesis of 3,4-dihydropyrimidine-2(1H)-ones (denoted as Biginelli compounds) and their derivatives31 is of importance due to the biological investigation of these various molecules via molecular manipulation which have shown several activities such as calcium channel blockers,32 anti-bacterial activity33, anti-tubercular. In view

---

of therapeutic and pharmacological importance, there is tremendous increase in activity, antitumor, anti-malarials, antihypertensive, and anti-inflammatory drugs. Further, 3,4-dihydropyrimidin-2(1H)-one type of structure has been found in natural marine alkaloid Batzelladine A and B which are the first low molecular weight natural products reported in the literature to inhibit the binding of HIV gp-120 to CD4 cells, so as to ensure a new field towards the development of AIDS therapy.

In search of more potent and effective medicinal important molecules, numerous Biginelli dihydropyrimidine related annulated or multifunctionalized pyrimidine heterocyclic compounds have been investigated or tested against different dangerous diseases. In the figure-3 only selective molecules have been presented showing significant activity against above mentioned diseases, and they are examined with clinically used drugs in vivo/in vitro and establishing QSAR.


3.10.2. Alternative synthetic routes of Biginelli reaction

Various modifications have been applied to Biginelli reaction to get better yield and to synthesize biologically active analogs using (i) different catalysts for increase in the
yield and rate of the reaction, (ii) use of microwave assisted synthetic strategies to shorten the reaction time and (iii) solid phase synthesis and combinatorial chemistry to generate library of DHPM analogs.

3.10.3. Microwave assisted synthesis

The major limitations of Biginelli reaction are lower yield, longer reaction time and work up, when different building blocks are used to synthesize library of compound. In organic synthesis the use of microwave as energy source for better yield and shorten the reaction time has been extensively investigated. Kappe et al have recently described a high yielding and rapid microwave-assisted protocol that allows the synthesis of DHPMs utilizing in gram quantities under controlled single-mode microwave irradiation using equimolar amount of benzaldehyde, ethyl acetoacetate, and urea react and Lewis acid (FeCl₃) as a catalyst to give the corresponding dihydropyrimidine. Using single-mode microwave irradiation, the reaction can be carried out on a 4.0 mmol scale in AcOH/EtOH 3:1 at 120 °C within 10 min, compared to 3-4 h using conventional thermal heating, providing DHPM in 88% isolated yield. (Scheme-2)

The antimony (III) chloride impregnated on alumina efficiently catalyses a one-pot, three component condensation reaction among an aldehyde, a β-ketoester, and urea or thiourea to afford the corresponding dihydropyrimidinones in good to excellent

---


yields. The reactions are probed in microwave (MW), ultrasonic, and thermal conditions and the best results are found using MW under solvent-free conditions. 42

3.10.4. Asymmetric Biginelli reactions

Biginelli compounds contain a stereogenic center, intensive researches have been started to develop methods for the synthesis of one or other of the enantiomers because it is common observation that individual enantiomers exhibit contrasting or even opposite biological activities,43 and the influence of the absolute configuration on the biological activity,44 has been investigated e.g. in SQ 32926 34 the (R)-enantiomer exhibits >400-fold more powerful antihypertensive activity than the (S)-isomer, and the (S)-enantiomer of Monastrol 44 has 15-fold more potent anti-cancer activity than (R)-Monastrol 63.45 It is also documented that the marine alkaloids i.e. Batzelladine A 59 and B 64 in enantiomerically pure state have potential anti-HIV activity46 as shown in (figure-4).

![Figure 4](image-url)
Several methods have been developed for the asymmetric synthesis of enantio-enriched dihydropyrimidines. Recently, M. A. Blasco et al. have reported biocatalytic highly enantioselective synthesis of (S) - monastrol of course they used enzymatic resolution employing enzymes lipase from *Candida antarctica* B and lipase from *Candida rugosa* yielding the (R) and (S) enantiomers. (Scheme-3).

![Scheme 3](image)

Optically active DHPMs have also been synthesized through auxiliary assisted asymmetric Biginelli synthesis by Dondoni et al. using chiral starting materials such as C-glycosyl substrates and in this investigation the synthesis of two diastereomers of monastrol analogs bearing the ribofuranosyl moiety has been successfully achieved via formation of diastereomeric N-3-ribofuranosyl amides from racemic monastrol and separation of both diastereomers and subsequent amide hydrolysis of the desired diastereomer (Scheme 4).

![Scheme 4](image)

### 3.10.5. Catalyst Variations

From literature review it is evident that dihydropyrimidinone moiety is pharmacologically important and many researchers are engaged to prepare their

---

libraries by using a variation of different catalysts. Some of the reported catalysts in the literature are Lewis acid,49 Cu(OTf)2/MWI,50 Yb(PFO)3,51 HCl/EtOH,52 H3PO4/Pd-Cat,53 TMSCI/CAN,54 silica immobilized Ni(II),55 Ca(OCl)2,56 Mg(NO3)2,57 chloroacetic acid,58 PPA-SiO2,59 gypsum,60 Nano-BF3, SiO2,61 Sulfated tungstate,62 Melamine trisulfonic acid,63 Silica-supported tin chloride and titanium tetrachloride,64 Cu(NO3)2·3H2O,65 Acidic ionic liquids,66 hexafluorophosphoratate (BMImPF6),67 Organocatalytic,68 H3PMO12O40,69 H3PW12O40,70 H6P2W18O62·24H2O,71 H4PMo11VO40,72 and other catalysts.

These chemical methods in spite of their potential utility, involve expensive / toxic reagents and adverse reaction conditions like, strong acidic conditions, long reaction times, high temperature, stoichiometric amount of catalysts, environmental concerns and poor yields. Therefore, in order to overcome these limitations, the discovery of a new and efficient catalyst with high catalytic activity, short reaction time, recyclability and simple work-up procedures for the preparation of 3,4-dihydropyrimidin-2(1H)-ones under neutral, mild and practical conditions is of prime

interest. Heteropolyacids that contain super acidic properties have found numerous applications during last three decades, as useful and versatile acid catalysts for some acid-catalyzed reactions. They are usually solids that are insoluble in non-polar solvents while as highly soluble in polar ones. They can be used in bulk or in supported forms, in both homogeneous and heterogeneous system. Furthermore, heteropolyacids have several advantages, including high flexibility in modification of the acid strength, ease of handling, environmental compatibility, non-toxicity, and experimental simplicity.\textsuperscript{73}

3.11. Results and Discussion

In the present study, MC Biginelli reaction using HPA-Montmorilonite-KSF (H\textsubscript{5}PV\textsubscript{2}W\textsubscript{10}O\textsubscript{40}) as a catalyst was explored. The strategy involves a three-component one-pot Biginelli-type reaction for the condensation of urea, ethylacetoacetate and aldehydes to corresponding pyrimidinones in presence of a catalytic amount of HPA-Montmorilonite-KSF (Scheme-5). The best conditions were achieved under solvent free conditions (neat) by using 2 mol\% of HPA, 1.5 equivalents of urea / thiourea and ethyl acetoacetate and 1 equivalent of aldehyde under reflux conditions for 1 h, affording the desired product in good yields (Table 1).

\begin{center}
\textbf{Scheme 5}
\end{center}

3.11.1. Optimization of reaction

The model reaction was carried out by using para-methoxy benzaldehyde (1eq.), urea (1.5eq.) and ethylacetoacetate (1.5eq.) in solvent ethanol and catalyst (H\textsubscript{5}PV\textsubscript{2}W\textsubscript{10}O\textsubscript{40}) under reflux conditions for the generation of dihydropyrimidinone (DHPM) products

in good yields, which were identified by spectral analysis (NMR, IR and MS) and compared with the reported literature.

Encouraged by the initial success with regard to the formation of 74 a comprehensive optimization study was performed with the objective of time, space and yields minimizing the temperature range, shrunken reaction times, and exploration study towards the use of other solvents and catalysts for better yields due to the biological and synthetic importance of the DHPMs as described earlier. In the first set of optimization experiments, the reaction was studied in different solvents that included acetonitrile, 1, 4-dioxane, toluene, ethanol at reflux conditions. The investigation was also extended for solvent free reaction. The use of solvent free condition had profound effect in terms of time saving as the reaction was complete in 1h and of the two set of proved better choice than the reaction in solvents. (Table 1)

The reaction was also optimized with respect to the use of appropriate/optimum amount of the catalyst (H$_5$PV$_2$W$_{10}$O$_{40}$) for obtaining better yields and minimizing reaction time. The optimum amount of catalyst was found to be 2 mol%. In order to improve the yields of the reaction products the reactions were performed using different quantities of reagents with catalyst concentration of 2.0 mol %. The best results were obtained when para-methoxy benzaldehyde, urea and ethylacetoacetate were used in the ratio of 1:1.5:1.5 respectively.

Next the reaction was monitored at different temperature conditions in microwave, moreover sonication methods were also tried. Microwave assisted reaction adsorbed on silica gel (mesh 60-120) for 5-6 minutes with (H$_5$PV$_2$W$_{10}$O$_{40}$) as catalyst furnish product 74 in low yield. However, under ultrasonication at 35°C with reaction time of 30 minutes yields were low as compared to that at reflux. Reaction was also performed at room temperature for stirring, it takes long time for the completion of the reaction and yields were very low as compared to the reaction at reflux. From the above results, it may be concluded that the temperature affects not only yield of the products, but also the reaction time. The results of the optimization of the reaction conditions are summarized in (Table-1).

We found that this method is effective with a variety of substituted aliphatic and aromatic aldehydes independent of the nature of substituent’s (electron donating or
electron withdrawing) in the aromatic ring, representing an improvement to the classical Biginelli’s methodology as shown in (Table-2).

Table 1: H$_2$PV$_2$W$_{10}$O$_{40}$ catalyzes synthesis of DHPM in different solvents and under solvent free conditions.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Solvent</th>
<th>Time (h)</th>
<th>Amount of catalyst (mol %)</th>
<th>Yield (%)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1,4-dioxane</td>
<td>6 h</td>
<td>10</td>
<td>65</td>
<td>Reflux ()</td>
</tr>
<tr>
<td>2.</td>
<td>Acetonitrile</td>
<td>5 h</td>
<td>10</td>
<td>77</td>
<td>reflux</td>
</tr>
<tr>
<td>3.</td>
<td>toluene</td>
<td>7 h</td>
<td>10</td>
<td>71</td>
<td>reflux</td>
</tr>
<tr>
<td>4.</td>
<td>Ethanol</td>
<td>2 h</td>
<td>10</td>
<td>87</td>
<td>reflux</td>
</tr>
<tr>
<td>5.</td>
<td>Solvent free</td>
<td>1 h</td>
<td>10</td>
<td>92</td>
<td>reflux</td>
</tr>
<tr>
<td>6.</td>
<td>Solvent free</td>
<td>1 h</td>
<td>2</td>
<td>96</td>
<td>reflux</td>
</tr>
<tr>
<td>7.</td>
<td>Solvent free</td>
<td>5 minutes</td>
<td>2</td>
<td>70</td>
<td>Microwave oven</td>
</tr>
<tr>
<td>8.</td>
<td>Solvent free</td>
<td>30 minutes</td>
<td>2</td>
<td>74</td>
<td>Sonication at 35 °C</td>
</tr>
<tr>
<td>9.</td>
<td>Ethanol</td>
<td>18 h</td>
<td>2</td>
<td>40</td>
<td>rt (25)</td>
</tr>
<tr>
<td>10.</td>
<td>Solvent free</td>
<td>14 h</td>
<td>2</td>
<td>52</td>
<td>rt</td>
</tr>
</tbody>
</table>

Para-methoxy benzaldehyde: urea: ethylacetoacetate in the ratio of 1:1.5:1.5

Table 3: Synthesis of 3,4-dihydropyrimidin-2(1H)-ones

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>R</th>
<th>X</th>
<th>Yield (%)</th>
<th>Mp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Found</td>
<td>reported</td>
</tr>
<tr>
<td>1</td>
<td>74a</td>
<td>C$_6$H$_5$</td>
<td>O</td>
<td>97</td>
<td>201-203</td>
</tr>
<tr>
<td>2</td>
<td>74b</td>
<td>C$_6$H$_5$</td>
<td>S</td>
<td>95</td>
<td>206-208</td>
</tr>
<tr>
<td>3</td>
<td>74c</td>
<td>4-(Cl)-C$_6$H$_4$</td>
<td>O</td>
<td>93</td>
<td>209-211</td>
</tr>
<tr>
<td>4</td>
<td>74d</td>
<td>2-(Cl)-C$_6$H$_4$</td>
<td>O</td>
<td>89</td>
<td>213-215</td>
</tr>
<tr>
<td>5</td>
<td>74e</td>
<td>4-(Br)-C$_6$H$_4$</td>
<td>O</td>
<td>93</td>
<td>211-214</td>
</tr>
<tr>
<td>6</td>
<td>74f</td>
<td>4-(MeO)-C$_6$H$_4$</td>
<td>O</td>
<td>96</td>
<td>201-204</td>
</tr>
<tr>
<td>7</td>
<td>74g</td>
<td>2,3-(OMe)$_2$-C$_6$H$_3$</td>
<td>O</td>
<td>94</td>
<td>176-177</td>
</tr>
<tr>
<td>8</td>
<td>74h</td>
<td>2,4-(OMe)$_2$-C$_6$H$_3$</td>
<td>O</td>
<td>95</td>
<td>157-160</td>
</tr>
<tr>
<td>9</td>
<td>74i</td>
<td>3,4,5-(OMe)$_2$-C$_6$H$_2$</td>
<td>O</td>
<td>97</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>74j</td>
<td>4-(NO$_2$)-C$_6$H$_4$</td>
<td>O</td>
<td>79</td>
<td>207-208</td>
</tr>
<tr>
<td>11</td>
<td>74k</td>
<td>4-(F)-C$_6$H$_4$</td>
<td>O</td>
<td>83</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>74l</td>
<td>2-(Br)-5-(OMe)-C$_6$H$_5$</td>
<td>O</td>
<td>87</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>74m</td>
<td>piperalal</td>
<td>O</td>
<td>94</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>74n</td>
<td>2-(NO$_2$)-C$_6$H$_4$</td>
<td>O</td>
<td>81</td>
<td>217-219</td>
</tr>
<tr>
<td>15</td>
<td>74o</td>
<td>4-(OH)-C$_6$H$_4$</td>
<td>O</td>
<td>78</td>
<td>226-228</td>
</tr>
<tr>
<td>16</td>
<td>74p</td>
<td>3-(OH)-4(OMe)-C$_6$H$_3$</td>
<td>O</td>
<td>93</td>
<td>98-99</td>
</tr>
<tr>
<td>17</td>
<td>74q</td>
<td>5-(Br)-2-(OMe)-C$_6$H$_5$</td>
<td>O</td>
<td>94</td>
<td>-</td>
</tr>
</tbody>
</table>

3.12. Synthesis of new 3,4-dihydropyrimidin-2(1H)-one derivatives

To explore/apply the newly designed present Biginelli reaction, versatile aldehydes (both aromatic and aliphatic aldehydes) were used, with some of them obtained by modification of natural products (Scheme-9) or through synthetic route (Scheme 6-8). Natural product osthol isolated from plant prangos *pabularia* was oxidized into corresponding aldehyde by using selenium dioxide. Other synthetic aldehydes (Scheme 7-9) were prepared by performing Gigrand and Vislimer reactions by following the procedure reported by Koul and co-workers. The purpose to use these aldehydes was to extend the scope of Biginelli reaction besides generation of new library of DHPM products not reported hitherto in the literature for their preparation and more importantly study their bioevaluation profiles.

---

Scheme 6
Scheme 7

\[
\begin{align*}
1. \text{CH}_3\text{MgI} & \rightarrow \text{CHO} \\
2. \text{H}_2\text{O} & \rightarrow \text{CHO}
\end{align*}
\]

83 → 84 → 85

\[a = R_1 = H, R_2 = \text{OCH}_3, R_3 = H, R_4 = H\]
\[b = R_1 = H, R_2 = \text{OCH}_3, R_3 = H, R_4 = \text{OCH}_3\]
\[c = R_1 = H, R_2 = \text{H}, R_3 = \text{OCH}_3, R_4 = \text{OCH}_3\]
\[d = R_1 = \text{OCH}_3, R_2 = \text{OCH}_3, R_3 = \text{OCH}_3, R_4 = \text{H}\]
\[e = R_1 + R_2 = \text{O} \, \text{O}, R_3 = H, R_4 = H\]
\[f = R_1 + R_2 = \text{O} \, \text{O}, R_3 = H, R_4 = H\]

Scheme 8

87 → 88 → 89

Scheme 9

90 → 91 → 92
The synthesized Bignilli products are summarized in the figure 5.

![Chemical structures of synthesized Bignilli products](image)

Figure 5

3.13. *In vitro* screening of the Synthesized Analogues on Human Cancer Cell Lines

All the newly synthesized analogues (figure-5) were subjected to *in vitro* cytotoxicity screening against various human cancer cell lines, colon, pancreatic, leukemia, and lung at 50µM concentration using MTT assay. Out of these 16 compounds screened, 11 compounds proved active. Compound 86a, showed anti-proliferative activity across the board, exhibiting highest inhibition of 90% against lung cancer. Compounds 76c, 82, and 86d showed inhibition against three out of four cancer cell lines and compounds 78, 86b and 86e showed inhibition against two cancer cell lines. Against Colo-205 cancer cell line, compound 76c showed maximum inhibition (80%)
followed by 86a (75%) with rest of the compounds (86a, 86d, 86f) showing moderate activity (52-60%).

Compound 86a exhibited maximum inhibition against Pc-5 cancer cell line (78%) and compounds 80a, 82, and 89 showed moderate activities.

Against THP-1, only five compounds exhibited inhibiting activity with maximum effect draw for 86a, followed by 82. Against lung cancer cell line 86a showed the maximum effect (90%) followed by 86d (69%). The other three compounds displayed moderate cytotoxic effect. The results are summarized in table-4.

**Table 4**: Anti-proliferative activity at 50 µM of new DHPM analogs against Colo-205, PC-3, THP-1 and A549, cell lines with MTT assay.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Colo-205</th>
<th>PC-3</th>
<th>THP-1</th>
<th>A549</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue</td>
<td>COLON</td>
<td>PROSTATE</td>
<td>LEUKEMEIA</td>
<td>Lung</td>
</tr>
<tr>
<td>Compd. No.</td>
<td>Conc.</td>
<td>Percentage inhibition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>74m</td>
<td>50µM</td>
<td>55</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>76a</td>
<td>50µM</td>
<td>-</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>76b</td>
<td>50µM</td>
<td>-</td>
<td>11</td>
<td>26</td>
</tr>
<tr>
<td>76c</td>
<td>50µM</td>
<td>80</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>78</td>
<td>50µM</td>
<td>19</td>
<td>72</td>
<td>54</td>
</tr>
<tr>
<td>80a</td>
<td>50µM</td>
<td>8</td>
<td>68</td>
<td>0</td>
</tr>
<tr>
<td>80b</td>
<td>50µM</td>
<td>12</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>82</td>
<td>50µM</td>
<td>21</td>
<td>58</td>
<td>76</td>
</tr>
<tr>
<td>86a</td>
<td>50µM</td>
<td>75</td>
<td>78</td>
<td>78</td>
</tr>
<tr>
<td>86b</td>
<td>50µM</td>
<td>60</td>
<td>43</td>
<td>6</td>
</tr>
<tr>
<td>86c</td>
<td>50µM</td>
<td>0</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>86d</td>
<td>50µM</td>
<td>58</td>
<td>49</td>
<td>19</td>
</tr>
<tr>
<td>86e</td>
<td>50µM</td>
<td>0</td>
<td>34</td>
<td>56</td>
</tr>
<tr>
<td>86f</td>
<td>50µM</td>
<td>60</td>
<td>23</td>
<td>-</td>
</tr>
<tr>
<td>89</td>
<td>50µM</td>
<td>52</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>92</td>
<td>50µM</td>
<td>46</td>
<td>38</td>
<td>-</td>
</tr>
<tr>
<td>BEZ-235</td>
<td>0.01 µM</td>
<td>35</td>
<td>-</td>
<td>31</td>
</tr>
</tbody>
</table>
3.14. Recycled use of the Catalyst was our Next Part of Optimization Study

It may be stated that due to the constantly rising environmental worry in the field of chemistry, it is sensible to use easily recovered and recycled catalysts, especially expensive or toxic metallic ones for the next use as, only few of them meet this criterion of green chemistry. For example, the recovery of ytterbium triflate from water seems cumbersome since water must be removed through heating and then drying under vacuum at 100 °C for 2 h, and In the case of polymer-supported Yb (III) resin, the activity of recycled resin is much lower than that of the original one thus limiting the recyclability. Therefore, there is still room for further search for recyclable catalysts to be used in the Biginelli type that can convert a variety of aldehydes to pyrimidinones in high yields under mild reaction conditions.

In order to show the merit of the present work in comparison with some reported protocols, we compared the results of the synthesis of 5-ethoxycarbonyl-4-phenyl-6-methyl-3, 4-dihydropyrimidin-2(1H)-one in the presence of montmorillonite KSF, sulfuric acid, zeolite, silica sulfuric acid, BF$_3$ /OEt$_2$/CuCl, H$_3$PMo$_{12}$O$_{40}$ with HPA-Montmorillonite-KSF with respect to the reaction times. The yield of product in the presence HPA-Montmorillonite-KSF is comparable with these catalysts. However, the reaction in the presence of these catalysts required longer reaction times than HPA-Montmorillonite-KSF. The catalyst could be re-used several times (six times) and did not show any significant negative influence on the overall yields of the reaction (Figure-6).

---

Figure 6: Recyclability of the catalyst

Table 4: Comparison the results of the synthesis of 4-aryl-3,4-dihydropyrimidin-2(1H)-one using different catalysts.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Montmorilonite KSF \textsuperscript{85}</td>
<td>48</td>
<td>82</td>
</tr>
<tr>
<td>2.</td>
<td>Sulphuric acid \textsuperscript{86}</td>
<td>18</td>
<td>71</td>
</tr>
<tr>
<td>3.</td>
<td>Zeolite \textsuperscript{87}</td>
<td>12</td>
<td>80</td>
</tr>
<tr>
<td>4.</td>
<td>Silica sulphur acid \textsuperscript{88}</td>
<td>16</td>
<td>91</td>
</tr>
<tr>
<td>5.</td>
<td>BF$_3$OEt$_2$/CuCl \textsuperscript{89}</td>
<td>18</td>
<td>71</td>
</tr>
<tr>
<td>6.</td>
<td>H$<em>3$PMo$</em>{12}$O$_{40}$ \textsuperscript{90}</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>7.</td>
<td>HPA-Montmorilonite-KSF (present work)</td>
<td>1</td>
<td>96</td>
</tr>
</tbody>
</table>

3.15. Conclusions

We have reported a catalytic method for the synthesis of dihydropyrimidinones using HPA-Montmorilonite-KSF as an efficient and eco-friendly heterogeneous inorganic catalyst. It is noteworthy to mention that the catalyst is reusable and even after five

runs, the catalytic activity of $\text{H}_5\text{PV}_2\text{W}_{10}\text{O}_{40}\cdot10\text{H}_2\text{O}$ was retained as that of the freshly used catalyst. The short reaction times, simple work-up and isolation of the products in high yields with high purity, and mild reaction conditions, are some of the features of the current procedure. A library of other DHPM products has been synthesized by using the above catalyst, for the production of drug like molecules that is the main motive of the Institute. Among the synthesized analogues, eleven compounds displayed impressive anti-proliferative effect at 50µM, with best cytotoxic effect observed for 86a exhibiting the inhibition effect against all the cell lines. The bioactive compounds were being evaluated at lower concentration to gauge their cytotoxic potential, so as to take up them for further detailed investigation (cell death and mode of action etc.).

3.16. Standard Experimental Procedure

NMR spectra’s were recorded on Bruker 200, 400 and 500 MHz spectrometers with TMS as the internal standard. Chemical shifts were expressed in parts per million ($\delta$ ppm). Reagents and solvents used were mostly LR grade. Silica gel coated aluminum plates from M/s Merck were used for TLC. MS were recorded on High Resolution Mass Spectrometer MS Q-TOF LC/MS, Agilent Technologies 6540. Optical rotations were measured on Perkin-Elmer 241 polarimeter at 25°C using sodium D light. Melting points were determined on Buchi B-542 apparatus by open capillary method and are uncorrected. Chemicals were purchased from M/s Aldrich Chemicals, Mumbai. All anhydrous reactions were carried out under nitrogen atmosphere using freshly dried solvents. The organic extracts were dried over anhydrous $\text{Na}_2\text{SO}_4$.

3.16.1. Preparation of Catalyst $\text{H}_5\text{PV}_2\text{W}_{10}\text{O}_{40}\cdot10\text{H}_2\text{O}$ (HPA)

Disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4$, 1.775 g, 25 mmol) was dissolved in 50 mL of water and mixed with Vanadium pentoxide ($\text{V}_2\text{O}_5$6.1 g, 100 mmol) dissolved in 20 mL of 1M $\text{Na}_2\text{CO}_3$ and the solution so formed was boiled for 30 min (colour = green) and then cooled to room temperature. To this Sodium tungstate dihydrate ($\text{Na}_2\text{WO}_4\cdot2\text{H}_2\text{O}$, 41.25 g, 250 mmol) dissolved in 20 mL of water (colour = black). This solution was kept at 90°C for 30min (colour = bluish green), cooled and to it was added 50% sulfuric acid (50 mL) drop wise, a wine red solution (pH=2) was obtained.
Extraction of the solution with diethyl ether (100 mL) afforded the orange-red \( \text{H}_5\text{PV}_2\text{W}_{10}\text{O}_{40}.10\text{H}_2\text{O} \) product.

The HPA so formed was dissolved in 150 ml water and added dropwise to 500 mL aqueous suspension of 10 gm Montomorillonite KSF (stirred for 3 h). This mixture was stirred for 5 h and the water was evaporated over water bath to get a dry powder, which was kept overnight in air oven at 110 °C. A portion of this solid was then calcined at 425 °C for 3h to get a heteropoly acid clay nano hybrid and was named as HCNH.

3.16.2. Catalyst characterization

The crystallinity of the sample (HCNH₄) was studied by recording X-ray powder diffraction patterns on a Rigaku Miniflex diffractometer, using Ni-filtered Cu Ka (0.15418 nm) radiation source (Figure 7). The sample was scanned over the range 2.00-79.99 on 2\( \theta \) scale with steps 0.011° and step time 13.6 s. From the XRD prototype, it has been confirmed that the synthesized catalysts are well crystalline in nature. The powder XRD patterns of the HCNH₄ were more crystalline and show additional reflections which are characteristics of HPA. This confirmed that HPA is well supported on Montomorillonite and also improves the crystallinity of the supported catalysts. The XRD pattern of montomorillonite shows a very low intensity reflection at 2\( \theta \)=9.9, which may be due to the residual 2:1(T-O-T) structure. The increase in specific surface area and formation of mesopores results because of delamination during the process of preparation. HPA loading on montomorillonite increases the phase crystallinity, which increases the available active acidic sites for the reactions. It is obvious that the peaks are very sharp which provide evidence that the sample is exceedingly crystalline. The B.E.T. surface area of the catalyst was determined using the instrument SMART SORB 92/93 under the liquid Nitrogen. The surface area of the catalyst is 80.4762 m²/g. To study the morphology SEM of sample (Figure 8) was carried out using JEOL.JEM100CXII ELECTRON MICROSCOPE with ASID Accelerating Voltage 40KV. The SEM image is a confirmation for coarse surface (thus elevated surface area), which is able to absorb substrate and/ or reagent to a high extent. The SEM image of the gross morphology of the HPA/KSF is displayed in (Figure 8). It was observed that HPA particles were randomly
distributed over the support surface. It should be noted that HPA layer formed in the present work was constituted by several aggregates of HPA particles and not by a continuous film.

**Figure 7**

Powder X-ray diffraction for heteropolyacid supported on montmorillonite

**Figure 8**

SEM image of Heteropolyacids clay nanocomposite
3.16.3. General procedure for the synthesis of 4-aryl-3,4-dihydropyrimidin-2(1H)-one

A mixture of aldehyde (10 mmol), 1,3-dicarbonyl compound (15 mmol), urea or thiourea (15 mmol) and HPA-Montmorillonite-KSF. (2 mol %) was refluxed for 1 h and the progress of the reaction was monitored by TLC using petroleum ether: ethylacetate as eluent. Upon completion of the reaction, the mixture was cooled to room temperature and the catalyst was then removed by filtration and the solution poured on to ice-water (30 mL). The resulting solid product was filtered and recrystallized from ethanol to give the pure products. After removing the reaction product by filtration and washing the solid catalyst with ethanol, it could be reused and subjecting to a second run of the Biginelli reaction and it can be reused more than ten times, not too much effect on the yield of the reaction.

3.16.4. Preparation of α-methyl substitutes benzyl alcohol: 84(a-f) (Scheme 7)

To an ethereal solution of Mg metal (110mmol) and methyl iodide (130mmol) added, after the generation of the Gigrand reagent, an ethereal solution of different substituted aldehydes (110mmol) and the contents were stirred for 2 hours at 0-5 °C. The reaction mixture was worked up by adding saturated solution aqueous solution of ammonium chloride, (10ml), followed by dilution with water, separation of organic layer followed by extraction of aqueous layer with solvent ether (2×100ml). The combined organic layer washed with water (2×20ml), dried over anhydrous sodium sulphate and concentrated in vacuo to give a gummy mass. (95% yield).

3.16.5. Preparation of 3-(4-substituted phenyl)-2E-propenal: 85(a-f) (Scheme 7)

To a chilled solution of scheme 3 products in DMF added phosphoryl chloride slowly at 0-5 °C for 1 hour. The contents were stirred further for 2 hours and then allowed to attain room temperature followed by heating on a water bath for 3 hours. The reaction mixture was cooled and a saturated solution of sodium acetate (15 ml) added, followed by dilution with water. The contents of the reaction mixture were extracted with ethylacetate (4×100ml), the organic layer washed with water (3×20 ml) and dried over anhydrous sodium sulphate to give crude product which on column chromatography pet. ether: ethyl acetate (9:1) as eluent to give the products. 78

3.16.6. Preparation of 2-methyl-4-(7-methoxy-2-oxo-2H-chromen-8-yl)-but-2-en-1-al : 88 (Scheme 8)

Compound 88 was prepared by adding selenium dioxide (1 eq.) to a solution of osthol (1 eq.) dissolved in glacial acetic acid (7 ml) and stirred for about 3 h. On completion of the reaction (monitored by TLC), the contents were poured into crushed ice and extracted with dichloromethane (3×50 ml), the organic layer washed with water (2×10 ml) dried over sodium sulfate and concentrated on rot-vapor to give crude product, which on silica gel column chromatography, using pet. Ether-ethyl acetate as the eluent, yielded pure aldehyde in 50% yield.

3.16.7. Preparation of 2,2-dimethyl-3,4-dihydro-6-formyl : 91 (Scheme 8)

To a stirring solution of 4- hydroxyl benzaldehyde and orthophosphoric acid in hexane, a slow addition of freshly distilled isoprene dissolved in hexane made in 8-9 hours at room temperature and the reaction mixture stirred further for 24 hours. The reaction was worked up by dilution with water followed by solvent ether. The contents of the reaction mixture were extracted with ether (3×50ml), the organic layer washed with water (3×20ml) and dried over anhydrous sodium sulphate and concentrated on rot-vapor to give crude product, which on column chromatography, using hexane: benzene (4:1) as the eluent, to give the corresponding aldehyde in 40% yield. 80

3.17. Screening of synthesized molecules for inhibitory effect on cell proliferation by MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] assay (Cell viability assay)

3.18. Cell culture methodology

A panel of human cancer cell lines was selected for evaluating the antiproliferative/cytotoxic potential of molecules synthesized.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>PC-3</td>
</tr>
<tr>
<td>Colon</td>
<td>Colo-205</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>THP-1</td>
</tr>
<tr>
<td>Lung</td>
<td>A-549</td>
</tr>
</tbody>
</table>
Sources of cell lines
National Center for Cell Science, Ganeshkhind, Pune- 4111007 (India). National Cancer Institute, Biological Testing Branch DTP/ DCTD/ NCI, Frederick Cancer Research and Development Center, Fairview Center, Suite 205, 1003 West 7th Street, Frederick, MD 21701-8527 (USA).

3.18.1. Materials and methodology

Chemicals required
Growth medium (RPMI, DMEM), Fetal calf serum (Sigma), Trypsin (SIGMA), PBS (Merck), Tryphan blue, Ethanol, Penicillin (Sigma), Streptomycin (Sigma), Gentamycin (Sigma), DMSO (Sigma), Double distilled water.

Complete growth medium: Contains 10 % FCS and 1% Penicillin. The amount of FCS may vary depending upon the requirements of cell lines used. Freezing Medium for cryopreservation contains 20 % FCS and 10 % DMSO in growth medium (RPMI or MEM).

Phosphate Buffer Saline (PBS): Dissolve 9.6 gm/lit. in distilled water. PBS is used to prepare solutions of Penicillin and Trypsin EDTA.

Penicillin Solution: Dissolve 100 units/ml or 625 µg/ ml in PBS. Penicillin is mixed in RPMI Medium to avoid contamination.

Trypsin EDTA: 0.05% Trypsin and 0.02% EDTA (disodium salt) are dissolved in PBS. Trypsin EDTA is used to detach the cells while sub-culturing and splitting the cell line.

3.18.2. Principle
MTT assay was developed by Alley et al.\textsuperscript{92} MTT is a yellow, water-soluble tetrazolium dye, which crosses both plasma and mitochondrial membranes. In the mitochondria of viable and glycolytically active cells, MTT is reduced by the action of NADH- or NADPH-dependent dehydrogenases to form an insoluble, purple formazan. The amount of the formazan product that results from this reaction is dependent on the number of viable cells. As this reaction goes to completion, residual viable cells die, the formazan product can be solubilized and the optical density (OD)

can be measured, which reflects the number of cells present at the end of the assay period. In an assay using the MTT dye, in which cells were added to replicate wells of 96-well plates. The plates were placed in an incubator for a sufficient time interval (usually 48 h) to allow cells to undergo several cell division cycles. MTT was added to each well followed by an additional incubation period of at least 4h to allow for complete bioreduction of MTT to the final formazan product. The OD of each well was determined in a plate reader, allowing a high throughput, accurate and sensitive method to estimate cell numbers.

3.18.3. MTT assay procedure

1. The MTT assay is useful for measuring the effect of compounds on the proliferation of cell lines. The assay was set up in a 96-well, flat-bottomed polystyrene micro titer plate.
2. 5000-15000 cells were seeded per well per 100 µl in appropriate growth medium. After seeding of cells, plates were incubated for two hours (suspension cell lines) or for overnight at 37°C, 5% CO₂ and 100 % humidity.
3. Test molecules were added at defined concentrations in triplicate wells. Compounds were dissolved in Dimethyl Sulfoxide (DMSO) for the final addition.
4. Cells treated with test molecules were incubated for 48 hours under standard conditions in incubator.
5. Add 20µl of MTT stock solution (2.5µg/ml) directly to each well with a multichannel pipette.
6. Return the plates to the incubator for a period of at least 4 h.
7. In case of suspension cell lines, centrifuge the plates at 1000g for 10 min at ambient temperature, followed by inversion of the plates and blotting off excess medium. In adherent cell lines, the plates can be inverted and excess media blotted off without centrifugation.

Added 150µl of working DMSO to solubilize the MTT formazan product and after shaking read at 570nm.
3.19. Spectral Data

5-Ethoxycarbonyl-4-(4-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one: 74f;
M. P. (202 °C), \(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta\) 1.21 (3H, t, \(J=7.12\)Hz), 2.34 (3H, s), 3.79(3H, s), 4.09 (2H, q, \(J=7.16\)Hz), 5.06 (1H, s), 6.86 (2H, d, \(J=8.7\)Hz), 7.26 (2H, d, \(J=8.9\)Hz), 7.66 (1H, s); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 14.18, 18.65, 55.20, 55.28, 59.99, 101.68, 114.02, 127.82, 136.15, 145.93, 153.36, 159.28, 165.71; IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 3225, 3097, 2928, 1710, 1651, 1514, 1462, 1097; MS at \(m/z\) 290.13 (M\(^+\)+Na).

Ethyl 4-(benzo[d][1,3]dioxol-6-yl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxylate: 74m;
\(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta\) 1.18 (3H, t, \(J=7.08\)Hz), 2.34 (3H, s), 4.01 (2H, q, \(J=7.08\)Hz), 5.34 (1H, s), 5.66 (1H, s), 5.94 (2H, s), 6.70 (1H, d, \(J=1.61\)Hz), 6.75 (1H, m), 6.80 (1H, d, \(J=8.71\)Hz), 7.92 (1H, s); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 14.20, 18.55, 55.45, 60.02, 101.12, 101.35, 107.09, 108.18, 120.0, 137.92, 146.33, 147.23, 147.94, 153.65, 155.67; IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 3225, 3097, 2928, 1710, 1651, 1514, 1462, 1097; MS at \(m/z\) 304.31 (M\(^+\)+Na).

Ethyl 1,2,3,4-tetrahydro-6-methyl-2-oxo-4-propylpyrimidine-5-carboxylate: 76a;
M. P. (158 °C), \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 0.95 (3H, t, \(J=6.9\)Hz), 1.28-1.30 (4H, m), 1.48 (2H, m), 2.29 (3H, s), 4.20 (2H, q, \(J=6.7\)Hz), 4.27 (1H, m); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 14.24, 14.68, 18.19, 18.45, 40.32, 51.97, 61.10, 102.23, 149.21, 156.04, 167.85; IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 1744, 1717, 1705, 1637, 1617, 1440, 1333, 1215, 1107; MS at \(m/z\) 226.13 (M\(^+\)+Na).

Ethyl 4-butyl-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxylate: 76b;
M. P. (148 °C), \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 0.93 (3H, t, \(J=7.4\)Hz), 1.28 (7H, m), 1.41 (2H, m), 2.24 (3H, s), 4.17 (2H, q,
$J=7.1\text{Hz}$, 4.22 (1H, m); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 14.04, 14.35, 22.56, 24.03, 31.45, 36.87, 51.49, 59.92, 101.58, 146.83, 154.85, 167.41; IR (KBr) $\nu_{\text{max}}$cm$^{-1}$: 1744, 1717, 1637, 1617, 1460, 1333, 1215, 1107; MS at $m/z$ 240.15 (M$^+$+Na).

**Ethyl-methyl-2-oxo-4-pentyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate: 76c**

M. P. (152 °C), $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 0.80 (3H, t, $J=7.6\text{Hz}$), 1.19 (6H, m), 1.40 (2H, m), 2.15 (3H, s), 3.24 (1H, s), 4.07 (2H, q, $J=7.3\text{Hz}$), 4.14 (1H, m), 4.88 (1H, s); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 14.04, 14.35, 18.52, 22.56, 24.03, 31.45, 51.49, 59.92, 101.58, 146.83, 154.85, 167.41; IR (KBr) $\nu_{\text{max}}$cm$^{-1}$: 1744, 1717, 1637, 1617, 1460, 1333, 1215, 1107; MS at $m/z$ 254.33 (M$^+$+Na).

**Ethyl4-(3-hydroxystyryl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxylate: 78**

M. P. (137 °C), $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.33 (3H, t, $J=7.3\text{Hz}$), 2.32 (3H, s), 4.19 (2H, q, $J=7.4\text{Hz}$), 4.27 (1H, m), 6.23 (1H, dd, $J=3.9$ & 16Hz), 6.52 (1H, d, $J=16\text{Hz}$), 7.22 (1H, m), 7.25 (1H, m), 7.31 (1H, m), 7.39 (1H, m); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 14.90, 18.44, 53.76, 59.75, 101.81, 109.72, 118.34, 127.62, 128.85, 129.78, 130.17, 130.60, 144.60, 154.50, 158.4, 167.65; IR (KBr) $\nu_{\text{max}}$cm$^{-1}$: 3240, 3117, 2960, 1693, 1514, 1462, 1097; MS at $m/z$ 302.33 (M$^+$+Na).

**Ethyl4-(3,4-dihydronaphthalen-2-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate: 80a**

M. P. (183 °C), $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.24 (3H, t, $J=7.5\text{Hz}$), 2.29 (2H, m), 2.31 (3H, s), 2.80(2H, t, $J=7.67\text{Hz}$), 4.14 (2H, q, $J=7.6\text{Hz}$), 5.05 (1H, s), 5.35 (1H, s), 6.35 (1H, s), 7.12-7.26 (4H, m); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 14.80, 18.36, 23.95, 29.25, 61.16, 99.85, 125.02, 127.51, 128.47, 128.23, 135.24, 136.19, 142.32, 149.51, 155.73, 167.78; IR (KBr) $\nu_{\text{max}}$cm$^{-1}$: 3240, 3117, 2960, 1693, 1728, 1681, 1608, 1514, 1497, 1097; MS at $m/z$ 312.3665 (M$^+$+Na).
Ethyl 4-(1-chloro-3,4-dihydronaphthalen-2-yl)-6-methyl-2-oxo-1,2,3,4-
etrahydroxypyrimidine-5-carboxylate: 80b;

M. P. (165 °C), $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.17 (3H, t, $J$ = 7.3Hz), 2.45 (2H, m), 2.31 (3H, s), 2.80 (2H, m), 4.12(2H, q, $J$ = 6.7Hz), 5.24 (1H, s), 5.30 (1H, s), 5.85 (1H, s), 7.11 (1H, m), 7.24-7.26 (2H, m), 7.65 (1H, m); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 15.24, 18.99, 25.18, 29.22, 61.44, 98.63, 126.26, 127.48, 127.99, 128.30, 129.54, 134.14, 137.47, 137.70, 150.35, 154.87, 167.50; IR (KBr) $\nu_{\max}$ cm$^{-1}$: 3235, 3107, 2936, 1693, 1700, 1681, 1608, 1596; MS at $m/z$ 346.79 (M$^+$+Na).

Ethyl 4-((Z)-3-(methoxycarbonyl)-1-chloro-1-(3,4-dimethoxyphenyl)prop-1-en-2-
yl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxylate: 82;

M. P. (127 °C), $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.31 (3H, t, $J$ = 6.9Hz), 2.25 (3H, s), 2.72 (2H, s), 3.63 (3H, s), 3.66 (6H, s), 4.01 (2H, q, $J$=7.2Hz), 4.59 (1H, s), 6.94 (1H, d, $J$ = 8.3Hz), 7.04 (1H, dd, $J$= 2.3 & 8.3Hz), 7.14 (1H, d, $J$ = 2.7Hz); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 14.70, 16.30, 36.34, 51.82, 56.76, 56.79, 61.50, 95.14, 113.03, 113.80, 121.68, 123.19, 134.30, 143.46, 148.52, 150.19, 151.33, 155.73, 169.99, 174.08; IR (KBr) $\nu_{\max}$ cm$^{-1}$: 3195, 2860, 1693, 1514, 1462, 1097; MS at $m/z$ 452.19.33 (M$^+$+Na).

(E)-Ethyl 4-(4-methoxystyryl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-

3-carboxylate: 86a;

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.27 (3H, t, $J$ = 7.1Hz), 2.4 (3H, s), 3.78(3H, s), 4.19 (2H, q, $J$ = 7.3Hz), 4.59 (1H, d, $J$ = 4Hz), 5.5 (1H, s), 5.96 (1H, dd, $J$= 3.2 & 16Hz), 6.20 (1H, d, $J$ = 16Hz), 6.80 (2H, d, $J$=8Hz), 7.01 (2H, d, $J$=7.92Hz), 7.27 (1H, s); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 14.44, 19.57, 36.46, 55.29, 59.75, 85.39,
101.81, 109.72, 118.34, 127.35, 127.43, 129.38, 129.77, 132.91, 144.60, 154.50, 167.65; IR (KBr) \( \nu_{\text{max}} \) cm\(^{-1}\): 3240, 3117, 2960, 1693, 1514, 1462, 1097; MS at \( m/z \) 316.3465 (M\(^+\)+Na).

Ethyl 4-(2,4-dimethoxystyryl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxylate: 86b;

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.27 (3H, t, \( J = 7.1 \)Hz), 2.4 (3H, s), 3.78(6H, s), 4.19 (2H, q, \( J = 7.3 \)Hz), 4.59 (1H, d, \( J = 4 \)Hz ), 5.5 (1H, s), 5.96 (1H, dd, \( J = 3.2 \) & 16Hz), 6.20 (1H, d, \( J = 1.8 \)Hz), 6.25 (1H, m), 6.80 (2H, d, \( J = 16 \)Hz), 7.02 (1H, d, \( J = 7.8 \)Hz), 7.27 (1H, s); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 14.34, 19.54, 36.46, 55.39, 55.39, 59.70, 93.85, 101.93, 104.55, 119.96, 122.58, 127.17, 130.09, 144.60, 154.26, 156.73, 159.83, 167.78; IR (KBr) \( \nu_{\text{max}} \) cm\(^{-1}\): 3240, 3117, 2960, 1693, 1514, 1462, 1097; MS at \( m/z \) 346.15 (M\(^+\)+Na).

Ethyl 4-(2,3-dimethoxystyryl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxylate: 86c;

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.27 (3H, t, \( J = 7.1 \)Hz), 2.4 (3H, s), 3.73(6H, s), 4.19 (2H, q, \( J = 7.4 \)Hz), 4.59 (1H, d, \( J = 4 \)Hz ), 5.5 (1H, s), 5.96 (1H, dd, \( J = 3.2 \) & 16Hz), 6.5-6.65 (3H, m), 6.80 (2H, d, \( J = 16 \)Hz), 7.27 (1H, s); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 14.34, 19.54, 36.46, 55.39, 55.39, 59.70, 93.85, 101.93, 104.55, 119.96, 122.58, 127.17, 130.09, 144.60, 154.26, 156.73, 159.83, 167.78; IR (KBr) \( \nu_{\text{max}} \) cm\(^{-1}\): 3240, 3117, 2960, 1693, 1514, 1462, 1097; MS at \( m/z \) 346.15 (M\(^+\)+Na).

Ethyl 4-(3,4,5-trimethoxystyryl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxylate: 86d;

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.27 (3H, t, \( J = 7.1 \)Hz), 2.4 (3H, s), 3.73(9H, s), 4.19 (2H, q, \( J = 7.6 \)Hz), 4.59 (1H, d, \( J = 3.4 \)Hz ), 5.5 (1H, s), 5.96 (1H, dd, \( J = 3.2 \) & 16Hz), 6.23 (2H, s), 6.58 (2H, d, \( J = 16 \)Hz), 7.27 (1H, s); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 14.34,
19.54, 36.46, 55.39, 55.39, 55.39, 59.70, 101.93, 104.55, 122.58, 127.17, 130.09, 144.60, 150.26, 155.73, 156.83, 167.78; IR (KBr) \( \nu_{\text{max}} \text{cm}^{-1} \): 3240, 3117, 2960, 1693, 1514, 1462, 1097; MS at \( m/z \) 366.16 (M\(^+\)+Na).

**2-Methyl-4-(7-methoxy-2-oxo-2H-chromen-8-yl)-but-2-en-1-al:** 88;

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.95 (3H, s), 3.94 (3H, s), 3.89 (2H, d, \( J = 7.69 \) Hz), 6.28 (1H, d, \( J = 9.46 \) Hz), 6.53 (1H, t, \( J = 7.34 \) Hz), 7.39 (1H, d, \( J = 9.5 \) Hz). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 195.4, 160.79, 160.30, 153.0, 150.74, 143.64, 139.81, 127.38, 114.27, 113.33, 113.10, 107.4, 50.19, 22.8, 9.27; IR (KBr) \( \nu_{\text{max}} \text{cm}^{-1} \): 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510; EIMS \( m/z \): 258.2482 [M\(^+\)].

**Ethyl 4-(4-(6-methoxy-2-oxo-2H-chromen-7-yl) but-2-en-2-yl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate:** 89;

M. P. (145 °C), \(^1\)H NMR (200 MHz, CDCl\(_3\)): \( \delta \) 1.25 (3H, t, \( J = 8 \) Hz), 1.94 (3H, s), 2.3 (3H, s), 3.89 (2H, d, \( J = 7.69 \) Hz), 3.94 (3H, s), 4.15 (2H, q, \( J = 8 \) Hz), 4.90 (1H, s), 6.28 (1H, d, \( J = 9.46 \) Hz), 6.53 (1H, t, \( J = 7.34 \) Hz), 6.88 (1H, d, \( J = 8.6 \) Hz), 7.27 (1H, s), 7.39 (1H, d, \( J = 8.62 \) Hz), 7.66 (1H, d, \( J = 9.5 \) Hz). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 14.19, 18.62, 22.54, 26.89, 55.22, 59.98, 61.50, 92.80, 106.85, 114.72, 119.33, 119.81, 126.17, 133.26, 134.58, 142.84, 149.39, 151.54, 154.61, 155.73, 161.82, 169.49; IR (KBr) \( \nu_{\text{max}} \text{cm}^{-1} \): 3340, 3012, 1728, 1681, 1608, 1497, 1072, 832, 774, 510; EIMS \( m/z \): 412.4566 [M\(^+\)].

**Ethyl 4-(2,2-dimethylchroman-6-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate:** 92b;

M. P. (137 °C), \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.20 (3H, t, \( J = 7.6 \) Hz), 1.32 (6H, s), 1.78 (2H, t, \( J = 8 \) Hz), 2.34 (3H, s), 2.75 (2H, t, \( J = 7.5 \) Hz), 4.12 (2H, q, \( J = 8 \) Hz), 5.30 (1H, s), 5.46 (1H, s), 6.68 (1H, d, \( J = 2 \) Hz), 7.00 (1H, s), 7.03 (1H, m), 7.55 (1H, s); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 14.19, 18.62, 22.54, 26.89, 32.69, 55.22, 59.98,
74.34, 101.64, 117.24, 120.95, 125.73, 127.72, 135.06, 145.96, 153.72, 165.86; IR (KBr) $\text{cm}^{-1}$: 3335, 3156, 1693, 1700, 1681, 1608, 1576, 1488, 1384, 1362, 1414, 1114; MS at $m/z$ 344.71 ($M^+\text{Na}$).
3.20. Spectra of Some Selected Compounds

$^1$H & $^{13}$C NMR spectra of compound (74f)
\( ^1H \text{ and } ^{13}C \text{ NMR spectra of compound (74m)} \)
\(^1\text{H}\) \& \(^{13}\text{C}\) NMR spectra of compound (76c)
Chapter 3: Section B

Biginelli Reaction

$^1$H & $^{13}$C NMR spectra of compound (80a)
\(^1\)H & \(^{13}\)C NMR spectra of compound (80b)
$^1$H & $^{13}$C NMR spectra of compound (89)
$^1$H & $^{13}$C NMR spectra of compound (92)
Summary of thesis

Bio-Prospection of Natural Products and Synthesis of Bioactive Molecules for Lead Development and Generation of Novel Synthetic Methodologies

Chapter-1: This chapter is divided into two sections:

**Section A:** Bio-prospection of Natural Products:

The section A comprises of three parts:

- **Part A** provides an overview regarding the historical role of medicinal/natural products in drug discovery and development especially anti-cancer and anti-microbial.

- **Part B** comprises comprehensive literature review on *Prangos pabularia*, which includes its various uses, medicinal properties especially of couramins and mechanism of action.

- **Part C** describes the isolation of phytochemicals from *Prangos pabularia* (Apiaceae), and structure elucidation of twenty compounds from the roots of the plant, osthol (PE-1) imperatorin (PE-2), isoimperatorin (PE-3), xanthotoxin (PE-4), bergapten (PE-5), merangin (PE-6), heraclenin (PE-7), oxypeucedanin (DME-1), psorlen (DME-2), isopsorlen (DME-3), 4-(2-hydroxy-3-methylbut-3-enyloxy)-7H-furo[3,2-g]chromen-7-one (DME-4), 4-hydroxy-9-(3-methylbut-2-enyl)-7H-furo[3,2-g]chromen-7-one (DME-5), oxypeucedanin hydrate monoacetate (DME-6), oxypeucedanin hydrate (DME-7), heraclenol (DME-8), xanthotoxol (DME-9), bergaptol (DME-10), 3-tetrahydro-2,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-3-yloxy)-1-(7-oxo-7H-furo[3,2-g]chromen-4-yloxy)-3-methylbutan-2-yl acetate (DME-11), (-)-4-[3-(β-D-glucopyranosyloxy)-2-hydroxy-3-methyl butoxy]-7Hfuro[3,2-g][1]benzopyran-7-one (DME-12), 7-hydroxocormarin (DME-13). (Figure-1)
Section B: This section comprises of two parts:

Part A constitutes literature review on chromatography, especially HPLC which includes its various applications in natural products.

Part B presents a simple, accurate, and validated RP-HPLC method developed for the simultaneous determination of twelve marker bioactive methanol soluble compounds isolated from *P. Pabularia*in (section A), 6-hydroxocouramin (1), umbelliferone (2), heraclenol glycoside(3), xanthotoxal (4), heraclenol (5), oxypeucedanin hydrate (6),
8-((3,3-dimethyloxiran-2-yl)methyl)-7-methoxy-2h-chromen-2-one (7), oxypeucedanin hydrate monoacetate (8), xanthotoxin (9), 4-((2-hydroxy-3-methylbut-3-en-1-yl)oxy)-7h-furo[3,2-g]chromen-7-one (10), imperatorin (11), osthol (12) which have been successfully resolved on single chromatogram of the root extract of *P. pabularia* by RP-HPLC. Through quantification of the DCM: methanol extract, heraclenol 5 and osthol 12 are found as the major constituents. These molecules are of importance because of their anticancer and antibacterial activities. Validation results demonstrate that the developed method is sufficiently reliable and sensitive to evaluate the quality of *P. pabularia*. This first RP-HPLC fingerprint developed for this plant can be helpful for the rapid analysis of its phytomolecules in various herbs/herbal formulation and plant products.

Structure of marker compounds isolated and quantified on RP- HPLC

![Structure of marker compounds](image_url)

**Figure 2**
Table-1: Quantification of marker compounds 1-12 (%w/w) in *Prangos pabularia* determined by HPLC

<table>
<thead>
<tr>
<th>Marker compounds</th>
<th>DCM:MeOH (1:1) extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-hydroxy-coumarin (1)</td>
<td>0.123± 0.001</td>
</tr>
<tr>
<td>Umbelliferone (2)</td>
<td>0.150± 0.001</td>
</tr>
<tr>
<td>Heraclenol glycoside(3)</td>
<td>0.460± 0.002</td>
</tr>
<tr>
<td>Xanthotoxol (4)</td>
<td>0.050±0.001</td>
</tr>
<tr>
<td>Heraclenol (5)</td>
<td>0.801±0.002</td>
</tr>
<tr>
<td>Oxypeucedanin hydrate (6)</td>
<td>0.368±0.003</td>
</tr>
<tr>
<td>8-((3,3-dimethyloxiran-2-yl)methyl)-7-methoxy-2H-chromen-2-one (7)</td>
<td>0.623±0.002</td>
</tr>
<tr>
<td>Oxypeucedanin hydrate monoacetate (8)</td>
<td>0.022±0.001</td>
</tr>
<tr>
<td>Xanthotoxin (9)</td>
<td>0.325±0.001</td>
</tr>
<tr>
<td>4-((2-hydroxy-3-methylbut-3-en-1-yl)oxy)-7H-furo[3,2-g]chromen-7-one (10)</td>
<td>0.101±0.001</td>
</tr>
<tr>
<td>Imperatorin (11)</td>
<td>0.455±0.002</td>
</tr>
<tr>
<td>Osthol (12)</td>
<td>0.680±0.003</td>
</tr>
</tbody>
</table>
Chapter-2: Studies on Modification and Bio-Evaluation of Plant Molecules

The biological studies of osthol and related molecules containing coumarin ring, carried out in last few years have provided an additional dimension to the bioactivity profile of the title compound osthol. The potential of osthol and other coumarins has not been fully exploited despite its biological importance; therefore, more efforts toward the building of diverse libraries around its chemical structure and their biological profile are in demand.

This chapter describes the following parts:

**Part A** describes the isolation of major constituents like osthol and other coumarins from *P. pabularia* in quantitative amounts to carry out different chemical transformations.

**Part B** includes the synthetic modification of osthol and other coumarins for improved anti-cancer and antimicrobial activity. Identification of lead molecules and detailed investigations related to their mechanism of action and structure activity relationship (SAR).

![Figure 4](image)

At the very outset, two susceptible sites (I, II) of osthol were identified for the structural modification as shown in Figure-4. The idea behind the selection was to study the contribution of different functionalities towards anticancer activity, which would help in the establishment of the structure activity relationship (SAR). The transformations were carried out by different organic reactions to get structurally different analogues encompassing modification at all the sites (I, II).
Scheme 1

Reagents and conditions: (a) SeO$_2$/AcOH, (b) HNO$_3$/Urea/AcOH, Ac$_2$O, -10°C, (c) CH$_3$Cl$_2$, mCPBA, 0°C (d) aq. NaOH/DMSO, R$_1$I, room temp. (e) Hg (OAc)$_2$/THF:H$_2$O (1:1), NaBH$_4$, room temp. (f) (C$_2$H$_5$)$_2$P(O)CH$_2$COOC$_2$H$_5$/ NaH/ dry ether, N$_2$-atm., 0°C, stir.

Scheme 2

Reagents and conditions: a. SOCl$_2$/DCM, reflux, Amines.
Further modification at site I was carried out to prepare the triazole derivatives of osthol.

\[ \text{Scheme 3} \]

**Reagents and conditions:**

(a) NaOH/DMSO, propargyl bromide, room temp.

(b) RN₃, t-butanol: H₂O (1:1), CuSO₄, Sod. Ascorbate, rt.

Similarly different triazole analogues of 7-hydroxy coumarins were prepared by propargylation followed by click reaction with different azides.

\[ \text{Scheme 4. Reagents and conditions:} \]

(a) NaOH/DMSO, propargyl bromide, room temp.

(b) RN₃, t-butanol: H₂O (1:1), CuSO₄, Sod. Ascorbate, rt.

Encouraged by the SAR results of osthol at site I and site II, we were interested in the chemical modification of similar coumarins for the said biological activity.
Scheme 5

Reagents and conditions: (a): aq. NaOH/DMSO, alkyl halides, rt.

Scheme 6

Reagents and conditions: (a): aq. NaOH/DMSO, alkyl halides, rt.
Part C comprises in vitro screening of all synthesised novel analogues on different human cancer cell lines, and antimicrobial activity (i.e., anti-bacterial and anti-fungal activity) against microbial strains.

Chapter-3: This chapter is divided into two sections

Section A: CuCN Catalyzed One Pot Synthesis of $\gamma$-Keto Diesters: Domino Double Michael Addition Followed by Nef Reaction

Synthesis of biologically significant cyclic compounds rely on the elaboration of polycarbonyl compounds among which 1,4-dicarbonyls have proven particularly useful. A few approaches towards the synthesis of such functional arrangements involve multicomponent coupling of siloxycyclopropane and carbon monoxide, carbonylative dimerisation of siloxycyclopropane, carbonylative arylation of siloxycyclopropane, conjugate addition of nitroalkanes to enones followed by reduction of nitro group to carbonyl group. Most of the previously reported methods suffer from drawbacks. Surprisingly, apart from these valuable strategies for the generation of $\gamma$-keto diesters, there exists no general one pot method available for the synthesis of the said class. Hence, more efficient and practical alternative methods using inexpensive and easily available reagents are warranted. Herein, we unveil one pot synthesis of $\gamma$-keto diesters in presence of Cs$_2$CO$_3$ through CuCN catalyzed domino double Michael reaction followed by Nef reaction to afford the target products, achieved in few minutes, with moderate to good yields.

Reaction of ethyl acrylate and nitromethane

![Reaction of ethyl acrylate and nitromethane](image)

Scheme 7
Reaction of different alkyl acrylates and nitromethane

\[
\text{O} \quad \text{O} \quad \text{O} \
\begin{align*}
1 & \quad + \quad \text{CH}_3\text{NO}_2 \\
\text{R}^1 & = \text{H}, \text{ R} = \text{C}_2\text{H}_5 \\
\text{R}^1 & = \text{H}, \text{ R} = \text{CH}_3 \\
\text{R}^1 & = \text{H}, \text{ R} = \text{C}_4\text{H}_9 \\
\text{R}^1 & = \text{CH}_3, \text{ R} = \text{C}_2\text{H}_5 \\
\text{R}^1 & = \text{CH}_3, \text{ R} = \text{CH}_3 \\
\text{R}^1 & = \text{CH}_3, \text{ R} = \text{C}_4\text{H}_9 \\
\end{align*}
\]

\[
\begin{align*}
\text{Cs}_2\text{CO}_3, \\
\text{CuCN} \\
\text{DCM, rt,} \\
0.5 \text{ hr.} \\
\end{align*}
\]

\[
\begin{align*}
3a & \quad + \quad 3b \\
\text{R}^1 & = \text{H}, \text{ R} = \text{C}_2\text{H}_5 \\
\text{R}^1 & = \text{H}, \text{ R} = \text{CH}_3 \\
\text{R}^1 & = \text{H}, \text{ R} = \text{C}_4\text{H}_9 \\
\text{R}^1 & = \text{CH}_3, \text{ R} = \text{C}_2\text{H}_5 \\
\text{R}^1 & = \text{CH}_3, \text{ R} = \text{CH}_3 \\
\text{R}^1 & = \text{CH}_3, \text{ R} = \text{C}_4\text{H}_9 \\
\end{align*}
\]

Scheme 8.

Reaction of different alkyl acrylates and nitroethane:

\[
\begin{align*}
\text{O} \quad \text{O} \quad \text{O} \
\begin{align*}
1 & \quad + \quad \text{CH}_3\text{C}_2\text{H}_4\text{NO}_2 \\
\text{R}^1 & = \text{H}, \text{ R} = \text{C}_2\text{H}_5 \\
\text{R}^1 & = \text{H}, \text{ R} = \text{CH}_3 \\
\text{R}^1 & = \text{H}, \text{ R} = \text{C}_4\text{H}_9 \\
\text{R}^1 & = \text{CH}_3, \text{ R} = \text{C}_2\text{H}_5 \\
\text{R}^1 & = \text{CH}_3, \text{ R} = \text{CH}_3 \\
\text{R}^1 & = \text{CH}_3, \text{ R} = \text{C}_4\text{H}_9 \\
\end{align*}
\]

\[
\begin{align*}
\text{Cs}_2\text{CO}_3, \\
\text{CuCN} \\
\text{DCM, rt,} \\
0.5 \text{ hr.} \\
\end{align*}
\]

\[
\begin{align*}
5a & \quad + \quad 5b \\
\text{R}^1 & = \text{H}, \text{ R} = \text{C}_2\text{H}_5 \\
\text{R}^1 & = \text{H}, \text{ R} = \text{CH}_3 \\
\text{R}^1 & = \text{H}, \text{ R} = \text{C}_4\text{H}_9 \\
\text{R}^1 & = \text{CH}_3, \text{ R} = \text{C}_2\text{H}_5 \\
\text{R}^1 & = \text{CH}_3, \text{ R} = \text{CH}_3 \\
\text{R}^1 & = \text{CH}_3, \text{ R} = \text{C}_4\text{H}_9 \\
\end{align*}
\]

Scheme 9

Conclusion

We have developed a copper catalyzed one pot synthesis of γ-keto diesters using simple available substrates in desirable yields. The procedure described is simple and involves relatively mild reaction conditions.
Section B: An efficient approach towards the synthesis of dihydropyrimidinones using heteropolyacid Montmorilonite-KSF catalyst

Cancer is presently the main cause of death in the world. The present treatment strategy includes alkylating agents, steroids, vinca alkaloids, anti-metabolites, taxanes and antibiotics. There is a need of anticancer drugs which are safe and more effective. Besides exploitation of natural resources (plants, microbes and fungi) for the discovery of new anticancer drugs or drug candidates, preparation of synthetic new chemical entities with potent therapeutic profiles can be equally explored. In this direction, Biginelli reaction catalysed by heteropolyacids can be one of the sources for the synthesis of large number of new molecules. Heteropolyacid (HPA) catalysts are widely exploited for the production of fine organic chemicals, health care products, pharmaceuticals and agrochemical products. HPAs are more reactive catalysts than conventional inorganic and organic acids for reactions in solution. Heteropolyacids have been used as catalysts for many of the organic transformations, such as synthesis of acylals, tetrahydropyranilation of phenols, thioacetalization and transacetalization reactions.

The work embodied in this section of the chapter relates to the synthesis of dihydropyrimidinone analogues by Biginelli reaction and there in vitro screening against different cancer cell lines. The methodology involves the multicomponent reaction catalyzed by “HPA-Montmorilonite-KSF” as a reusable and heterogeneous catalyst.

In the present study, MC Biginelli reaction using HPA-Montmorilonite-KSF ($\text{H}_{5}\text{PV}_{2}\text{W}_{10}\text{O}_{40}$) as a catalyst was explored. The strategy involves a three-component one-pot Biginelli-type reaction for the condensation of urea, ethylacetoacetate and aldehydes to corresponding pyrimidinones in presence of a catalytic amount of HPA-Montmorilonite-KSF.
To explore/apply the newly designed present Biginelli reaction, versatile aldehydes (both aromatic and aliphatic aldehydes) were used, with some of them obtained by modification of natural products or through synthetic route. The purpose to use these aldehydes was to extend the scope of Biginelli reaction besides generation of new library of DHPM products not reported hitherto in the literature for their preparation and more importantly study their bioevaluation profiles.

The synthesized Biginelli products are summarized in the figure 5.
Conclusions

We have reported a catalytic method for the synthesis of dihydropyrimidinones using HPA-Montmorillonite-KSF as an efficient and eco-friendly heterogeneous inorganic catalyst. It is noteworthy to mention that the catalyst is reusable and even after five runs, the catalytic activity of $\text{H}_5\text{PV}_2\text{W}_{10}\text{O}_{40}.10\text{H}_2\text{O}$ was retained as that of the freshly used catalyst. The short reaction times, simple work-up and isolation of the products in high yields with high purity, and mild reaction conditions, are some of the features of the current procedure. A library of other DHPM products has been synthesized by using the above catalyst, for the production of drug like molecules which currently is the important area of research in the institute.
List of Publications


