Computer-guided Design and Synthesis of IL-2 Inhibitors as Immunomodulating Agents

A dissertation submitted to the Department of Chemistry, Quaid-i-Azam University, Islamabad, in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Organic Chemistry

by

Saima Kalsoom

Department of Chemistry
Quaid-i-Azam University
Islamabad, Pakistan
2016
Dedicated
to
My Parents
and
My Sons
(Muhammad Jibran & Muhammad Ayan)
DEDICATED

TO

My loving Parents & My Sons
(Muhammad Jibran & Muhammad Ayan)
In the name of Allah, the Beneficent, the most Merciful
CONTENTS

Acknowledgments ........................................................................................................................................... i
List of Abbreviations and Symbols .................................................................................................................. iii
Abstract ............................................................................................................................................................ vi
List of Schemes ................................................................................................................................................... vii
List of Figures ..................................................................................................................................................... viii
List of Tables .................................................................................................................................................... x

1. INTRODUCTION ........................................................................................................................................ 1-44

1.1. Disorders of human immunity .................................................................................................................. 1
  1.1.1. Immunodeficiencies ............................................................................................................................... 2
  1.1.2. Autoimmunity ........................................................................................................................................ 2
  1.1.3. Hypersensitivity ..................................................................................................................................... 3

1.2. Interleukin-2 .............................................................................................................................................. 4
  1.2.1. Cellular sources and production of IL-2 .............................................................................................. 4

1.3. Interleukin-2 receptor ............................................................................................................................... 5

1.4. IL-2/IL-2 Receptor binding ...................................................................................................................... 6

1.5. IL-2 inhibitors ........................................................................................................................................... 7

1.6. Computer-aided drug design ................................................................................................................... 9

1.7. Strategies of CADD .................................................................................................................................. 10
  1.7.1. Ligand based drug designing ............................................................................................................... 10
    1.7.1.1. 2D & 3D similarity search ................................................................................................................. 10
    1.7.1.2. Pharmacophore modeling ................................................................................................................ 11
    1.7.1.3. Quantitative structure activity relationship ................................................................................... 12
  1.7.2. Structure based drug designing .......................................................................................................... 13
    1.7.2.1. Homology Modeling ....................................................................................................................... 14
    1.7.2.2. Molecular docking .......................................................................................................................... 15
      1.7.2.2.1. Types of molecular docking ..................................................................................................... 16
      1.7.2.2.2. Docking Algorithm ................................................................................................................... 17
    1.7.2.3. Molecular dynamic simulations ..................................................................................................... 19

1.8. Virtual screening ..................................................................................................................................... 19
1.9. Wet Lab .......................................................................................................................... 20

1.9.1. Chalcones .................................................................................................................. 21
    1.9.1.1. Synthesis of chalcones ..................................................................................... 22

1.9.2. Dihydropyrimidines .............................................................................................. 23
    1.9.2.1. Synthesis of dihydropyrimidines ................................................................... 24

1.9.3. Heteroazepines ...................................................................................................... 27
    1.9.3.1. Synthesis of heteroazepines ........................................................................... 28

1.9.4. Bezimidazoles ....................................................................................................... 30
    1.9.4.1. Synthesis of bezimidazoles .......................................................................... 31

1.9.5. Anthraquinone sulfonamide derivatives ............................................................. 32
    1.9.5.1. Synthesis of anthraquinone sulfonamide derivatives ................................... 33

1.9.6. Schiff bases ........................................................................................................... 35
    1.9.6.1. Synthesis of Schiff base derivatives .............................................................. 36

1.9.7. Pyrazoles .............................................................................................................. 37
    1.9.7.1. Synthesis of pyrazoles .................................................................................. 38

1.9.8. Benzamides .......................................................................................................... 41
    1.9.8.1. Synthesis of benzamides ............................................................................. 41

1.10. Aim of the study ...................................................................................................... 43

2. RESULTS AND DISCUSSION ....................................................................................... 45-90

2.1. In silico identification of IL-2 inhibitors (Dry lab) .................................................. 45
    2.1.1. Target identification ......................................................................................... 45
    2.1.2. Determination of active site ............................................................................. 46
    2.1.3. Designing a training set .................................................................................... 46
    2.1.4. Generation of structure based pharmacophore model ................................... 50
    2.1.5. Validation of pharmacophore model ............................................................... 52
    2.1.6. Pharmacophore based virtual screening ......................................................... 53
    2.1.7. High throughput docking ................................................................................ 54
    2.1.8. Binding mode analysis of the Novel IL-2 Inhibitors ....................................... 55

2.2. Synthesis of analogs of different heterocycles from PBVS .................................... 69
2.2.1. Synthesis of chalcones (1-7)........................................................................................................69
   2.2.1.1. Characterization of chalcones derivatives..............................................................................70
2.2.2. Synthesis of dihydropyrimidines (8-13).........................................................................................70
   2.2.2.1. Characterization of dihydropyrimidines..............................................................................71
2.2.3. Synthesis of heteroazepines (14-19)..............................................................................................72
   2.2.3.1. Characterization of heteroazepines......................................................................................73
2.2.4. Synthesis of pyrazole carbaldehydes (20-25).................................................................................74
   2.2.4.1. Characterization of pyrazole carbaldehydes........................................................................74
2.2.5. Synthesis of pyrazole carbothioamides (26-28)............................................................................76
   2.2.5.1. Characterization of pyrazole carbothioamides..................................................................76
2.2.6. Synthesis of benzimidazoles (29-32)............................................................................................77
   2.2.6.1. Characterization of benzimidazoles...................................................................................78
2.2.7. Synthesis of anthraquinone sulfonamide derivatives (33-36).........................................................78
   2.2.7.1. Characterization of anthraquinone sulfonamide derivatives ..............................................79
2.2.8. Synthesis of Schiff base derivatives (37-41)..................................................................................80
   2.2.8.1. Characterization of Schiff base derivatives........................................................................80
2.2.9. Synthesis of pyrazoles (42-43)......................................................................................................81
   2.2.9.1. Characterization of pyrazoles............................................................................................82
2.2.10. Synthesis of benzamides (44-46)................................................................................................83
   2.2.10.1. Characterization of benzamides....................................................................................84
2.3. IL-2 Inhibition assay.........................................................................................................................84
   2.3.1. Compounds (Chalcones) 1-7 ......................................................................................................84
2.3.2. Compounds (Heterocycles derived from chalcones) 8-28.............................................................85
2.3.3. Compounds (Benzimidazoles and anthraquinone sulfonamide derivatives)
      29-36...............................................................................................................................................87
2.3.4. Compounds (Schiff base derivatives) 37-41 ..............................................................................87
2.3.5. Compounds (Pyrazole and benzamide derivatives) 42-46 .........................................................88
3. EXPERIMENTAL.................................................................................................................................91-120
3.1. In silico guided identification of IL-2 inhibitors (Dry lab).................................................................91
   3.1.1. Hardware specifications..........................................................................................................91
3.1.2. Selection and preparation of protein protein complex ................................................. 91
3.1.3. Determination of active site of protein ....................................................................... 91
3.1.4. Pharmacophore generation ......................................................................................... 91
3.1.5. Excluded volumes ........................................................................................................ 92
3.1.6. Generation of training set (S-1 to S-30) ...................................................................... 93
3.1.7. Building of compounds .................................................................................................. 93
3.1.8. Validation of pharmacophore model ........................................................................... 97
3.1.9. Virtual screening ........................................................................................................... 98
3.1.10. Docking accuracy ........................................................................................................ 98
3.1.11. Descriptor based filtering of the database ................................................................. 99
3.1.12. 3D pharmacophore based filtering of database ......................................................... 100
3.1.13. Receptor based virtual screening of hits ....................................................................... 100
3.2. Hits optimization ................................................................................................................ 101
  3.2.1. Pharmacophore mapping .............................................................................................. 101
  3.2.2. Molecular docking ........................................................................................................ 101
3.3. Synthesis of hit compounds (Wet lab) ........................................................................... 101
  3.3.1. Chemicals used ............................................................................................................. 101
3.4. Purification of solvents ..................................................................................................... 102
  3.4.1. Acetone ........................................................................................................................ 102
  3.4.2. Absolute ethanol and methanol .................................................................................. 102
  3.4.3. Ethyl acetate ................................................................................................................ 102
  3.4.4. Chloroform and dichloromethane .............................................................................. 102
  3.4.5. Absolute methanol ....................................................................................................... 102
  3.4.6. Dimethyl formamide (DMF) ....................................................................................... 103
  3.4.7. Dimethyl sulfoxide (DMSO) ...................................................................................... 103
3.5. Instruments used ................................................................................................................ 103
  3.5.1. Melting point ................................................................................................................. 103
  3.5.2. NMR-Spectrometer ....................................................................................................... 103
  3.5.3. Mass Spectrometer ....................................................................................................... 103
3.6. Chromatographic Techniques .................................................................................................................. 103
  3.6.1. Thin layer chromatography (TLC) ...................................................................................................... 103

3.7. Synthesis of chalcones (1-7) .................................................................................................................... 104
  3.7.1. 3-Phenyl-1-phenylprop-2-en-1-one (1) .......................................................................................... 104
  3.7.2. 3-(4-Fluorophenyl)-1-phenylprop-2-en-1-one (2) .......................................................................... 104
  3.7.3. 3-(4-Chlorophenyl)-1-phenylprop-2-en-1-one (3) .......................................................................... 104
  3.7.4. 3-(4-Bromophenyl)-1-phenylprop-2-en-1-one (4) .......................................................................... 105
  3.7.5. 1-(3-Hydroxyphenyl)-3-(3-nitrophenyl)prop-2-en-1-one (5) ...................................................... 105
  3.7.6. 3-(2-Hydroxyphenyl)-1-(3-hydroxyphenyl)prop-2-en-1-one (6) .............................................. 105
  3.7.7. 1-(4-Aminophenyl)-3-(3-chlorophenyl)prop-2-en-1-one (7) ...................................................... 105

3.8. Synthesis of dihydropyrimidines (8-13) .................................................................................................. 105
  3.8.1. 4-(3-Hydroxyphenyl)-6-(3-nitrophenyl)-5,6-dihydropyrimidine-2(1H)-thione (8) ...................... 105
  3.8.2. 6-(2-Hydroxyphenyl)-4-(3-hydroxyphenyl)-5,6-dihydropyrimidine-2(1H)-thione (9) .............. 106
  3.8.3. 4-(4-Aminophenyl)-6-(3-chlorophenyl)-5,6-dihydropyrimidine-2(1H)-thione (10) ................. 106
  3.8.4. 4-(3-Hydroxyphenyl)-6-(3-nitrophenyl)-5,6-dihydropyrimidin-2(1H)-one (11) ....................... 106
  3.8.5. 6-(2-Hydroxyphenyl)-4-(3-hydroxyphenyl)-5,6-dihydropyrimidin-2(1H)-one (12) ............... 107
  3.8.6. 4-(4-Aminophenyl)-6-(3-chlorophenyl)-5,6-dihydropyrimidin-2(1H)-one (13) ....................... 107

3.9. Synthesis of heteroazepines (14-19) ....................................................................................................... 107
  3.9.1. 4-(4-(3-Chlorophenyl)-4,5-dihydro-3H-benzo[b][1,4]diazepin-2-yl)-benzenamine (14) ............. 108
  3.9.2. 3-(4-(3-Nitrophenyl)-4,5-dihydro-3H-benzo[b][1,4]diazepin-2-yl)-phenol (15) ................. 108
  3.9.3. 3-(4-(2-Hydroxyphenyl)-4,5-dihydro-3H-benzo[b][1,4]diazepin-2-yl)-Phenol (16) ......... 108
3.9.4. 4-(2-(3-Chlorophenyl)-2,3-dihydrobenzo[1,4]thiazepin-4-yl)benzenamine (17) .................................................................................................................................................108

3.9.5. 3-(2-(3-Nitrophenyl)-2,3-dihydrobenzo[b][1,4]thiazepin-4-yl)phenol (18) ........109

3.9.6. 3-(2-(2-Hydroxyphenyl)-2,3-dihydrobenzo[b][1,4]thiazepin-4-yl)- phenol (19). 109

3.10. Synthesis of pyrazole carbaldehydes (20-25) and pyrazole carbothioamides (26-28) ....109

3.10.1. 3-(3-Hydroxyphenyl)-5-(3-nitrophenyl)-4,5-dihydropyrazole-1-carbaldehyde (20) .........................................................................................................................................................109

3.10.2. 5-(2-Hydroxyphenyl)-3-(3-hydroxyphenyl)-4,5-dihydropyrazole-1-carbaldehyde (21) .................................................................................................................................................................110

3.10.3. 3-(4-Aminophenyl)-5-(3-chlorophenyl)-4,5-dihydropyrazole-1-carbaldehyde (22) .................................................................................................................................................................110

3.10.4. 5-(4-Bromophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole (23) ..................................110

3.10.5. 1-(3,5-Diphenyl-4,5-dihydropyrazol-1-yl)ethanone (24) ........................................111

3.10.6. 1-(5-(4-Bromophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)ethanone(25) ..........111

3.10.7. 3,5-Diphenyl-4,5-dihydropyrazole-1-carbothioamide (26) ....................................111

3.10.8. 5-(4-Fluorophenyl)-3-phenyl-4,5-dihydropyrazole-1-carbothioamide(27) ......112

3.10.9. 5-(4-Chlorophenyl)-3-phenyl-4,5-dihydropyrazole-1-carbothioamide(28) ......112

3.11. Synthesis of benzimidazoles (29-32) ..............................................................................112

3.11.1. 2-(1-(4-Isobutylphenyl)ethyl)-1H-benzo[d]imidazole (29) .......................................112

3.11.2. 2-(2-(4-(4-Chlorophenyl)(phenyl)methyl)piperazin-1-yl)-ethoxy)methyl-1H- benzo[d]imidazole (30) ..................................................................................................................113

3.11.3. N,N-dimethyl-4-(6-nitro-1H-benzo[d]imidazol-2-yl)benzenamine(31) .............113

3.11.4. 4-(1H-benzo[d]imidazol-2-yl)-N,N-dimethylbenzenamine (32) ........................113


3.12.1. 2-(2-(4-(Dimethylamino)phenyl)-1H-benzo[d]imidazol-1-ylsulfonyl)- anthracene- 9,10-dione (33) .............................................................................................................................114

3.12.2. 2-(2-(4-(Dimethylamino)phenyl)-6-nitro-1H-benzo[d]imidazol-1- ylsulfonyl)- anthracene-9,10-dione (34) ......................................................................................................................115

3.12.3. 2-(2-(1-(4-Isobutylphenyl)ethyl)-1H-benzo[d]imidazol-1-ylsulfonyl)-anthracene- 9,10-dione (35) .............................................................................................................................115
3.12.4. 2-(2-(4-Chlorophenyl)(phenyl)methyl)piperazin-1-yl)ethoxy)-methyl)-1H-benzoimidazol-1-ylsulfonylanthracene-9,10-dione(36)…………………………115

3.13. Synthesis of Schiff bases (37-41)........................................................................................................116
3.13.1. 1,5-bis-(3-Hydroxybenzylideneamino)anthracene-9,10-dione (37).................................................116
3.13.2. 1,5-bis-(4-Fluorobenzylideneamino)anthracene-9,10-dione (38).................................................116
3.13.3. 3-(3-(Naphthalen-1-ylimino)prop-1-enyl)-5-nitrophenol (39).........................................................117
3.13.4. bis-N-3-phenylallylidene)biphenylamine (40).................................................................................117
3.13.5. bis-N-(2,4-dichlorobenzylidene)biphenylamine (41).............................................................117

3.14.1. 1-(4-((3-Nitrophenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)-2-(quinolin-8-yloxy)ethanone (42)........................................................................................................................................118
3.14.2. 1-(4-((4-Hydroxyphenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)2-(quinolin-8-yloxy)ethanone (43)........................................................................................................................................118

3.15. Synthesis of benzamides (44-46).................................................................................................118
3.15.1. 2-(4-Isobutylphenyl)-N-(naphthalen-2-yl)propanamide (44).......................................................119
3.15.2. 2-(2-(4-Chlorophenyl)(phenyl)methyl)piperazin-1-yl)ethoxy)-N-(naphthalen-2-yl)acetamide (45)........................................................................................................................................119
3.15.3. bis-N-biphenylacetamide (46)..................................................................................................119

3.16. Biological assay...............................................................................................................................120
3.16.1. IL-2 production and quantification..........................................................................................120

4. CONCLUSION AND FUTURE PLAN....................................................................121-122

4.1. Conclusion........................................................................................................................................121
4.2. Future Recommendations.............................................................................................................122

REFERENCES....................................................................................................................................123-140

ANNEXURE.........................................................................................................................................141-152

LIST OF INTERNATIONAL PUBLICATIONS..................................................................................153-155
ACKNOWLEDGEMENTS

All praises to ALMIGHTY ALLAH, who showered upon me all His blessings throughout my life especially for giving me the strength for the completion of this research work. Many thanks to Him as He blessed us the Holy Prophet, Hazrat Muhammad (PBUH) for whom the whole universe is created and who enabled us to worship only ALLAH. He (PBUH) brought us out of darkness and enlightened the way of Heaven.

I feel great pleasure in expressing my ineffable thanks to my encouraging and motivating Co-Supervisor, Prof. Dr. Farzana Latif Ansari (TI) for her excellent supervision, and guidance. Her vision, patience and motivation at every step of this study made it possible for me to work in this exciting and interesting field of research.

My research for this dissertation was made more extensive and proficient by the use of resources at Department of Pharmacy, University of Padova, Italy and I wish to express my gratitude to Prof. Dr. Stefano Moro and his research group, with whom I completed a part of my research work. In continuation, my heartfelt gratitude goes to Higher Education Commission (HEC) Pakistan for providing me fellowship under International Research Support Initiative Program (IRISP) during my six months stay at Italy.

I am deeply indebted to my Supervisor, Prof. Dr. Humaira Siddiqi, for her excellent administrative guidance and opportunity to undertake research within her group. Without her cooperation, it would have been impossible to complete this research.

I owe my sincere gratitude to Dr. Zaheer-ul-Haq Qasmi and Dr. Ahmed Mesaiik and Dr. Almas Jabeen, PCMD, Karachi, for doing in vitro IL-2 inhibition activity.

I am thankful to Prof. Dr. Muhammad Siddiq, the Chairman, Department of Chemistry, Quaid-i-Azam University, Islamabad and Prof. Dr. Shahid Hameed, Head of Organic Section, for providing necessary research facilities. Many thanks are due to all the faculty members of Chemistry Department, especially to all teachers of Organic Section for being a source of inspiration and enlightenment.

I also owe my recognition to all my lab fellows/friends, especially Dr. Awais Shaukat and Dr. Iram Batool for their help at crucial times of my research work. I am also very thankful to my friend Uzma Bilal for her well wishes during my thesis write-up.

I am very obliged to the supporting staff of the department for their all-time help.
I have no words to acknowledge the sacrifice, efforts, prayers, guidance, support, encouragement and firm dedication of my family members. Their endless prayers contributed to the successful completion of this thesis. Words become meaningless when I look at them as icons of strength for being what I am today. I pay especial gratitude to my Mother, elder brother Tariq Mehmood who not only supported me but also boosted my morale during the toughest time of my life. My apologies to my husband Zulfqar Ali Fraz and my kids Jibran and Ayan for their sufferings when I had to but ignore them.

Finally I would like to thank everybody who directly or indirectly supported me in the completion of my thesis. I extend my apology to those whom I could not mention individually by name in this acknowledgement.

Saima Kalsoom,
Chak No, 90WB, Tehsil : Melsi,
District: Vehari,
<table>
<thead>
<tr>
<th>Abbrev.</th>
<th>Symbols</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Θ</td>
<td>Angle</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Hydrogen bond acceptor interaction site</td>
<td></td>
</tr>
<tr>
<td>Å</td>
<td>Angstrom ($10^{-10}$m)</td>
<td></td>
</tr>
<tr>
<td>Acc</td>
<td>Hydrogen bond acceptors feature</td>
<td></td>
</tr>
<tr>
<td>Acc&amp;ML</td>
<td>Hydrogen bond acceptor and metal ligation feature</td>
<td></td>
</tr>
<tr>
<td>ADME</td>
<td>Absorption, distribution, metabolism and excretion</td>
<td></td>
</tr>
<tr>
<td>ADMET</td>
<td>Absorption, distribution, metabolism, excretion &amp; toxicity</td>
<td></td>
</tr>
<tr>
<td>amu</td>
<td>Atomic mass unit</td>
<td></td>
</tr>
<tr>
<td>Aro</td>
<td>Aromatic center</td>
<td></td>
</tr>
<tr>
<td>Aro</td>
<td>Hyd</td>
<td>Aromatic and hydrophobic feature</td>
</tr>
<tr>
<td>AutoDOCK</td>
<td>Software</td>
<td></td>
</tr>
<tr>
<td>bs</td>
<td>Broad singlet</td>
<td></td>
</tr>
<tr>
<td>CADD</td>
<td>Computer-aided drug designing</td>
<td></td>
</tr>
<tr>
<td>CART</td>
<td>Classification and regression trees</td>
<td></td>
</tr>
<tr>
<td>°C</td>
<td>Celsius</td>
<td></td>
</tr>
<tr>
<td>$^{13}$C NMR</td>
<td>Carbon Nuclear Magnetic Resonance Spectroscopy</td>
<td></td>
</tr>
<tr>
<td>CATALYST</td>
<td>Software</td>
<td></td>
</tr>
<tr>
<td>2D</td>
<td>Two dimensional</td>
<td></td>
</tr>
<tr>
<td>3D</td>
<td>Three dimensional</td>
<td></td>
</tr>
<tr>
<td>Δ</td>
<td>Triangle</td>
<td></td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
<td></td>
</tr>
<tr>
<td>DDD</td>
<td>Drug Discovery and Development</td>
<td></td>
</tr>
<tr>
<td>DHFR</td>
<td>Dihydrofolate reductase</td>
<td></td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
<td></td>
</tr>
<tr>
<td>DISCO</td>
<td>Distance comparison</td>
<td></td>
</tr>
<tr>
<td>DOCK</td>
<td>Software</td>
<td></td>
</tr>
<tr>
<td>Don</td>
<td>Hydrogen bond donor feature</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Hydrogen bond donor interaction site</td>
<td></td>
</tr>
<tr>
<td>Et$_3$N</td>
<td>Triethylamine</td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Pharmacophoric feature</td>
<td></td>
</tr>
<tr>
<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
<td></td>
</tr>
<tr>
<td>FlexX</td>
<td>Software</td>
<td></td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier transform infrared spectroscopy</td>
<td></td>
</tr>
<tr>
<td>GOLD</td>
<td>Software</td>
<td></td>
</tr>
<tr>
<td>Et$_3$N</td>
<td>Triethylamine</td>
<td></td>
</tr>
<tr>
<td>$^1$H NMR</td>
<td>Proton Nuclear Magnetic Resonance Spectroscopy</td>
<td></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Hydrophobic interaction site</td>
<td></td>
</tr>
<tr>
<td>HBA</td>
<td>Number of hydrogen bond acceptors</td>
<td></td>
</tr>
<tr>
<td>HBD</td>
<td>Number of hydrogen bond donors</td>
<td></td>
</tr>
<tr>
<td>Hyd</td>
<td>Hydrophobic feature</td>
<td></td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>50% inhibitory concentrations</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>Interlukin 2</td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant</td>
<td></td>
</tr>
<tr>
<td>JAK/STAT</td>
<td>Janus kinase/Signal Transducer and Activator of Transcription</td>
<td></td>
</tr>
<tr>
<td>kcal</td>
<td>Kilo Calories</td>
<td></td>
</tr>
<tr>
<td>LBDD</td>
<td>Ligand-based drug design</td>
<td></td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid chromatography-mass spectrometry</td>
<td></td>
</tr>
<tr>
<td>LIGANDSCOUT</td>
<td>Software</td>
<td></td>
</tr>
<tr>
<td>LogP</td>
<td>Lipophilicity</td>
<td></td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
<td></td>
</tr>
<tr>
<td>m.p.</td>
<td>Melting point</td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>Monte Carlo</td>
<td></td>
</tr>
<tr>
<td>MD</td>
<td>Molecular dynamics</td>
<td></td>
</tr>
<tr>
<td>ML</td>
<td>Metal ligation feature</td>
<td></td>
</tr>
<tr>
<td>MMFF94x</td>
<td>Merck Molecular Force Field 94x</td>
<td></td>
</tr>
<tr>
<td>MOE</td>
<td>Molecular Operating Environment Software</td>
<td></td>
</tr>
<tr>
<td>MW</td>
<td>Microwave</td>
<td></td>
</tr>
<tr>
<td>M.wt</td>
<td>Molecular weight</td>
<td></td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
<td></td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance Spectroscopy</td>
<td></td>
</tr>
<tr>
<td>PCl&lt;sub&gt;5&lt;/sub&gt;</td>
<td>Phosphorus pentachloride</td>
<td></td>
</tr>
<tr>
<td>PDB</td>
<td>Protein Data Bank</td>
<td></td>
</tr>
<tr>
<td>PHA</td>
<td>Phytoheamagglutinin</td>
<td></td>
</tr>
<tr>
<td>PMA</td>
<td>Phorbol myristate acetate</td>
<td></td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
<td></td>
</tr>
<tr>
<td>q&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Correlation coefficient between predicted and observed</td>
<td></td>
</tr>
<tr>
<td>QSAR</td>
<td>Quantitative Structure Activity Relationship</td>
<td></td>
</tr>
<tr>
<td>MD</td>
<td>Molecular dynamics</td>
<td></td>
</tr>
<tr>
<td>ML</td>
<td>Metal ligation feature</td>
<td></td>
</tr>
<tr>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Correlation coefficient</td>
<td></td>
</tr>
<tr>
<td>R&lt;sub&gt;f&lt;/sub&gt;</td>
<td>Retardation factor</td>
<td></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>RMS</td>
<td>Root mean square</td>
<td></td>
</tr>
<tr>
<td>RMSD</td>
<td>Root mean square deviation</td>
<td></td>
</tr>
<tr>
<td>Ro5</td>
<td>Lipinski’s rule of five</td>
<td></td>
</tr>
<tr>
<td>s</td>
<td>Singlet</td>
<td></td>
</tr>
<tr>
<td>SBDD</td>
<td>Structure-based drug design</td>
<td></td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
<td></td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethyl silane</td>
<td></td>
</tr>
<tr>
<td>TPSA</td>
<td>Total polar surface area</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Excluded volumes</td>
<td></td>
</tr>
<tr>
<td>δ</td>
<td>Chemical shift</td>
<td></td>
</tr>
<tr>
<td>μ</td>
<td>$10^{-6}$ (one millionth)</td>
<td></td>
</tr>
<tr>
<td>u</td>
<td>Frequency</td>
<td></td>
</tr>
</tbody>
</table>
Cytokine Interleukin-2 (IL-2) has a prevalent role in the growth, activation, and differentiation of T-cells. To suppress immune responses associated with organ transplant rejection and other autoimmune diseases, it is important to disrupt the interaction of IL-2 with its receptor system. IL-2 is now emerging as a target in the discovery of novel therapeutics for addressing the problems related to immune system. The main goal of this study was to establish an effective in silico protocol for identification of IL-2 inhibitors. It describes a pharmacophore based virtual screening combined with docking study as a rational strategy for identification of novel IL-2 inhibitors. Structure based pharmacophore model was developed using the crystal structure of IL-2/IL-2Ra (PDB ID: 1Z92) complex.

The predictive pharmacophore model consisted of three features, two hydrophobic and one cationic feature with three excluded volumes. The pharmacophore was validated using a training set of thirty known IL-2 inhibitors. Pharmacophore model as a 3D search query was searched against ZINC and MOE database, in order to retrieve new chemical scaffolds that may be potent IL-2 inhibitors. The hits retrieved from this search were filtered based on their RMSD values and pharmacophoric features. Hits that were retained were used in a molecular docking study to find the binding mode and molecular interactions with crucial residues at the active site of the target. Pharmacophore based molecular docking was carried out on virtually screened compounds using 1Z92 as target by MOE software that led to the identification of 15 hits belonging to diverse classes of heterocycles. These hits were further optimized and a library of forty six compounds including 5-6 membered azaheterocycles namely dihydropyrimidines, heteroazepines, pyrazoles and benzimidazoles besides some compounds such as chalcones and Schiff bases, was designed and synthesized.

All newly synthesized compounds were characterized by their MS and NMR spectral analysis. IL-2 inhibition studies on the members of the synthesized library led to the identification of novel IL-2 inhibitors with IC\textsubscript{50} values ranging from \textless 2\textgreater >50 μg/ml using cyclosporine as a standard drug. This entire set of experiments in both dry and wet labs led to a successful designing and synthesis of a variety of compounds as novel scaffolds that may be developed into interesting immunomodulators.
<p>| Scheme 2.x: | Synthesis of chalcones (1-7) | 69 |
| Scheme 2.2: | Synthesis of dihydropyrimidines (8-13) | 71 |
| Scheme 2.3: | Synthesis of heteroazepines (14-19) | 72 |
| Scheme 2.4: | Synthesis of pyrazole carbaldehydes (20-25) | 74 |
| Scheme 2.5: | Synthesis of pyrazole carbothioamides (26-28) | 76 |
| Scheme 2.6: | Synthesis of benzimidazoles (29-32) | 77 |
| Scheme 2.7: | Synthesis of (benzimidazolylsulfonyl)anthracene-dione derivatives (33-36) | 79 |
| Scheme 2.8: | Synthesis of Schiff-base derivatives (37-41) | 80 |
| Scheme 2.9: | Synthesis of pyrazole derivatives (42-43) | 82 |
| Scheme 2.10: | Synthesis of benzamides (44-46) | 83 |</p>
<table>
<thead>
<tr>
<th>List of Figures</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1: Immune system fight against different pathogens</td>
<td>1</td>
</tr>
<tr>
<td>Figure 1.2: Bone marrow with weak immune system resulting different diseases</td>
<td>2</td>
</tr>
<tr>
<td>Figure 1.3: Types of Hypersensitivity disorders</td>
<td>3</td>
</tr>
<tr>
<td>Figure 1.4: Top view of interleukin-2</td>
<td>5</td>
</tr>
<tr>
<td>Figure 1.5: 3D structure of Human IL-2/IL-2R receptor (α, β and γ)</td>
<td>7</td>
</tr>
<tr>
<td>Figure 1.6: Drug discovery and development pipeline</td>
<td>10</td>
</tr>
<tr>
<td>Figure 1.7: Ligand based drug designing</td>
<td>11</td>
</tr>
<tr>
<td>Figure 1.8: Pharmacophore of morphine a) Pharmacophoric features b) Distances between pharmacophoric features</td>
<td>12</td>
</tr>
<tr>
<td>Figure 1.9: Structure based drug designing</td>
<td>13</td>
</tr>
<tr>
<td>Figure 1.10: Algorithm used for Molecular docking</td>
<td>18</td>
</tr>
<tr>
<td>Figure 1.11: Designing and synthesis of IL-2 inhibitors by CADD</td>
<td>42</td>
</tr>
<tr>
<td>Figure 2.1: Different steps used in dry &amp; wet labs for identification of IL-2 inhibitors</td>
<td>44</td>
</tr>
<tr>
<td>Figure 2.2: 3D Structure of protein-protein complex, IL-2 (red), IL-2Rα (green ribbon)</td>
<td>45</td>
</tr>
<tr>
<td>Figure 2.3: A close-up view of the binding pocket showing hydrophobic (green) and hydrophilic surface (magenta)</td>
<td>46</td>
</tr>
<tr>
<td>Figure 2.4: 3D view of structure based pharmacophore in the active site of 1Z92. Blue sphere indicates cationic feature and yellow spheres indicate hydrophobic feature. Excluded volumes shown as white meshed spheres</td>
<td>51</td>
</tr>
<tr>
<td>Figure 2.5: 3D view of structure based pharmacophore model for 1Z92, showing a) distances b) angles in the active site</td>
<td>52</td>
</tr>
<tr>
<td>Figure 2.6: The superimposition of standards docked compounds in binding site of 1Z92</td>
<td>53</td>
</tr>
<tr>
<td>Figure 2.7: Chemical structures of top 15 compounds retrieved from PBVS</td>
<td>56</td>
</tr>
<tr>
<td>Figure 2.8: Superimposed view of pharmacophore based docked synthesized compounds (9, 22 &amp; 45) in active site of 1Z92. All interacting amino acids are in red color</td>
<td>60</td>
</tr>
<tr>
<td>Figure 2.9: The binding mode analysis of compounds 5, Hydrogen bond is displayed in blue dotted line and π-π interactions are in green color. A) 3D and B) 2D docked view</td>
<td>61</td>
</tr>
<tr>
<td>Figure 2.10: The binding mode analysis of compound 22, Hydrogen bonds and π-π interaction is displayed in green dotted lines. A) 3D and B) 2D docked view</td>
<td>63</td>
</tr>
<tr>
<td>Figure 2.11: The binding mode analysis of compounds 35, Hydrogen bonds and distances are displayed in blue dashed line and π-π interactions are in green color. A)3D &amp; B)2D docked view</td>
<td>65</td>
</tr>
</tbody>
</table>
Figure 2.12: The binding mode analysis of compounds 37, A) 3D B) 2D ..............................................................66
Figure 2.13: Bar graphs for in vitro IL-2 inhibition assay of chalcones (1-7) ..........................................................85
Figure 2.14: Bar graphs for in vitro IL-2 inhibition assay of compounds (8-28) .........................................................86
Figure 2.15: Bar graphs for in vitro IL-2 inhibition assay of compounds (29-36) .........................................................87
Figure 2.16: Bar graphs for in vitro IL-2 inhibition assay of compounds (37-41) .........................................................88
Figure 2.17: Bar graphs for in vitro IL-2 inhibition assay of compounds (42-46) .........................................................88
Figure 2.18: Active compounds identified for IL-2 inhibition .................................................................................90
Figure 3.1: Schematic presentation of virtual screening protocol ..............................................................................99
Figure 3.2: Receptor based virtual screening protocol ..............................................................................................100
LIST OF TABLES

Table 2.1: The structures and inhibitory activities of IL-2 inhibitors used in training set........................................47
Table 2.2: Distances of pharmacophoric features................................................................................................51
Table 2.3: Structure of 46 novel and potent IL-2 inhibitors.....................................................................................56
Table 2.4: Binding interactions observed in docked compounds (1-7) with 1Z92...................................................61
Table 2.5: Binding interactions observed in hits optimized from PBVS (8-28) with 1Z92.................................62
Table 2.6: Binding interactions observed in docked compounds (29-36) with 1Z92.............................................64
Table 2.7: Binding interactions observed in docked compounds (37-41) with 1Z92..........................................66
Table 2.8: Binding interactions observed in docked compounds (42-46) with 1Z92...........................................67
Table 2.9: Drug likeness of the synthesized compounds (Lipinski’s ROS)...............................................................68
Table 2.10: Physical data of synthesized chalcones (1-7).......................................................................................70
Table 2.11: Physiochemical & Spectral data of synthesized dihydropyrimidine derivatives (8-13)..............72
Table 2.12: Physiochemical & Spectral data of synthesized heteroazepines (14-19).............................................73
Table 2.13: Physiochemical & Spectral data of synthesized pyrazole derivatives (20-25).............................75
Table 2.14: Physiochemical & Spectral data of synthesized pyrazole carbothioamides (26-28).................77
Table 2.15: Physiochemical & Spectral data of synthesized benzimidazoles (29-32)........................................78
Table 2.16: Physiochemical & Spectral data of synthesized sulfonamides (33-36).............................................80
Table 2.17: Physiochemical & Spectral data of synthesized Schiff base derivatives (37-41).........................81
Table 2.18: Physiochemical & Spectral data of synthesized pyrazole derivatives (42-43).................................83
Table 2.19: Physiochemical & Spectral data of synthesized benzamides (44-46)................................................84
Table 2.20: The IL-2 inhibitory activities of hits (1-7)..............................................................................................85
Table 2.21: The IL-2 inhibitory activities of optimized hits (8-28)......................................................................86
Table 2.22: The IL-2 inhibitory activities of optimized hits (29-36).................................................................87
Table 2.23: The IL-2 inhibitory activities of optimized hits (37-41).................................................................88
Table 2.24: The IL-2 inhibitory activities of optimized hits (42-46).................................................................88
Chapter 1

Introduction
Human health is directly influenced by immune system and its performance which are fundamentally designed for the protection against the attack of foreign invaders. However the beginning of almost all infectious and degenerative diseases is largely due to inadequate or hyperactive immune system\(^1\). The immune system is the body's defense against infectious organisms and other invaders. Through a series of steps called the immune response, the immune system attacks organisms and substances that invade body systems and cause disease. In order to function properly, an immune system must detect a wide variety of agents, known as pathogens, viruses, bacteria and parasitic worms and distinguish them from the organism's own healthy tissue\(^2\) as shown in Figure 1.1\(^3\). The immune system is made up of a network of cells, tissues and organs that work together to protect the body. The cells involved are white blood cells or leukocytes, which come in two basic types that combine to seek out and destroy disease causing organisms or substances\(^4\).

![Figure 1.1: Immune system fight against different pathogens\(^3\)](image)

**1.1 Disorders of human immunity**

Disorders of the immune system can result in immune deficiency, hypersensivity\(^5\) autoimmune diseases that occurs when the immune system is resulting in recurring and life-threatening infections.
1.1.1 Immunodeficiencies

Immunodeficiencies occur when one or more of the components of the immune system are inactive. The ability of the immune system to respond to pathogens is diminished in both the young and the elderly, with immune responses beginning to decline at around 50 years of age due to immunosenescence\(^6\). In developed countries, obesity, alcoholism, and drug use are common causes of poor immune function. However, malnutrition is the most common cause of immune deficiency in developing countries\(^7\). Additionally, the loss of the thymus at an early age through genetic mutation or surgical removal results in severe immune deficiency and a high susceptibility to infection\(^8\). Immunodeficiencies can also be inherited or acquired\(^9\). Chronic granulomatous disease, where phagocytes have a reduced ability to destroy pathogens, is an example of an inherited or congenital immune deficiency as shown in Figure 1.2\(^{10}\). AIDS and some type of cancer [particularly bone marrow and blood cells] cause acquired immunodeficiency\(^{11-12}\).

![Figure 1.2: Bone marrow with weak immune system resulting different diseases\(^{10}\)](image)

1.1.2 Autoimmunity

Over active immune responses include the other end of immune dysfunction, particularly the autoimmune disorders. Here, the immune system fails to properly distinguish between self and non-self and starts attacking part of the body. Under normal circumstances, many T-cells and antibodies react with self-peptides\(^{13}\). One of the functions of specialized cells is to present young lymphocytes with self-antigens produced throughout the body and to eliminate those cells that
recognize self-antigens, preventing autoimmunity\textsuperscript{14}. Ankylosing spondylitis, rheumatoid arthritis, type 1 diabetes mellitus, adrenal insufficiency and multiple sclerosis are some common autoimmune diseases\textsuperscript{15}.

\textbf{1.1.3 Hypersensitivity}

Hypersensitivity is an immune response that damages the body's own tissues. They are divided into four classes [Type I–IV] as shown in \textbf{Figure 1.3}\textsuperscript{16} based on the mechanisms involved and the time course of the hypersensitive reaction. Type-I hypersensitivity is an immediate or anaphylactic reaction, often associated with allergy. Symptoms can range from mild discomfort to death. Type-I hypersensitivity is mediated by Ig E, which triggers degranulation of mast cells and basophiles when cross-linked by antigen. Type-II hypersensitivity occurs when antibodies bind to antigens on a patient's own cells, marking them for destruction. This is also called antibody-dependent hypersensitivity\textsuperscript{17}. Immune complexes deposited in various tissues trigger Type-III hypersensitivity reactions. Type-IV hypersensitivity usually takes between two and three days to develop. Type-IV reactions are involved in many autoimmune and infectious diseases. These reactions are mediated by T-cells, monocytes and macrophages\textsuperscript{18}.

\begin{center}
\textbf{Figure 1.3: Types of hypersensitivity disorders}\textsuperscript{16}
\end{center}
1.2 Interleukin-2

Interleukin 2 \([IL-2]\) is an interleukin, a type of cytokine signaling molecule in the immune system. It is a protein that regulates the activities of white blood cells (leukocytes) that are responsible for immunity. IL-2 is part of the body's natural response to microbial infection, toxin and pollutants\(^9\). IL-2 is a 15.5kD glycoprotein cytokine secreted by activated T-cells as a growth factor for T lymphocytes, natural killer cells, and lymphokine-activated killer cells\(^{20}\). The gene for IL-2 was cloned in 1983 by Degrave \textit{et al.}\(^{21,22}\) and its crystal structure was determined in 1987 by Brandhuber \textit{et al.}\(^{23}\). X-ray crystallographic studies revealed that human IL-2 molecule contains four \(\alpha\) helices A,B,C and D, which are connected by a long downward A-B loop that also contains a short helical segment, a B-C loop and a long C-D cross over loop (\textbf{Figure 1.4})\(^{24}\). IL-2 plays an important role in regulating lymphocytes that has led to exciting new directions for use in cancer immunotherapy. IL-2 is a key mediator of the T-helper 1 [Th1] immune response\(^{25-26}\). Irregular Th1 immune responses play a central role in the development of autoimmune disorders such as rheumatoid arthritis, multiple sclerosis, psoriasis and graft rejection\(^{27}\). It prevents autoimmune diseases by promoting the differentiation of certain immature T-cells into regulatory T-cells, which kill off other T-cells that are primed to attack normal healthy cells in the body. IL-2 also promotes the differentiation of T cells into effectors T-cells and into memory T-cells when the initial T-cells are also stimulated by an antigen, thus helping the body for fight against infections. Its expression and secretion is tightly regulated and functions as part of both transient positive and negative feedback loops in rising immune responses and forcing them down. Through its role in the development of T-cell immunologic memory, which depends upon the expansion of the number and function of antigen-selected T-cell clones, it also plays a key role in enduring cell-mediated immunity\(^{22}\).

1.2.1 Cellular sources and production of IL-2

IL-2 is exclusively produced by T lymphocytes, CD4+ and CD8+ T-cells. TH1 and TH2 can produce IL-2, although TH1 cells may produce higher levels than TH2 cells. T-cells require stimulus with antigen or mutagenic lectins to secrete IL-2. Activation of mature, resting T-cells initiates a complex cascade of signaling pathways that leads to cellular responses including induction of genes essential for T-cell activation and induction of genes that encode IL-2,
IL-2Rα and IL-2Rβ. Rapid induction of IL-2 gene expression activates a number of signaling pathways including protein Kinase C [PKC] and calcium pathways which are required for regulation of IL-2 gene expression\textsuperscript{24-25}.

**Figure 1.4: Top view of IL-2\textsuperscript{24}**

### 1.3 IL-2 receptor

The receptor for IL-2 was initially characterized as a single 55-kDa chain. Subsequent studies demonstrated the existence of a second 70-75-kDa IL-2R subunit, termed the β chain, and it was established that IL-2Rs existed in low, intermediate, and high-affinity forms consisting of α,β and αβ subunits, respectively\textsuperscript{28-29}. However, whence DNAs for the α and β subunits were cloned and stated in both lymphoid and non-lymphoid cells, it became evident that some additional, lymphoid cell-specific component was required to completely reconstitute the intermediate and high-affinity forms of IL-2R\textsuperscript{30-32}. Takeshita et al\textsuperscript{33} first observed a 64-kD a molecule in high-affinity IL-2R complexes that was only detectable in the presence of IL-2. This molecule was termed as IL-2Rγ chain. The co-structure of IL-2, IL-2Rα was determined in 2005\textsuperscript{34} and confirmed the IL-2 hotspot. IL-2Rα is an elbow-shaped protein consisting of two β-sheet sushi. The IL-2/IL-2Rα interactions are defined by an ear parallel packing of IL-2 and IL-2Rα secondary structures, with twenty IL-2 ligand side chains and twenty one IL-2Rα receptor residues burying an area of ~1,900Å\textsuperscript{35}. 
The IL-2/IL2Rα hotspot is composed of hydrophobic patches, including IL-2 side chains Phe42 and Leu72, projecting into a complementary cavity formed by Leu42, Tyr43, and Met25 on the surface of IL-2Rα and a buried salt-bridge between Glu62 [IL-2] and Arg36 [IL-2Rα]. Numerous polar and salt-bridge interactions surround the hotspot. Site-specific mutagenes are identified a set of surface residues [Lys35, Arg38, Phe42, and Lys 43] critical for receptor binding; these residues lie on a concave face of IL-2. The structure of the quaternary high-affinity and biologically active complex [IL-2/IL-2Rα/IL-2Rβ/IL-2γ] was reported five months after the IL-2/IL-2Rα structure. Structural studies of IL-2 have shown that the binding interface for the α-chain of IL-2R contains both rigid and flexible portions of protein structure.

**Figure 1.5:** 3D structure of human IL-2/IL-2R receptor (α, β and γ)  

### 1.4 IL-2/IL-2 receptor binding

To modulate immune responses, IL-2 needs to interact with its cognate receptor on the effector cells. The IL-2Rα binds IL-2 with low affinity [kd10-8M], while the IL-2Rβ shows even lower affinity for IL-2 (kd10-7M). The combination of all three chains results in increased affinity for IL-2. Co-expression of hetero-dimer of βγ or αγ chains shows intermediate affinity for IL-2. However, the affinity of hetero-trimmers of αβγ chains for IL-2 is dramatically increased and the expression of receptors constitute high affinity binding. The high affinity receptors are only
detected on activated T-cells. The low and intermediate affinity receptors are expressed on T-cells and resting lymphocytes, respectively\(^40\).

Small, organic molecules that bind to IL-2 at the IL-2R\(\alpha\) site take advantage of this flexibility. Present work focuses on studies of IL-2/IL-2R\(\alpha\), which is considered to be an important therapeutic target for immune disease and training ground for developing approaches toward small-molecule drug discovery at protein-protein interfaces.

### 1.5 IL-2 Inhibitors

IL-2 inhibitors are drugs that inhibit the action of IL-2. The first small molecule shown to inhibit the IL-2/IL-2R\(\alpha\) interaction was reported by Roche\(^39\). Compound a, was an enantiomer-specific, competitive inhibitor of IL-2R\(\alpha\) with an IC50 = 6 \(\mu\)M.

![Chemical Structure](a)

A fragment-minded approach was used to evolve compound a into a more potent and drug-like inhibitor. Braisted et al reported a compound (b, IC\(_{50}\) = 6 \(\mu\)M) that binds to IL-2, preventing its association with IL-2R\(\alpha\)\(^41\).

![Chemical Structure](b)

Optimization of compound b included the introduction of a furanoic acid fragment onto the dichlorophenyl ring (c, IC\(_{50}\) = 0.060 \(\mu\)M) which offered a 30-fold increase in activity.
Some natural products were isolated from lindolefiastylosa which showed the ability to modulate the immune response\(^42\).

For the development of new immunomodulators, current therapies target IL-2 production or the IL-2 signaling pathway\(^43\). For instance, several IL-2 drugs such as corticosteroids, cyclosporine and tacrolimus inhibit IL-2 production by antigen-activated T-cells and are used in the treatment of autoimmune diseases and the suppression of graft rejection. Similarly, sirolimus blocks IL-2R signaling, thereby preventing the clonal expansion and function of antigen-selected T-cells. Clinical data with antibodies directed against IL-2R\(\alpha\) suggest that specific inhibition of the IL-2/IL-2R\(\alpha\) interaction targets the Th1 response without causing toxicity which has been observed with general immunomodulators\(^44\). Therapeutic antibodies have several drawbacks, including high cost and lack of oral bioavailability. A small molecule inhibitor of IL-2/IL-2R\(\alpha\) interaction could offer a significant improvement in immunomodulating therapy. Thus far, small molecule inhibitors of IL-2/IL-2R interaction have been difficult to identify\(^45\). In contrast, computational drug design approaches can be effectively followed resulting in lower cost and less screening time at onset\(^46\).

\textit{In silico} protocol was also used for identification of IL-2 inhibitors. VS of a database of 6,000 compounds resulted in the identification of three novel and moderately active hits. Furthermore, the effect of these three compounds on the expression of IL-2R\(\alpha\) was measured. The three active hits showed dose-dependent inhibitory effects on the expression of IL-2R\(\alpha\) with an IC\(_{50}\) ranging from 5.8-140 \(\mu\)M\(^43\). The structures of these compounds (d-f) are shown below. 

\begin{center}
\includegraphics[width=\textwidth]{structures.png}
\end{center}
1.6 Computer-aided drug design (CADD)

For the success of a drug research, the choice of a right bioassay is essential. The chosen test should be simple, quick and relevant. Human testing is not possible at such an early stage and so the test has to be done in vitro or in vivo. In general in vitro tests are preferred over in vivo tests. In vivo tests on animals often involve a clinical condition in an animal to produce observable symptoms. The animal is then treated to see whether the drug alleviates the problem by eliminating the observable symptoms. There are several problems associated with in vivo testing. Such testing is slow and also causes animal suffering. In vitro tests do not involve live animals, instead, specific tissues, cells or enzymes are used. Besides in vitro and in vivo testing of pharmacologically active compounds, in silico studies, are also carried out on interesting compounds in search of potential drug candidates. In silico studies, also described as computational studies, have played a very significant role in drug discovery and development.

The drug discovery process is both time-consuming and expensive\(^1\) yet new drugs are required to satisfy the numerous unmet clinical needs in many disease indications. A discovery of safe and effective drug molecule and bringing it to market takes around12-15 years. A vast range of technologies and specialties are involved to expedite the drug process which cost from 800 million to more than 1 billion\(^2\). Computational and molecular modeling tools have become a close counterpart to experiment in the understanding of molecular aspects of biological systems\(^3\). The computational approaches like homology modeling, molecular docking, and quantitative structure activity relationships (QSAR) and molecular dynamics (MD) are widely employed to discover the novel hits for various therapeutic targets\(^4\). Generally the following eight steps are undertaken for the identification of drugs for any disease as shown in Figure 1.6\(^5\). Among the eight steps, mentioned in Figure1.6. The lead characterization is the most crucial step in the process. A lead is obtained by the screening of thousands of chemicals in a chemical library.
1.7 Strategies of CADD

Computational screening has become a popular tool in the search for the new drug leads which have potential to amplify other drug like properties. There are two approaches towards CADD.

1.7.1 Ligand based drug designing (LBDD)

LBDD, also called indirect drug design, relies on the knowledge of other molecules that bind to the biological target of interest. Ligand based drug design efforts are based on the analysis of the biological and chemical properties of a set of ligands and are often used when little or no information about the structure of the target protein is available as shown in Figure 1.7\textsuperscript{54}.

2D & 3D similarity searching, pharmacophore modeling and quantitative structure activity relationship (QSAR) are most popular techniques used in LBDD\textsuperscript{52}.

1.7.1.1 2D & 3D similarity searches

2D chemical similarity analysis methods are used to scan a database of molecules again stone or more active ligand structure in LBDD. It is based on searching molecules with shape similar to that of known actives, as such molecules will fit the target's binding site and hence will be likely to bind to the target. There are a number of prospective applications of this class of techniques in
the literature\textsuperscript{55-56}. Various successful examples of 2D similarity searches are available. Topological indices, 2D fingerprints and maximum common sub graph similarity methods are used 2D similarity method\textsuperscript{57}.

![Figure 1.7: Ligand based drug designing\textsuperscript{54}](image)

\textbf{1.7.1.2 Pharmacophore modeling}

One of the most enduring concepts of CADD is that of a ‘pharmacophore’. A pharmacophore is the spatial arrangement of functional groups essential for biological activity; it is a three dimensional pattern that emerges from a set of biologically active molecules. The concept of a pharmacophore was introduced by Monty Kier in a series of papers which were published\textsuperscript{1967-1971}.\textsuperscript{58}

According to IUPAC, "a pharmacophore is the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger its biological response"\textsuperscript{59}. The pharmacophore of morphine is given in \textbf{Figure 1.8} to illustrate concept of pharmacophore.
Figure 1.8: Pharmacophore of morphine a) Pharmacophoric features b) Distances between pharmacophoric features

Typical pharmacophore features include hydrophobic centroids, aromatic rings, hydrogen acceptors or donor, cations and anions. These pharmacophoric points may be located on the ligand itself or may be the projected points presumed to be located in the receptor. The features need to match different chemical groups with similar properties, in order to identify novel ligands. Ligand-receptor interactions are typically “polar positive”, “polar negative” or “hydrophobic”. A well-defined pharmacophore model includes both hydrophobic volumes and hydrogen bond vectors. In 2D search usually similar compounds are searched while pharmacophore searching is conducted with a goal to discover new chemical series structurally different from known chemical series. Ligand Scout, Catalyst, PHASE, MOE, GASP and GALAHAD softwares are mainly used for pharmacophore modeling.

1.7.1.3 Quantitative structure activity relationship (QSAR)

QSAR is a descriptor based technique used to explain the biological activities of structurally similar compounds to their physicochemical properties. The nature of the molecular properties used and the extent to which they describe the structural features of molecules can be related to its biological activity, which is an important part of QSAR study. QSAR studies use chemometric methods to describe how a given biological activity or a physicochemical property varies as a function of the molecular descriptors. Thus, it is possible to replace costly biological tests or experiments using a given physicochemical property with calculated descriptors that can, in turn, be used to predict the responses of interest for new compounds. Two main steps are involved in QSAR.
1) A wide variety of molecular descriptors (hydrophobic, steric and electronic) is computed for each drug like candidate in the data set.

2) A quantitative correlation between the activity and molecular descriptors is derived\textsuperscript{64}.

QSAR may be 2D or 3D QSAR. 2D-QSAR methods were devised in the early days of drug designing by Free, Wilson, Hansch, and Fujita in 1964. In 2D-QSAR methods, 2D molecular substituents and their physicochemical properties are used to perform quantitative predictions. They do not require any substituent parameters or descriptors to be defined; only activity is needed. In 3D-QSAR methods, macroscopic properties of target are correlated with atom-based descriptors derived from the spatial 3D arrangement of the molecular structure\textsuperscript{65}.

1.7.2 Structure based drug designing (SBDD)

Structure based drug design (SBDD), also known as receptor based drug design or direct drug design, applies when a protein with its 3D structure is known and the structure of the proposed ligand is under investigation as shown in Figure 1.9\textsuperscript{54}.

![Figure 1.9: Structure based drug designing\textsuperscript{54}](image)

If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein\textsuperscript{66}. The first computational structure-based drug design methods came into existence in the early 1980s. There have been a few successes to date. Dihydrofolate reductase (DHFR) was the first target...
protein solved in complex with a drug methotrexate (g), bound to a bacterial enzyme; it was an important first step\textsuperscript{67}. Three-dimensional structures of dihydrofolate reductase have been the basis for the design of several improved inhibitors\textsuperscript{68}. Another example of the role of a defined molecular target coupled with structure-based drug design was the discovery of imatinib mesylate (h), as elective tyrosine kinase inhibitor approved for the treatment of chronic myelogenous leukemia\textsuperscript{69}.

![Chemical structures](image)

The ability to work at higher solution with both proteins and drug compounds makes SBDD one of the most powerful methods in drug design. Chemists may be guided to subsets of compounds with desired features to complement 3D shape of the site. From the geometry and functional features of the binding site, complementary structures of a drug are so designed as to have high binding affinity with the target molecule. It is a powerful technique to design a corresponding drug specifically interacting with the target, particularly for the development of a novel therapeutic through stimulation or inhibition of the receptor protein\textsuperscript{70}. Homology modeling, molecular docking, structure based pharmacophore docking, MD simulations are most popular techniques used in SBDD.

1.7.2.1 Homology Modeling

Homology modeling is the process by which one or more template proteins with known structures, with sequences similar to a protein of interest that lacks a known structure, is used to model the unknown structure introduced in 1970s\textsuperscript{71}. Molecular modeling has become an essential tool in several fields of science, including chemistry, physics, drug discovery, and biochemistry. If the 3D structure of a protein is resolved and the sequence of a related protein of interest is known, the approach of homology modeling becomes applicable. In particular, the structural information of the template protein can then be used as a scaffold for the generation of a model of the protein of interest (target protein). Thus knowledge-based approaches were developed to predict the 3D structure of proteins based on experimental data of the 3D structure.
of homologous reference proteins. The accuracy of the model is justified by the sequence identity between the model and template structure. A sequence identity of <30% suggests that the model have serious errors, while >50% identity suggests that the model is reliable.

1.7.2.2 Molecular docking

Molecular docking is a computational procedure performed on structure-based rational drug design to identify correct conformations of small molecule ligands and also to estimate the strength of the protein-ligand interactions, usually one receptor and one ligand. The most common docking programs and software include Autodock, Autodock Vina, GOLD, FlexX and MOE. Docking calculations allow the prediction of the structures of all the complexes between the enzymes and ligands, thereby, suggesting different kinds of interactions. Two approaches are particularly popular within the molecular docking community. One approach is shape complementarity that uses a matching technique which describes the protein and the ligand as complementary surfaces. The second approach is simulation which is the actual docking process in which the ligand-protein pairwise interaction energies are calculated. Both approaches have significant advantages as well as some limitations. These are outlined below.

- Shape complementarity methods describe the protein and ligand as a set of features that make them dockable. These features may include molecular surface/complementary surface descriptors. In this case, the receptor’s molecular surface is described in terms of its solvent-accessible surface area and the ligand’s molecular surface is described in terms of its matching surface description. The complementarity between the two surfaces amounts to the shape matching description that may help finding the complementary pose of docking the target and the ligand molecules. Another approach is to describe the hydrophobic features of the protein using turns in the main-chain atoms. Yet another approach is to use a Fourier shape descriptor technique. Shape complementarity methods can quickly scan through several thousand ligands in a matter of second sand actually figure out whether they can bind at the protein’s active site, and are usually scalable to even protein-protein interactions. They are also much more cooperative to pharmacophore based approaches, since they use geometric descriptions of the ligands to find optimal binding.
• Simulating the docking process as such is much more complicated. In this approach, the protein and the ligand are separated by some physical distance, and the ligand finds its position into the protein’s active site after a certain number of moves in its conformational space. Simulation applies the simplification of Newton’s equation of motion. There are two simulation methods available: molecular dynamics (MD) and pure energy minimization methods. MD has wide range of applications. MD methods are gaining popularity in docking because they consider flexibility of protein during docking but it takes lengthy timeframe for sampling the conformational space.\textsuperscript{90} The obvious advantage of docking simulation is that ligand flexibility is easily incorporated, whereas shape complementarity techniques must use in genius methods to incorporate flexibility in ligands. Also, it more accurately models reality, whereas shape complimentary techniques are more of an abstraction. Clearly, simulation is computationally expensive, having to explore a large energy landscape. Grid-based techniques, optimization methods and increased computer speed have made docking simulation more realistic.\textsuperscript{91}

1.7.2.2.1 Types of molecular docking

• Protein-ligand docking

The goal of protein–ligand docking is to predict the position and orientation of a ligand when it is bound to a protein receptor or enzyme.\textsuperscript{92} The protein–ligand docking procedure can be typically divided into two parts: rigid body docking and flexible docking. Rigid docking treats both the ligand and the receptor as rigid and explores only six degrees of translational and rotational freedom, hence excluding any kind of flexibility. Most of the docking suites employ rigid body docking procedure as a first step.\textsuperscript{93} Flexible docking is a more common approach to model the ligand flexibility while assuming having a rigid protein receptor, considering thereby only the conformational space of the ligand. Ideally, however, protein flexibility should also be taken into account and some approaches in this regard have been developed. There are three general categories of algorithms to treat ligand flexibility: systematic methods, random or stochastic methods and simulation methods.\textsuperscript{94}

• Protein-protein docking
Macromolecular docking is the computational modelling of the quaternary structure of complexes formed by two or more interacting biological macromolecules. Protein–protein complexes are the most commonly attempted targets of such modelling. In 1996 the results of the first blind protein-protein docking were published in which six research groups attempted to predict the complex structure of TEM-1 beta-lactamase with beta-lactamase inhibitor protein (BLIP). The goal of protein–protein docking is to determine the structure of a complex from the separately determined coordinates of its component proteins. Since the molecule unbound structures generally differ from bound structures in the complex, docking is not simply the rigid-body geometric problem of fitting together two complementary shapes. The quality of the predicted complex in test problems is usually assessed in terms of the following measures:

1) Fraction of native contacts: defined as the number of correct residue-residue contacts in the predicted complex divided by the number of contacts in the target complex. A pair of residues on different sides of the interface is considered to be in contact if any of their atoms are within 10Å.

2) Ligand root-mean-square deviation (RMSD): The RMSD of the backbone atoms of the ligand in the predicted target complex versus target complex after superimposition of the receptor.

3) Binding site RMSD: Ligand RMSD is calculated only for those ligand residues in contact with the receptor.

The goal of rigid-body search is to generate as many hits as possible, whereas the re-scoring and ranking step differentiates true hits among the large number of structures obtained in the first step. The discrimination might require flexible refinement of selected structures.

1.7.2.2.2 Docking algorithm

The strength of a successful docking program depends on two components, i.e. A) Search algorithm, B) Scoring function, explained in Figure.1.10.
A. Search algorithm

It consists of all possible orientations and conformations of the protein paired with the ligand. The search algorithm should create an optimum number of configurations that include the experimentally determined binding modes. Although a rigorous searching algorithm would go through all possible binding modes between the two molecules, this search would be impractical due to the size of the search space and amount of time it might take to complete. As a consequence, only a small amount of the total conformational space can be sampled, so a balance must be reached between the computational expense and the amount of the search space examined. Some common searching algorithms include molecular dynamics, Monte Carlo methods, and genetic algorithms, fragment-based, point complementary and distance geometry methods, Tabu and systematic searches.

B. Scoring function

The scoring function takes a pose as input and returns a number indicating the likelihood that the pose represents a favorable binding interaction. Scoring function consists of a number of mathematical methods used to predict the strength of the non-covalent interaction called the binding affinity. In molecular docking scoring functions are classified into empirical, force field and knowledge based scoring functions. In all computational methodologies, one important problem is the development of an energy scoring function that can rapidly and accurately describe...
the interaction between the protein and ligand. Several reviews on scoring are available in the literature\textsuperscript{100-101}.

### 1.7.2.3 Molecular dynamics simulations (MDS)

MD is a versatile and powerful computational technique used to simulate the dynamics of complex biochemical, chemical and physical systems. The availability of powerful computers and algorithms contributes in the success of MD. The thermodynamic properties, dynamic aspects and kinetics of organic and bio-molecules, molecular recognition process and macromolecular stability can be explained by MD simulation. Statistical mechanics gives strength to MD simulations which connect microscopic simulation with macroscopic observables\textsuperscript{102}(97). The distribution and motion of atoms are related with macroscopic observables such as temperature; pressure, heat capacity and free energies by using mathematical expressions provided by statistical mechanics. The binding free energy of a protein ligand complex and the conformational changes of protein can be explored in this way. We have already reported BChE inhibition activities of 2,3-dihydro-1,5-benothiazepines through MD simulation studies. It was reported that the availability of BChE to catalyze various substrates depends on the difference between amino acid residues that line the enzyme cleft at about 20Å depth. Such structural information allow the medicinal chemist to explore the region specific for BChE so that a combination therapy can be employed\textsuperscript{103}. AMBER, CHARMM and GROMACS are widely used for MD simulation\textsuperscript{104}.

### 1.8 Virtual screening

It is commonly accepted that there are several steps in the drug discovery process; including disease selection, target hypothesis, lead identification, lead optimization, pre-clinical trials, clinical trials and pharmacological optimization. Traditionally, these steps are carried out sequentially and if one of the steps is slow, the entire process is delayed. Because it is not possible to speed-up clinical trials, it seems that the only way to accelerate the process of DD is to act on the preclinical steps. Among the various techniques used to facilitate hit identification, high throughput screening (HTS) represents probably the most investigated one. The perspective of screening millions of compounds on a specific target can be powerful to identify hits\textsuperscript{105}.

In virtual database screening, computational techniques are used to search databases of compounds for small molecules predicted to bind to a drug target\textsuperscript{106}. Such predictions are not
meant to replace experimental affinity determination, but virtual screening method scan complement the experimental methods by producing an enriched subset of a large chemical database; the enriched subset is one in which the proportion of compounds that actually bind to the drug target of interest is increased, compared to the proportion within the whole database\(^\text{107}\). Thus, compounds from the subset that pass the initial virtual screening are found to be pharmacologically interesting at a higher rate and at a lower cost. In principle, the methods used in virtual screening may be applied to any number of conceivable compounds, but in practice one usually focuses on curated libraries of purchasable or synthesizable compounds or close analogues of such compounds. Some examples include Accelrys available Chemical Directory, eMolecules Database and free ZINC database\(^\text{108}\).

There are two general types of virtual screening, ligand-based virtual screening (LBVS) and structure-based virtual screening (SBVS). In ligand-based virtual screening, properties of a set of ligands known to bind to the drug target of interest are used to build a model for common features believed to be important for a ligand’s biological effect. This model can then be used to find new ligands that share these common features\(^\text{109}\). In SBVS, the ligands are modeled as physical entities and scoring functions are used to predict the affinity of the ligand for the binding site of interest\(^\text{110}\). Structure-based virtual screening typically employs docking software that is designed to explore the possible binding modes of a ligand within a binding site of interest\(^\text{92,111-113}\).

1.9 Wet Lab

Having identified some hits as small molecules of simple and easy to synthesize scaffolds in wet lab were selected. Some important scaffolds that possess diverse pharmaceutical applications have been discussed in the following sections.

Their methods of synthesis are already reported in literature along with their biological potential. Chalcones, for example is one of the most widely studied scaffold, synthesized by Claisen Schmidt condensation reaction, while dihydropyrimidines and benzimidazoles can be synthesized by Biginelli and Philip reaction respectively.

1.9.1 Chalcones

One of the most easily synthesized scaffolds for DD or structure optimization is chalcone.
Chalcones, also known as α,β-unsaturated ketones are not only important precursors for synthetic manipulations but also form a major component of the natural products. Chalcones as well as their synthetic analogues display enormous number of biological activities such as IL-2 inhibitors i\textsuperscript{43}, anticancer agents ii\textsuperscript{114}, iii\textsuperscript{115}, anti-malarial iv\textsuperscript{116}, v\textsuperscript{117}, antimicrobial vi\textsuperscript{118}, vii\textsuperscript{119}, anti-inflammatory viii\textsuperscript{120}, anti-protozoal ix\textsuperscript{121}, antioxidant x\textsuperscript{122}. 

![Chemical Structures](image-url)
1.9.1.1 Synthesis of chalcones

Bandgaret al synthesized 3-(2,4-dimethoxy-phenyl)-1-phenyl-propenone by reacting substituted 1-phenyl ethanone and 2,4-dimethoxy-benzaldehyde or 3,4,5 trimethoxybenzaldehyde using NaOH as catalyst (Scheme 1.1)\textsuperscript{123}.

Hans et al synthesized acetylenic chalcones from commercially available hydroxyl-acetophenone or benzaldehyde and commercially available vanillin or acetovanillone by O-alkylation followed by Claisen-Schmidt condensation as shown in Scheme 1.2\textsuperscript{124}.

Kumar et al synthesized indolyl chalcones by reacting indol-3-carboxaldehyde and appropriate acetophenone in the presence of piperidine under reflux (Scheme 1.3)\textsuperscript{125}.
1.9.2 Dihydropyrimidines

Another important scaffold in CADD is a pyrimidine nucleus. Pyrimidines are the most important six member heterocyclic compounds containing two nitrogen atoms at 1 and 3 positions, called 1,3-diazines B. Other structurally isomeric diazines are pyridazine A (1,2-diazine) and pyrazine C (1,4-diazine). The numbering of 3-isomers of diazines is shown below.

The chemistry of pyrimidines has become increasingly important as a result of recent developments in medicinal chemistry. Pyrimidine derivatives possess a broader spectrum of biological activities such as antiproliferative xi, antibacterial xii, anticancer xiii, cytotoxic xiv, antifolates xv, inhibitor of dihydrofolate reductase (DHFR) of malarialplasmodia xvi, and antifungal xvii etc.
1.9.2.1 Synthesis of dihydropyrimidine derivatives

Mainly, pyrimidine synthesis can be classified according to the fundamental nature of fragments, which combine together to form pyrimidine nucleus. Some of the synthetic routes to dihydropyrimidinones are given below.

One of the prominent multicomponent reactions that produces an interesting class of nitrogen heterocycles is the “Biginelli dihydropyrimidines synthesis.” 3,4-dihydropyrimidinones and 3,4-dihydropyrimidine-2-thiones have been synthesized by Biginelli condensation of differently substituted aryl aldehydes with different 1,3 dicarbonyl compounds in the presence of urea or thiourea and stannous chloride by conventional heating or at reflux temperature as shown in Scheme 1.4.

![Scheme 1.4](image-url)
The reaction of substituted aromatic aldehydes, 4-chloro acetanilide ethanol, and potassium hydroxide solution resulted in the formation of chalcone derivatives which was then treated with guanidine nitrate in presence of ethanol and 40% sodium hydroxide solution to give pyrimidine derivatives as shown in Scheme 1.5\textsuperscript{134}.

\[ \text{Scheme 1.5} \]

The reaction of aromatic hydrazine and ethyl 3-oxobutanoate resulted in the formation of an intermediate which was then reacted with substituted benzaldehyde to form another intermediate which when treated with urea and KOH in DMF under microwave irradiations yielded pyrimidine derivatives (Scheme 1.6).\textsuperscript{135}

\[ \text{Scheme 1.6} \]
Pyrimidine derivatives have been synthesized by the reaction of 2-amino-3-carbonitrile, triethylorthoformate and acetic anhydride in 1,4-dioxane followed by the reaction of an intermediate formed with ammonia or primary amine in ethanol as described in Scheme 1.7\textsuperscript{136}.

\begin{center}
\includegraphics[width=0.8\textwidth]{Scheme1.7.png}
\end{center}

Scheme 1.7

The reaction of 6-methyl-5-(2-oxobutanoyl)-4-phenyl-3,4-dihydropyrimidine derivative (prepared from the reaction of ethyl ester with thiosemicarbazide in acetone) with carbothioamide at reflux temperature resulted an intermediate (Carbothioamide)-3,4-dihyro-6-methyl-4-phenylpyrimidin-2(1H)-one. Then gradually adding the Iodine solution in KI and heating the reaction mixture continuously for 5 hr gave desired pyrimidine derivatives as shown in Scheme 1.8\textsuperscript{137}.

\begin{center}
\includegraphics[width=0.8\textwidth]{Scheme1.8.png}
\end{center}

Scheme 1.8

Another reported method for the synthesis of dihydropyrimidine derivatives involves the condensation of separately synthesized chalcone (3-(4-substitutedphenyl)-1-(pyridine-4-yl)prop-2-
en-1-one) with urea or guanidine hydrochloride in the presence of acetic acid as shown in Scheme 1.9.\textsuperscript{138}

\[
\begin{align*}
\text{Scheme 1.9}
\end{align*}
\]

1.9.3 Heteroazepines

Heteroazepines are important seven membered heterocyclic compounds used as tranquilizers, such as Librium and Valium. 1,5-benzothiazepines have recently emerged as important target molecules due to their pharmacological properties such as p53-MDM2 Inhibitors \textsuperscript{xviii, xix}, antimicrobial \textsuperscript{xx}, antioxidiant \textsuperscript{xxi}, acetylcholinesterase inhibitors \textsuperscript{xxii}.\textsuperscript{142}

\[
\begin{align*}
\text{xviii} \\
\text{xx} \\
\text{xxi} \\
\text{xxii}
\end{align*}
\]
1.9.3.1 Synthesis of heteroazepine derivatives

Synthesis of a series of some new 1,5-benzodiazepines has been reported by the condensation of o-phenylenediamine and various substituted chalcones in the presence of DMF as solvent (Scheme 1.10).¹⁴³

![Scheme 1.10](image)

Chalcone derivatives have also been prepared by the Claisen-Schmidt condensation reaction of 1-(4-chlorophenyl)-1H-pyrazole-4-carbaldehyde and variously substituted o-hydroxyacetophenones in the basic medium followed by Michael addition of o-amino thiophenol to chalcones in acetic acid/ethanol (Scheme 1.11).¹⁴⁴
The reaction of gem-acylnitrostyrenes with o-aminothiophenol afforded gem-benzoynitrostyrenes which on heating under reflux in EtOH in the presence of methanolicHCl gave 2-aryl-3-nitro-4-phenyl-2,5-dihydro-1,5-benzothiazepine derivatives (Scheme 1.12).\textsuperscript{145}

A mixture of 2-aminothiophenol or 2-aminophenol or o-phenylenediamine, 1,3-diketone and \(N,N\) -dimethylformamide dimethyl acetal in distilled water was stirred well and subjected to microwave
irradiation at 130-140 °C that resulted in the formation of heteroazepines as illustrated in Scheme 1.13.

\[
\begin{align*}
\text{OTs} & \quad \text{Microwave} \quad \text{N} \quad X \\
\text{O} & \quad \text{N} \quad \text{O} \\
\end{align*}
\]

\[X=O,S,NH\]

Scheme 1.13

1.9.4 Benzimidazoles

Benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds, they are remarkably effective compounds both with respect to their inhibitory activity and their favorable selectivity ratio. The most prominent benzimidazole in nature is N-ribosyl-dimethyl benzimidazole, which serves as an axial ligand for cobalt in vitamin B12. Benzimidazoles exhibit a range of pharmaceutical activities like antiulcer xxiii, xxiv proton pump inhibitorxxv, antihelmenthic xxvi, antifungal xxvii, antimicrobial and antibacterial activity xxviii, xxix, antiviral xxx, antihelmintic xxxi, HIV inhibitors xxxii, antihypertensive xxxiii, anti-inflammatory xxxiv, analgesic xxxv.
1.9.4.1 Synthesis of benzimidazole derivatives

Pyridinyloxyphenyl benzimidazoles have been obtained by reacting chloropyridinyloxy benzaldehyde (prepared by reacting 2,3,5,6-tetrachloropyridine with m-hydroxy benzaldehyde using anhydrous K$_2$CO$_3$ in DMF medium) with o-phenylene diamine in presence of sulfonium bromide catalyst (Scheme 1.14)$^{161}$. 
The o-Phenylenediamine was reacted with benzaldehyde followed by condensation with aryl aldehydes afforded corresponding benzimidazoles in good yield (Scheme 1.15).  

\[ \text{Scheme 1.14} \]

1.9.5 Anthraquinone sulfonamide derivatives

Sulfonamides (sulfa drugs) were the first drugs largely employed and systematically used as preventive and chemotherapeutic agents against various diseases\(^\text{163}\). These compounds are well known for their use as cytotoxic against human cancer cells \(\text{xxxvi}\)\(^\text{164}\), antimicrobial \(\text{xxxvii, xxxviii}\)\(^\text{165}\), and are known to be involved in a number of biological reactions.

\[ \text{xxxvi} \]
1.9.5.1 Synthesis of anthraquinone sulfonamide derivatives

Due to the broad applicability of sulfonamides, it is desirable to find general and effective methods for their synthesis. Recently, a direct oxidative conversion of thiols into sulfonamides with H$_2$O$_2$-SOCl$_2$ was reported by Bahrami et al.\textsuperscript{166} leading to corresponding sulfonamides were obtained in excellent yields in very short reaction time (Scheme 1.16).\textsuperscript{167}

\begin{center}
\begin{align*}
RSH & \overset{\text{aq. H}_2\text{O}_2, \text{SOCl}_2}{\text{MeCN}} \rightarrow R\underset{\text{O}}{\text{S}}\underset{\text{O}}{\text{Cl}} \quad \overset{\text{R}_1\text{NH}_2}{\text{Pyridine}} \rightarrow R\underset{\text{O}}{\text{S}}\underset{\text{N}}{\text{H}}
\end{align*}
\end{center}

Scheme 1.16

A method of formation of sulfonamides from thiols was reported by Wright et al (Scheme 1.17)\textsuperscript{168}, requiring \textit{in situ} synthesis of a sulfonyl chloride using sodium hypochlorite-mediated oxidation of thiol.

\begin{center}
\begin{align*}
RSH & \overset{\text{NOCl}}{\rightarrow} \left[ \begin{array}{c} \text{R-S-Cl} \\ \text{O} \end{array} \right] \quad \overset{\text{H}_2\text{N}\text{C}_{6}\text{H}_4}{\rightarrow} \text{R-SO-} \text{NH-C}_{6}\text{H}_4
\end{align*}
\end{center}

Scheme 1.17

Another innovative example for the synthesis of sulfonamides was illustrated by the synthesis of 2-amino-9H-purin-6-sulfonamide (Scheme 1.18). Mild and selective oxidants were used by Revankare et al.\textsuperscript{169} They reported the oxidation of 2-amino-9H-purin-6-sulfonamide using one equivalent of \textit{m}-chloro para-benzoic acid in 48% yield.
Chavasiret et al.\textsuperscript{170} reported the use of trichloroacetonitrile-triphenylphosphine complex (Cl\textsubscript{3}CCN/PPh\textsubscript{3}) for sulfonamide construction (Scheme 1.19).

\[
\begin{align*}
\text{R-SO}_{\text{OH}} & \xrightarrow{\text{Cl\textsubscript{3}CCN, PPh\textsubscript{3}, DCM}} \text{R-SO-NHR} \\
& \hspace{1cm} \text{RNH\textsubscript{2}, 4-Picoline}
\end{align*}
\]

Scheme 1.19

An effective method for \textit{N}-arylation on sulfonamide using copper (II) acetate in air and arylboronic acid to get \textit{N}-arylsulfonamide is given in Scheme 1.20.\textsuperscript{171}

\[
\begin{align*}
\text{NH}_2\text{SO}_\text{phen} & + \text{HO-B} & \xrightarrow{\text{Cu(OAc)}_2, \text{TEMPO}} & \text{O-SO_NH} \\
& \text{CH\textsubscript{3}} & & \text{CH\textsubscript{3}}
\end{align*}
\]

Scheme 1.20

The sulfonamide derivatives have also been synthesized by reacting substituted benzene sulphonamide and naphthoic acid, phosphorus oxychloride at 40-45 °C (Scheme 1.21).\textsuperscript{172}

\[
\begin{align*}
\text{R}\text{-} & \text{COOH} & \xrightarrow{\text{H}_2\text{N-SO}_{\text{R}_1}} & \text{R}\text{-} & \text{SO}\text{-} & \text{HN} \\
\text{POCl\textsubscript{3}/Heat}, 10 \text{ min,} & 40-45 ^\circ\text{C} & & \text{R}\text{-} & \text{S} & \text{HN}
\end{align*}
\]

Scheme 1.21

The reaction of celecoxib (prepared according to the reported method\textsuperscript{173} and 2-chloropropanoyl chloride, or chlorobutyryl chloride resulted in the formation of an intermediate which was then subjected to condensation with 2-aminoanthraquinone to give alkanamide derivatives (Scheme 1.22).\textsuperscript{174}
1.9.6 Schiff base derivatives

Schiff bases and their metal complexes have been extensively investigated due to their wide range of applications including antifungal xxxix\textsuperscript{175}, antitumor xl\textsuperscript{176}, anti-inflammatory xli\textsuperscript{177}, antiviral xlii\textsuperscript{178}, antioxidant xliii\textsuperscript{179}, angiotension-II receptor antagonist xliv\textsuperscript{180}, antibacterial xlv\textsuperscript{181}, antiglycation xlvi\textsuperscript{182}, anticonvulsant xlvii\textsuperscript{183} and anti-tuberculosis xlviii\textsuperscript{184}. 

xxxix xl xli xlii xliii
1.9.6.1 Synthesis of Schiff base derivatives

The reaction of primary aromatic amines with aryl aldehydes is found to be catalyzed by lemon juice as natural acid under solvent-free conditions to give the corresponding Schiff bases in good yield (Scheme 1.23).\(^{185}\)

\[
\begin{align*}
\text{R}_2 \text{HCO} &+ \text{R}_1 \text{NH}_2 \rightarrow \text{R}_2 \text{HCO-N}=\text{C} \text{H}_2 \text{R}_1 \\
\text{OHC} &+ \text{H}_2 \text{N-S} & & \rightarrow & & \text{H}_3 \text{CO} &+ \text{H}_2 \text{N-S} \\
\end{align*}
\]

Scheme 1.23

A mixture of 2-aminobenzothiazoles and different aromatic aldehydes were taken in mortar, added to conc. H\textsubscript{2}SO\textsubscript{4}, water and stirred at room temperature for 30 minutes (Scheme 1.24).\(^{186}\)

\[
\begin{align*}
\text{H}_3 \text{CO} &+ \text{H}_2 \text{N-S} & & \rightarrow & & \text{H}_3 \text{CO} &+ \text{H}_2 \text{N-S} \\
\text{OHC} &+ \text{R}_1 \text{NH}_2 & & \rightarrow & & \text{R}_2 \text{HCO-N}=\text{C} \text{H}_2 \text{R}_1 \\
\end{align*}
\]

Scheme 1.24

A simple and efficient method has been developed for the synthesis of some novel Schiff bases by the reaction of aromatic aldehydes with 2-aminobenzimidazole by using catalytic amount of M(NO\textsubscript{3})\textsubscript{2} \cdot xH\textsubscript{2}O in an organic solvent at room temperature (Scheme 1.25).\(^{187}\)
1.9.7 Pyrazoles

Pyrazoles are an important class of heterocyclic compounds with two adjacent nitrogens in a five-membered ring system. Among the two nitrogen atoms, one is basic and the other is neutral in nature.

Pyrazoles are widely found as the core structure in a large variety of compounds that possess important agrochemical and pharmaceutical activities such as antitumor xlix\textsuperscript{188}, antitubercular l\textsuperscript{189}, anticonvulsant li\textsuperscript{190}, lii\textsuperscript{191}, antihepatotoxic liii\textsuperscript{192}, anti-inflammatory liv\textsuperscript{193}, antimicrobial lv\textsuperscript{194}, antibacterial lvi\textsuperscript{195}, antioxidant lvii\textsuperscript{196} etc.
1.9.7.1 Synthesis of pyrazole derivatives

The synthetic strategy of pyrazole derivatives is illustrated in Scheme 1.26. The acetophenone and substituted phenyl hydrazine were exposed to microwave irradiations at 200 W.\(^{197}\)

![Scheme 1.26](image)

A series of novel indeno-pyrazole derivatives were synthesized by the reaction of \(\alpha\)- and \(\beta\)-unsaturated ketones with phenyl hydrazine in polyethylene glycol-400 (PEG-400) and few drops of acetic acid (Scheme 1.27).\(^{198}\)

![Scheme 1.27](image)

A convenient synthesis of 3-substituted pyrazole derivatives by a mixed anhydride method using isobutylchloroformate and \(N\)-methylmorpholine at -20°C in THF (Scheme 1.28).\(^{199}\)
A series of novel N’-[(aryl)methylene]-5-substituted-1H-pyrazole-3-carbohydrazide derivatives were synthesized by the reaction of substituted pyrazole carbohydrazide with functionalized aromatic aldehydes (Scheme 1.29).

A strategy based on the concept of acid-catalyzed dimerization was applied to the synthesis of methylene bis-salicylaldehyde from readily available salicylaldehyde, which then underwent reaction with substituted acetophenones, leading to the formation of methylene bis-chalcones. Construction of the pyrazole ring was accomplished by the reaction of chalcones with hydrazine hydrate under microwave irradiation with or without acetic acid as shown in Scheme 1.30.
In another method, 3,5-bis-trifluoroacetophenone was treated with diethyl oxalate and sodium hydride in toluene at rt. The intermediate ethyl ester was then reacted with 4-fluorophenyl hydrazine hydrochloride in ethanol:acetic acid (2:1) that resulted in the formation of ethyl ester which was added to the ethanolic NaOH solution and the mixture was stirred at rt for 4h to afford pyrazole derivatives (Scheme 1.31).
1.9.8 Benzamides

Benzamides are an important class of organic compound which are derived from benzoic acid. Benzamide and their heterocyclized products display diverse biological activities including enzyme inhibitors against BchE, AchE and lipoxygenase enzymes \textsuperscript{lviii} \textsuperscript{203}, antibacterial \textsuperscript{lix} \textsuperscript{204}, cell cycle inhibition in HEPG2 cells \textsuperscript{lx} \textsuperscript{205}, anti-inflammatory \textsuperscript{lii}, Synthesis, anticancer and antibacterial activity of salinomycin\textsuperscript{nx}-benzyl amides \textsuperscript{206}, angiotensin-i-converting enzyme inhibition, antioxidant \textsuperscript{lxii} \textsuperscript{207} and anticancer \textsuperscript{lxiii} \textsuperscript{208}.

\[
\text{N-(3-hydroxyphenyl) benzamide derivatives were synthesized by reacting 3-hydroxyaniline with benzoyl chloride in aqueous medium, which was then treated with different alkyl halides to synthesis new 3-O-derivatives via O-alkylation (Scheme 1.32).} \textsuperscript{203}
\]
The reaction of benzoyl chloride, ammonium hydroxide and water on stirring yielded the amide derivatives (Scheme 1.33). 204

\[
\begin{array}{c}
\text{Cl}^+ \text{C}=\text{O} \\
\text{NH}_3 \text{30\% NH}_4\text{OH} \\
\text{NH}_2 \text{HCHO, ethanol, reflux} \\
\text{NH} \text{CH}_2\text{-R}
\end{array}
\]

Scheme 1.33

Amide derivatives have been synthesized by a three-step reaction. First, a Buchwald-Hartwig C-N or C-O coupling was carried out by an ester hydrolysis reaction that lead to an intermediate. Next, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) and 1-hydroxybenzotriazole hydrate (HOBT) were used to catalyze amide formation as shown in Scheme 1.34. 205

\[
\begin{array}{c}
\text{R'}\text{NH}_2 + \text{OC}_{\text{C}2\text{H}_5} \text{F} \\
\text{HOBTEDCI} \\
\text{NH}
\end{array}
\]

Scheme 1.34

The reaction of methyl-2-aminobenzoxazole-5-carboxylate in DMF and NaH, substituted benzoyl chloride afforded the desired amide in 60-70% yield (Scheme 1.35). 206

\[
\begin{array}{c}
\text{H}_3\text{CO} \text{NH}_2 + \text{OC}_{\text{Cl}} \text{DMF} \\
\text{NaH} \\
\text{H}_3\text{CO} \text{NH}\text{R}
\end{array}
\]

Scheme 1.35
1.10 Aim of the study

With view to address the challenges of immunomodulating issues as a scoring health hazard it was planned to design some novel IL-2 inhibitors by making use of the most rapidly emerging computational tools commonly referred to as computer-aided drug designing, the proposed strategy would involve the initial study of a novel drug target i.e. IL-2-IL-2R, followed by detail study of its binding site. This would follow the extraction of pharmacophoric features expected to be important for IL-2 inhibitory using the structure based pharmacophore protocol. The analogs of the hits identified in the 1st round of experiments in dry lab will be synthesized for their potential as IL-2 inhibitors. Molecular Operating System (MOE) software will be used for the entire set of study (Figure 1.1).

Figure 1.1: Designing and synthesis of IL-2 inhibitors by CADD

The discovery of small-molecule inhibitors and the study of their interactions with IL-2/IL-2Ra will help in elucidating the inhibitors' mechanisms of action by using following steps.
1) Establishing structure-based 3D-Pharmacophore models for IL-2 inhibitors.
2) Establishing an appropriate virtual screening protocol.
3) Filtration of IL-2 inhibitors by virtual screening.
4) Identification of hits.
5) Determination of binding modes of known natural and synthetic IL-2 inhibitors by molecular docking method.
6) Synthesis and IL-2 inhibition studies on the analogs of hits structures identified in dry lab and synthesized later in wet lab.
The function of the immune system depends largely on the action of interleukins which are a group of cytokines that were first seen to be expressed by white blood cells. The majority of interleukins are synthesized by helper CH4T lymphocytes, as well as through monocytes, macrophages and endothelial cells. They stimulate the development and differentiation of T and B lymphocytes and hematopoietic cells. The rare deficiencies in the number of interleukins cause autoimmune diseases or immune deficiency. Hence, IL-2 holds considerable promise as a therapeutic target for the treatment of autoimmune disorders. For the development of new immunomodulators, current therapies target IL-2 production or the IL-2 signaling pathway. A small molecule inhibitor of the IL-2/IL-2Rα interaction could offer a significant improvement in immunosuppressive therapy. The present study describes the usefulness of in silico tools in identification of new IL-2 inhibitors. Such studies also help in the identification and improving the physiochemical properties of the existing drugs. Today, in silico screening has also become a popular tool in identifying novel drugs at a much faster rate. The identification of novel IL-2 inhibitors was carried out by the steps followed both in dry and wet labs in different rounds of experiments.

![Figure 2.1. Different steps used in dry & wet labs for identification of IL-2 inhibitors](image)
STEP 1 (Dry lab)

2.1. *In silico* identification of IL-2 inhibitors

In an attempt to identify novel *IL*-2 inhibitors, structure-based pharmacophore designing and virtual screening was conducted. A diverse group of the compounds that passed the VS steps was selected for optimization and retested by 3D pharmacophore mapping and molecular docking. The optimized VS hits were subjected to synthesis. All synthesized hits were tested for *IL*-2 inhibitory activity. MOE software has been used for carrying out all computational studies.

2.1.1. Target identification

The first step of any drug designing project is the target identification. The target was selected on the basis of the criteria:

1) The complex should be of human origin.
2) The target should have high quality of good resolution (<3.0° A).
3) The protein-protein or protein-ligands are interacted with each other through non-covalent interactions and the experimental binding data should be available.

X-ray structure of the drug target i.e. *IL*-2/*IL*-2Rα complex (1Z92) was obtained from Protein Data Bank. The structure of complex is given in *Figure 2.2*. It’s a dimer with two chains (A & B) and has a resolution 2.8 °A. Chain A represents *IL*-2 (red) while chain B is *IL*-2Rα (green).

![Figure 2.2. 3D Structure of protein-protein complex, *IL*-2 (red), *IL*-2Rα (green ribbon)](image)
2.1.2. Determination of active site

The active site of 1Z92 was determined through ‘Site Finder’ tool of MOE. It created α-spheres at the active site. The site having key contributing amino acids was selected as the active site which was usually larger in size. The key contributing amino acid residues in the active site were identified by literature\textsuperscript{33,206}. These amino acids were Lys35, Arg38, Met39, Thr41, Phe42, Phe44, Tyr45, Glu62, Lys65, Pro65, Glu68, Val69, Aln71, Leu72, Ala73, Thr111 and Thr113. The molecular surface of the binding pocket was visualized by calculating molecular surface and volume through the ‘surfaces and maps’ option present in MOE as shown in Figure 2.3. After identification of the active site of 1Z92 the next step was the generation of a training set for pharmacophore modeling.

![Figure 2.3. A close-up view of the binding pocket showing hydrophobic (green) hydrophilic (magenta)](image)

2.1.3. Designing a training set

For pharmacophore generation a training set of the compounds was built, which contains the standard drugs as well as drugs present in complex structure of \textit{IL}-2 complexes. These compounds were selected from literature\textsuperscript{41, 44, 210-212} and all were structurally diverse and were known to strongly and selectively inhibit IL-2. All these compounds have IC\textsubscript{50} values from 0.06-80 \text{ g/ml}. Firstly, all these structures were built using MOE Builder then hydrogen atoms were
added and energy minimized by using MOE. The minimized structures of all these compounds were added to the training database. The chemical structures and experimental IC\textsubscript{50} values of the training set are given in Table 2.1.

Table 2.1: Structures and IC\textsubscript{50} of IL-2 inhibitors used in training set

<table>
<thead>
<tr>
<th>No.</th>
<th>Code</th>
<th>Structure</th>
<th>IC\textsubscript{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-1</td>
<td>CHEMBL104594</td>
<td><img src="image" alt="Structure S-1" /></td>
<td>0.2</td>
</tr>
<tr>
<td>S-2</td>
<td>CHEMBL107320</td>
<td><img src="image" alt="Structure S-2" /></td>
<td>2</td>
</tr>
<tr>
<td>S-3</td>
<td>CHEMBL108123</td>
<td><img src="image" alt="Structure S-3" /></td>
<td>0.4</td>
</tr>
<tr>
<td>S-4</td>
<td>CHEMBL421412</td>
<td><img src="image" alt="Structure S-4" /></td>
<td>3</td>
</tr>
<tr>
<td>S-5</td>
<td>CHEBI:47417 (SP-4206)</td>
<td><img src="image" alt="Structure S-5" /></td>
<td>0.06</td>
</tr>
<tr>
<td>S-6</td>
<td>CHEMBL106951</td>
<td><img src="image" alt="Structure S-6" /></td>
<td>0.6</td>
</tr>
<tr>
<td>S-7</td>
<td>CHEMBL317898</td>
<td><img src="image" alt="Structure S-7" /></td>
<td>9</td>
</tr>
</tbody>
</table>

Continued
<table>
<thead>
<tr>
<th></th>
<th>Chemical ID</th>
<th>Structure</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-9</td>
<td>CHEMBL181983</td>
<td><img src="image" alt="Structure S-9" /></td>
<td>20</td>
</tr>
<tr>
<td>S-10</td>
<td>CHEMBL104746</td>
<td><img src="image" alt="Structure S-10" /></td>
<td>20</td>
</tr>
<tr>
<td>S-11</td>
<td>CHEMBL104914</td>
<td><img src="image" alt="Structure S-11" /></td>
<td>40</td>
</tr>
<tr>
<td>S-12</td>
<td>CHEMBL182704</td>
<td><img src="image" alt="Structure S-12" /></td>
<td>0.6</td>
</tr>
<tr>
<td>S-13</td>
<td>CHEMBL182098</td>
<td><img src="image" alt="Structure S-13" /></td>
<td>48</td>
</tr>
<tr>
<td>S-14</td>
<td>CHEMBL350354</td>
<td><img src="image" alt="Structure S-14" /></td>
<td>6.5</td>
</tr>
<tr>
<td>S-15</td>
<td>CHEMBL164641</td>
<td><img src="image" alt="Structure S-15" /></td>
<td>0.8</td>
</tr>
<tr>
<td>S-16</td>
<td>CHEMBL166149</td>
<td><img src="image" alt="Structure S-16" /></td>
<td>2</td>
</tr>
</tbody>
</table>

Continued
| S-17 | CHEMBL103894 | ![Chemical Structure](image) | 0.26 |
| S-18 | CHEMBL100390 | ![Chemical Structure](image) | 0.37 |
| S-19 | CHEMBL101202 | ![Chemical Structure](image) | 0.30 |
| S-20 | CHEMBL167745 | ![Chemical Structure](image) | 0.9 |
| S-21 | CHEMBL350780 | ![Chemical Structure](image) | 0.6 |
| S-22 | CHEMBL164498 | ![Chemical Structure](image) | 0.8 |
| S-23 | CHEMBL419362 | ![Chemical Structure](image) | 3.0 |
| S-24 | CHEMBL106262 | ![Chemical Structure](image) | 40 |

Continued
2.1.4. Generation of structure based pharmacophore model

The pharmacophore model was generated by the Pharmacophore Generating Program of MOE. In MOE, additional features were developed using the MOE Pharmacophore Query Editor. Several possible hypotheses were generated by considering key amino acids of IL-2Rα. After testing 60-70 hypotheses, three amino acids were identified as pharmacophoric features for IL-2 inhibition. One cationic center F2 (Cat.) and two hydrophobic groups F1 & F3 (Hyd) were concluded as key features for inhibitors.
Besides, the program automatically generated three excluded volumes in the model. The cationic feature (F2) was located on side chain amines of arginine (Arg36B). The hydrophobic features F1 and F3 were located on imidazole group of His120B and the methyl group of leucine (Leu2B) respectively. F1 and F3 were depicted by yellow spheres and F2 by blue sphere in Figure 2.4.

Figure 2.4. 3D view of structure based pharmacophore in the active site of 1Z92. Blue sphere indicates cationic feature, Yellow spheres indicate hydrophobic features. Excluded volumes are shown as white meshed spheres.

Prior to screening, it was necessary to make a number of adjustments in term of radii and distances. The radii of features were optimized for best hit search\textsuperscript{213}. F1 & F3 had radii 2.2 Å and 1.8 Å respectively while cationic feature F3 had a radius of 2.1 Å as shown in Figure 2.5. All possible distances among these features are shown in Table 2.2. The pharmacophore model for IL-2 inhibitors was of triangle shape, the following angles of three features were calculated as depicted in Figure 2.5.

<table>
<thead>
<tr>
<th>No.</th>
<th>Feature type</th>
<th>Distances (Å)</th>
<th>Feature type</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1-F2</td>
<td>12.07</td>
<td>(\Delta F1.F2.F3)</td>
<td>21.9°</td>
</tr>
<tr>
<td>2</td>
<td>F1-F3</td>
<td>5.96</td>
<td>(\Delta F2.F3.F1)</td>
<td>43.7°</td>
</tr>
<tr>
<td>3</td>
<td>F2-F3</td>
<td>16.97</td>
<td>(\Delta F3.F1.F2)</td>
<td>114.4°</td>
</tr>
</tbody>
</table>
2.1.5. Validation of pharmacophore model

For any conformation of compound to bind with the active site of target it should reasonably match the pharmacophoric features of the active site. In addition the conformation of the compound should never overlap with the excluded volume around the active site of target protein. To find out such conformations, the training set was thoroughly searched by using pharmacophore search through the in built option present in “Pharmacophore Query Editor” module of MOE and for this purpose a validation database of 30 compounds was screened after virtual screening. All these compounds correctly mapped by the modified pharmacophore model as shown in Figure 2.6. The RMSD values of these hits were in the acceptable range of 0.05 to 2.73. The results verified the validity of modified pharmacophore model that can be used for the screening of large databases.

The above validated pharmacophore was further used as a search query in virtual screening to screen all those compounds which exhibited the same pharmacophoric features. In the current study this virtual database was built by selecting compounds from ZINC & MOE databases.
2.1.6. Pharmacophore based virtual screening

The modified validated pharmacophore model was then used as in silico filter to screen the ZINC and MOE databases of commercially available compounds. ZINC database contains a total of 1,594,931 compounds out of which 1.5 million compounds passed the filtration criteria of Lipinski’s RO5 and 15,000 compounds were randomly selected for virtual screening in the next step. Enrichment studies were performed using 30 compounds including quite a few standard drugs selected from literature with IC$_{50}$ ranging from 0.06-80 μg/ml against human IL-2. These active compounds were seeded into 15,000 decoy molecules selected from ZINC database. The resulting data set of 15,030 compounds was imported into MOE database containing 10,000 compounds leading to a new set of database containing 25,030 compounds having a variety of chemical scaffolds. Geometry optimization was performed using protonate 3D command in MOE. The output files of compounds of both databases were loaded into MOE environment in SDF format where the 3D structure of each compound was modeled using MMFF94x force field. The Conformation Import methodology was applied to generate low-energy conformations for each compound. All these compounds and their respective conformations were saved in MOE database. The new and extended database was exhaustively searched through the Search option present in the ‘Pharmacophore Query Editor’ of MOE and all those conformations which matched the pharmacophoric features were selected as hits in the
output database. The output database was sorted on the basis of RMSD in ascending order and only those conformations having RMSD up to 2.0 were retained and those having RMSD above 2.0 were removed from the database. The next step i.e. pharmacophore-based virtual screening, reduced the number of compounds from 25,030 to 500 compounds that mapped on the developed pharmacophore model. The output database of the resulting 500 compounds was sorted out on the basis of RMSD in ascending order. These initially identified hits were selected for further evaluation using docking studies. To reduce the data of identified hits, they were docked into the identified binding pocket of IL-2/IL-2Ra complex.

2.1.7. High throughput docking

To prioritize the compounds for synthesis and biological testing, they were subjected to high-throughput docking using MOE. These initially identified 500 compounds from the output file were selected for further evaluation using molecular docking. The top ranked docked poses were re-scored and binding energies were calculated using LIGX option implemented in MOE to prioritize these compounds. Analysis of the docking results, on the basis of the scoring functions and visual inspection of docked poses of top 5% compounds led to identification of 15 hits (H1-H15) for optimization. The chemical structures of these 15 hits are shown in Figure 2.7.

These hits included the compounds such as chalcone (H-11), dihydropyrimidine (H-5), sulfonamide (H-13), Schiff base (H-14), benzamides (H-15). Moreover a variety of azaheterocycles were also identified as hits for example benzimidazole (H-3), benzodiazepine (H-8) and pyrazoles (H-10).

The next step was the synthesis of these hits in wet lab from readily available precursors by the methods reported in literature. Different analogs (1-46) of these hits identified in dry lab were synthesized in wet lab.
2.1.8. Binding mode analysis of novel IL-2 inhibitors

Using the previously presented VS strategy, forty six novel and potent IL-2 inhibitors were identified (Table 2.3) to explore the binding behavior of compounds (1-46) with amino acid residues of the binding pocket of 1Z92. All compounds of the library were successfully docked in the active site of 1Z92 as shown in Figure 2.8.
Figure 2.7. Chemical structures of top hits retrieved from PBVS.

Table 2.3: Structure of 46 novel and potent IL-2 inhibitors

<table>
<thead>
<tr>
<th>No.</th>
<th>Structure</th>
<th>Code</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Structure" /></td>
<td><img src="image2" alt="Code" /></td>
<td><img src="image3" alt="Structure" /></td>
</tr>
<tr>
<td>2</td>
<td><img src="image4" alt="Structure" /></td>
<td><img src="image5" alt="Code" /></td>
<td><img src="image6" alt="Structure" /></td>
</tr>
<tr>
<td>3</td>
<td><img src="image7" alt="Structure" /></td>
<td><img src="image8" alt="Code" /></td>
<td><img src="image9" alt="Structure" /></td>
</tr>
<tr>
<td>4</td>
<td><img src="image10" alt="Structure" /></td>
<td><img src="image11" alt="Code" /></td>
<td><img src="image12" alt="Structure" /></td>
</tr>
<tr>
<td>5</td>
<td><img src="image13" alt="Structure" /></td>
<td><img src="image14" alt="Code" /></td>
<td><img src="image15" alt="Structure" /></td>
</tr>
<tr>
<td>6</td>
<td><img src="image16" alt="Structure" /></td>
<td><img src="image17" alt="Code" /></td>
<td><img src="image18" alt="Structure" /></td>
</tr>
</tbody>
</table>

Continued
<table>
<thead>
<tr>
<th>7</th>
<th><img src="image" alt="Chemical Structure 7" /></th>
<th>8</th>
<th><img src="image" alt="Chemical Structure 8" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td><img src="image" alt="Chemical Structure 9" /></td>
<td>10</td>
<td><img src="image" alt="Chemical Structure 10" /></td>
</tr>
<tr>
<td>11</td>
<td><img src="image" alt="Chemical Structure 11" /></td>
<td>12</td>
<td><img src="image" alt="Chemical Structure 12" /></td>
</tr>
<tr>
<td>13</td>
<td><img src="image" alt="Chemical Structure 13" /></td>
<td>14</td>
<td><img src="image" alt="Chemical Structure 14" /></td>
</tr>
<tr>
<td>15</td>
<td><img src="image" alt="Chemical Structure 15" /></td>
<td>16</td>
<td><img src="image" alt="Chemical Structure 16" /></td>
</tr>
<tr>
<td>17</td>
<td><img src="image" alt="Chemical Structure 17" /></td>
<td>18</td>
<td><img src="image" alt="Chemical Structure 18" /></td>
</tr>
<tr>
<td>19</td>
<td><img src="image" alt="Chemical Structure 19" /></td>
<td>20</td>
<td><img src="image" alt="Chemical Structure 20" /></td>
</tr>
</tbody>
</table>

Continued
<table>
<thead>
<tr>
<th></th>
<th><img src="image" alt="Chemical Structure 21" /></th>
<th><img src="image" alt="Chemical Structure 22" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td><img src="image" alt="Chemical Structure 23" /></td>
<td><img src="image" alt="Chemical Structure 24" /></td>
</tr>
<tr>
<td>23</td>
<td><img src="image" alt="Chemical Structure 25" /></td>
<td><img src="image" alt="Chemical Structure 26" /></td>
</tr>
<tr>
<td>25</td>
<td><img src="image" alt="Chemical Structure 27" /></td>
<td><img src="image" alt="Chemical Structure 28" /></td>
</tr>
<tr>
<td>27</td>
<td><img src="image" alt="Chemical Structure 29" /></td>
<td><img src="image" alt="Chemical Structure 30" /></td>
</tr>
<tr>
<td>29</td>
<td><img src="image" alt="Chemical Structure 31" /></td>
<td><img src="image" alt="Chemical Structure 32" /></td>
</tr>
<tr>
<td>31</td>
<td><img src="image" alt="Chemical Structure 33" /></td>
<td><img src="image" alt="Chemical Structure 34" /></td>
</tr>
</tbody>
</table>

**Continued**
<table>
<thead>
<tr>
<th></th>
<th><img src="image1.png" alt="Chemical Structure 35" /></th>
<th><img src="image2.png" alt="Chemical Structure 36" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td><img src="image3.png" alt="Chemical Structure 37" /></td>
<td><img src="image4.png" alt="Chemical Structure 38" /></td>
</tr>
<tr>
<td>37</td>
<td><img src="image5.png" alt="Chemical Structure 39" /></td>
<td><img src="image6.png" alt="Chemical Structure 40" /></td>
</tr>
<tr>
<td>39</td>
<td><img src="image7.png" alt="Chemical Structure 41" /></td>
<td><img src="image8.png" alt="Chemical Structure 42" /></td>
</tr>
<tr>
<td>41</td>
<td><img src="image9.png" alt="Chemical Structure 43" /></td>
<td><img src="image10.png" alt="Chemical Structure 44" /></td>
</tr>
<tr>
<td>43</td>
<td><img src="image11.png" alt="Chemical Structure 45" /></td>
<td><img src="image12.png" alt="Chemical Structure 46" /></td>
</tr>
</tbody>
</table>
Some of the significant binding interactions such as hydrogen bonding, \(\pi-\pi\) interactions and hydrophobic interactions between different ligand-receptor complexes were observed. Amino acid residues Ile28, Leu36, Val69 and Phe42 were found having hydrophobic interactions. Hydrogen bonding interactions were observed with amino acid residues Phe42, Glu62, Pro63, Val69, Asn71 and Leu72. In addition to both these interactions strong \(\pi-\pi\) interactions were also observed with Phe42 and Tyr43 in close vicinity of docked complexes as shown in Table 2.4.

![Image](image1.png)

**Figure 2.8.** Superimposed view of pharmacophore based docked view of some compounds 9 (dihydropyrimidine), 22 (pyrazole) & 45 (amide) in the active site of 1Z92.

All interacting amino acids are shown in red color.

The binding mode of chalcone is shown in **Figure 2.9**. The ligand-receptor complex was stabilized by hydrogen bonding and \(\pi-\pi\) interactions. A hydrogen bond interaction was observed between the hydroxyl hydrogen of chalcone and amino acid residue Leu72 at a distance of 2.94 Å. The complex was further stabilized by the \(\pi-\pi\) interaction between phenyl rings of compound with Phe42 as shown in **Figure 2.10**.

The details of the binding modes of chalcones (1-7) are shown in **Table 2.4**. The strongest complex was formed between chalcone 7 and IL-2/IL-2R as evidence from its lowest dG value (-10.30 kcal/mol).
### Table 2.4: Binding interactions observed in chalcones (1-7) with 1Z92.

<table>
<thead>
<tr>
<th>No.</th>
<th>H-bonding interaction</th>
<th>Distance (Å)</th>
<th>Amino acid</th>
<th>Amino acid</th>
<th>π-π interaction</th>
<th>Hydrophobic interaction</th>
<th>London dG kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>Tyr43</td>
<td>Leu72</td>
<td>-</td>
<td></td>
<td>-7.613</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>2.92</td>
<td>Phe42</td>
<td>Phe42</td>
<td>2.92</td>
<td>Phe42,Met39</td>
<td>-8.766</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>3.08</td>
<td>Leu72</td>
<td>Phe42</td>
<td>3.08</td>
<td>Leu72</td>
<td>-8.936</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>2.71</td>
<td>Tyr43</td>
<td>Phe42</td>
<td>2.71</td>
<td>Ile28</td>
<td>-7.436</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>2.94</td>
<td>Leu72</td>
<td>Phe42</td>
<td>2.94</td>
<td>Val69</td>
<td>-6.662</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>1.83</td>
<td>Pro63</td>
<td>Phe42</td>
<td>1.83</td>
<td>Leu72,Val69</td>
<td>-9.486</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>2.81</td>
<td>Tyr43</td>
<td>Phe42</td>
<td>2.81</td>
<td>Leu72,Val69</td>
<td>-10.308</td>
</tr>
</tbody>
</table>

Figure 2.9. The binding mode analysis of chalcone 5
Hydrogen bonding (blue) π-π interactions (green). A) 3D and B) 2D docked view
Different azaheterocycles (8-28) (dihydropyrimidines, benzothiazepines and pyrazoles) derived from synthesized 3 different chalcones were also docked in the active site of 1Z92. **Figure 2.10** depicts the preferred docked orientation of compound 22 (pyrazole) in the binding cavity of receptor. The key residues have been highlighted in **Table 2.5**. The carbonyl group of pyrazole is involved in H-bonding with Arg38 at distance of 2.7Å. Phenyl ring was involved in π-π interactions.

**Table 2.5: Binding interactions observed in heterocycles derived from chalcones (8-28) with 1Z92.**

<table>
<thead>
<tr>
<th>No.</th>
<th>H-bonding interaction</th>
<th>π-π interaction</th>
<th>Hydrophobic interaction</th>
<th>London dG kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distance Å</td>
<td>Amino acid</td>
<td>Amino acid</td>
<td>Amino acid</td>
</tr>
<tr>
<td>Dihydropyrimidines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2.98</td>
<td>Phe42</td>
<td>Tyr43</td>
<td>Leu36</td>
</tr>
<tr>
<td>9</td>
<td>3.07</td>
<td>Glu62</td>
<td>Phe42</td>
<td>Ile28</td>
</tr>
<tr>
<td>10</td>
<td>3.12</td>
<td>Val69</td>
<td>Phe42</td>
<td>Phe42, Val69</td>
</tr>
<tr>
<td>11</td>
<td>2.00</td>
<td>Asn71</td>
<td>His120</td>
<td>Leu36</td>
</tr>
<tr>
<td>12</td>
<td>2.21</td>
<td>Phe42</td>
<td>Tyr43</td>
<td>Leu36</td>
</tr>
<tr>
<td>13</td>
<td>3.71</td>
<td>Val69</td>
<td>Phe42</td>
<td>Leu72, Val69</td>
</tr>
<tr>
<td>Heteroazepines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1.92</td>
<td>Val69</td>
<td>Phe42</td>
<td>Ile28</td>
</tr>
<tr>
<td>15</td>
<td>1.89</td>
<td>Pro63</td>
<td>His120</td>
<td>Val69</td>
</tr>
<tr>
<td>16</td>
<td>2.11</td>
<td>Glu62</td>
<td>Tyr43</td>
<td>Leu72</td>
</tr>
<tr>
<td>17</td>
<td>2.14</td>
<td>Glu62</td>
<td>Tyr43</td>
<td>Leu72, Leu36</td>
</tr>
<tr>
<td>18</td>
<td>3.72</td>
<td>Asn71</td>
<td>-</td>
<td>Phe42</td>
</tr>
<tr>
<td>19</td>
<td>2.01</td>
<td>Pro63</td>
<td>Phe42</td>
<td>Phe42</td>
</tr>
<tr>
<td>Pyrazoles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.99</td>
<td>Phe42</td>
<td>Tyr43</td>
<td>Phe42</td>
</tr>
<tr>
<td>21</td>
<td>1.96</td>
<td>Asn71</td>
<td>Tyr43</td>
<td>Leu36</td>
</tr>
<tr>
<td>22</td>
<td>2.7</td>
<td>Arg38</td>
<td>His120</td>
<td>Leu36</td>
</tr>
<tr>
<td>23</td>
<td>2.15</td>
<td>Pro63</td>
<td>Tyr43</td>
<td>Val69</td>
</tr>
<tr>
<td>24</td>
<td>2.36</td>
<td>Phe42</td>
<td>Phe42</td>
<td>Val69</td>
</tr>
<tr>
<td>25</td>
<td>2.00</td>
<td>Phe42</td>
<td>-</td>
<td>Val69</td>
</tr>
<tr>
<td>26</td>
<td>3.05</td>
<td>Phe42</td>
<td>Phe42</td>
<td>Val69</td>
</tr>
<tr>
<td>27</td>
<td>2.61, 1.95</td>
<td>Tyr43, Val69</td>
<td>-</td>
<td>Val69, Phe42</td>
</tr>
<tr>
<td>28</td>
<td>3.28</td>
<td>Glu62</td>
<td>Tyr43</td>
<td>Ile28</td>
</tr>
</tbody>
</table>

As a generalization, chalcones show stronger binding with 1Z92 compared to the derived dihydropyrimidines as reflected from their lower dG values (**Table 2.5**). Different classes of heterocycles (dihydropyrimidines, benzimidazoles, pyrazoles) were better in interacting with residues of the active site of 1Z92.
Figure 2.10. The binding mode analysis of pyrazole 22, Hydrogen bonds and $\pi-\pi$ interactions are displayed in green dotted lines. A) 3D and B) 2D docked view

Generally speaking, the residues around the docked compounds anthraquinone sulfonamides (29-36) remain almost the same in all compounds studied and the residues Phe42, Glu62, Pro63, Glu67, Val69, Asn71 and Leu72 located in the binding pocket may have catalytic role in inhibitory mechanism (Table 2.6). It may be seen the docking pattern of compound 35 (benzimidazole) in Figure 2.11. Its ability to interact with the binding pocket through H-bonding
interactions (Val69, Glu67) and $\pi$-$\pi$ Interactions (Phe42) with several residues including catalytic triad may account for the greater stability of the ligand-receptor complex.

Table 2.6: Binding interactions observed in benzimidazoles and anthraquinone sulfonamides (29-36) with 1Z92.

<table>
<thead>
<tr>
<th>No.</th>
<th>H-bonding interaction</th>
<th>$\pi$-$\pi$ interaction</th>
<th>Hydrophobic interaction</th>
<th>London dG kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distance Å</td>
<td>Amino acid</td>
<td>Amino acid</td>
<td>Amino acid</td>
</tr>
<tr>
<td><strong>Benzimidazoles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>2.86</td>
<td>Pro63</td>
<td>Tyr43</td>
<td>Phe42</td>
</tr>
<tr>
<td>30</td>
<td>2.93</td>
<td>Glu62</td>
<td>Phe42</td>
<td>Phe42</td>
</tr>
<tr>
<td>31</td>
<td>1.99</td>
<td>Glu62</td>
<td>Phe42</td>
<td>Phe42</td>
</tr>
<tr>
<td>32</td>
<td>1.05</td>
<td>Glu67</td>
<td>Phe42</td>
<td>Ile28</td>
</tr>
<tr>
<td><strong>Anthraquinone sulfonamides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>2.20</td>
<td>Pro63</td>
<td>-</td>
<td>Leu36</td>
</tr>
<tr>
<td>34</td>
<td>2.16</td>
<td>Pro63</td>
<td>Tyr43</td>
<td>Phe42</td>
</tr>
<tr>
<td>35</td>
<td>2.29, 2.94</td>
<td>Val69, Tyr43</td>
<td>Phe42</td>
<td>Phe42</td>
</tr>
<tr>
<td>36</td>
<td>2.19</td>
<td>Pro63</td>
<td>-</td>
<td>Leu72</td>
</tr>
</tbody>
</table>

Table 2.7 shows the interactions between Schiff bases (37-41) and receptor complex which is stabilized by strong hydrogen bonding and hydrophobic interactions. The observed binding mode of Schiff base 37 reveals H-bonding interactions between the carbonyl oxygen and hydroxyl group of Schiff bases with the hydroxyl group of Tyr43 at 2.61 Å and with their backbone carbonyl oxygen of Val69 at 1.95 Å, respectively as shown in Figure 2.12.
Figure 2.11. The binding mode analysis of anthraquinone sulfonamide 35, Hydrogen bonds and distances are displayed in blue dashed line and π-π interactions are in green color.

A) 3D and B) 2D docked view
Table 2.7: Binding interactions observed in Schiff bases (37-41) with 1Z92.

<table>
<thead>
<tr>
<th>No.</th>
<th>H-bonding interactions</th>
<th>π-π interactions</th>
<th>Hydrophobic interactions</th>
<th>London dG kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distance Å</td>
<td>Amino acid</td>
<td>Amino acid</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>1.95, 2.61</td>
<td>Tyr43</td>
<td>Val69</td>
<td>-8.271</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Val69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>1.93</td>
<td>Phe42</td>
<td>Tyr43</td>
<td>-9.328</td>
</tr>
<tr>
<td>39</td>
<td>2.16</td>
<td>Glu62</td>
<td>Val69</td>
<td>-7.299</td>
</tr>
<tr>
<td>40</td>
<td>2.05</td>
<td>Glu62</td>
<td>Phe42</td>
<td>-7.336</td>
</tr>
<tr>
<td>41</td>
<td>2.68</td>
<td>Glu62</td>
<td>Tyr43</td>
<td>-6.321</td>
</tr>
</tbody>
</table>

Figure 2.12. Binding mode analysis of Schiff base 37 A) 3D B) 2D
Table 2.8 shows the binding pattern of screened amides (42-46) as observed in almost all pharmacophore based virtually. In summary, the binding modes of these compounds reveal that each one is forming at least one interaction with amino acid residues Phe42, Tyr43, Pro63, Leu72, thereby stabilizing the ligand-target complexes.

Table 2.8: Binding interactions observed in pyrazoles and benzamides (42-46) with 1Z92.

<table>
<thead>
<tr>
<th>No.</th>
<th>H-bonding interaction</th>
<th>π-π interaction</th>
<th>Hydrophobic interaction</th>
<th>London dG kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distance Å</td>
<td>Amino acid</td>
<td>Amino acid</td>
<td>Amino acid</td>
</tr>
<tr>
<td>Pyrazoles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>1.95</td>
<td>Glu62</td>
<td>Tyr43</td>
<td>Leu72, Val69</td>
</tr>
<tr>
<td>43</td>
<td>2.19</td>
<td>Phe42</td>
<td>Tyr43</td>
<td>Ile28</td>
</tr>
<tr>
<td>Amides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>3.02</td>
<td>Leu72</td>
<td>Tyr43</td>
<td>Val69</td>
</tr>
<tr>
<td>45</td>
<td>2.24</td>
<td>Leu72</td>
<td>Tyr43</td>
<td>Leu36</td>
</tr>
<tr>
<td>46</td>
<td>2.63, Pro63</td>
<td>Phe42</td>
<td>Ile28</td>
<td></td>
</tr>
</tbody>
</table>

The drug-likeness of all the analogs of identified hits was evaluated by calculating their properties using MOE suit, these properties include molecular weight, hydrophobicity (logP), Hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), and number of rotatable bond (NOR) complete with full and short form and are embedded in Lipinski’s RO5 (Table 2.9) indicates that all these compounds follow RO5 and may serve as potential drug candidates. All these compounds comply cut off limits of 500, for mol.wt less than 500 except compound 36 while logP less than 5 with few exceptions. As far as hydrogen bond donating and accepting capability, all compounds follow the cut off limits of 5 and 10 respectively. The same is true for NOR (less than 10) without any exception. These results are evidence that these analogs of hits to be synthesized may serve as potential drug candidates.
Table 2.9: Lipinski’s RO5 compliance of the synthesized compounds (1-46)

<table>
<thead>
<tr>
<th>No.</th>
<th>M.wt</th>
<th>logP</th>
<th>HBA</th>
<th>HBD</th>
<th>NOR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chalcones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>208.26</td>
<td>3.58</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>236.25</td>
<td>3.72</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>242.70</td>
<td>4.23</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>287.15</td>
<td>4.34</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>290.25</td>
<td>5.10</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>240.25</td>
<td>2.99</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>257.27</td>
<td>3.41</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Dihydropyrimidines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>317.36</td>
<td>3.20</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>298.36</td>
<td>3.01</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>315.82</td>
<td>3.82</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>311.29</td>
<td>3.03</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>282.29</td>
<td>2.83</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>299.76</td>
<td>3.66</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Heteroazepines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>347.12</td>
<td>5.16</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>359.13</td>
<td>5.07</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>330.14</td>
<td>4.87</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>368.08</td>
<td>6.37</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>375.09</td>
<td>5.75</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>347.10</td>
<td>5.55</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Prazoles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>311.09</td>
<td>2.70</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>21</td>
<td>292.10</td>
<td>2.50</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>22</td>
<td>299.08</td>
<td>3.52</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>23</td>
<td>300.03</td>
<td>3.98</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>24</td>
<td>304.13</td>
<td>4.47</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>25</td>
<td>342.04</td>
<td>4.27</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>26</td>
<td>281.10</td>
<td>3.17</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>27</td>
<td>299.09</td>
<td>3.31</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>28</td>
<td>315.06</td>
<td>3.83</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Benzoimidazoles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>278.18</td>
<td>4.91</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>30</td>
<td>460.20</td>
<td>5.50</td>
<td>5</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>31</td>
<td>282.11</td>
<td>3.20</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>32</td>
<td>237.13</td>
<td>3.29</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sulfanamides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>507.13</td>
<td>4.83</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>34</td>
<td>552.11</td>
<td>4.74</td>
<td>5</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>35</td>
<td>548.18</td>
<td>6.19</td>
<td>5</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>36</td>
<td>710.20</td>
<td>6.98</td>
<td>8</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Schiff bases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>446.40</td>
<td>4.37</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>38</td>
<td>450.12</td>
<td>6.24</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>39</td>
<td>318.10</td>
<td>4.86</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>40</td>
<td>412.19</td>
<td>8.16</td>
<td>2</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>41</td>
<td>496.01</td>
<td>9.05</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>42</td>
<td>410.14</td>
<td>5.01</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>43</td>
<td>401.15</td>
<td>4.90</td>
<td>6</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Benzamides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>311.19</td>
<td>5.78</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>45</td>
<td>513.22</td>
<td>5.95</td>
<td>4</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>46</td>
<td>208.12</td>
<td>3.27</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
STEP 2 (Wet lab)

2.2. Synthesis of analogs of different heterocycles identified from PBVS

Chalcone derivatives, natural or synthetic, exhibit a variety of biological activities and therefore have been used as precursors for pharmacological activities such as anticancer agents\textsuperscript{114,115}, antimalarial\textsuperscript{116,117}, antimicrobial\textsuperscript{118,119}, anti-inflammatory\textsuperscript{120}, anti-protozoal\textsuperscript{121}, antioxidant\textsuperscript{122}. In addition, dihydropyrimidines and its derivatives are powerful antibacterial\textsuperscript{127} and anticancer\textsuperscript{128} agents. Similarly benzimidazoles also possess several bioactivities such as antiviral\textsuperscript{155}, anthelmintic\textsuperscript{156}, HIV inhibitors\textsuperscript{157}, antihypertensive\textsuperscript{158}, anti-inflammatory\textsuperscript{159}, analgesic\textsuperscript{160}. Some pyrazole derivatives have been shown to possess antitumor\textsuperscript{188}, antitubercular\textsuperscript{189}, anticonvulsant\textsuperscript{190,191}, antihepatotoxic\textsuperscript{192}, anti-inflammatory\textsuperscript{193}, antimicrobial\textsuperscript{194}, antibacterial\textsuperscript{195}, antioxidant\textsuperscript{196} etc. In continuation of ongoing chemistry research and \textit{in silico} studies on these classes of compounds, some optimized hits were synthesized for IL-2 inhibition studies.

2.2.1. Synthesis of chalcones (1-7)

One of the important hits identified in dry lab is an interesting scaffold i.e chalcone (H-11) which, alongwith 6 analogs was synthesized.

![Chemical structure of chalcone (H-11)](https://example.com/chalcone_structure.png)

The synthesis of chalcones (1-7) was carried out by simply adding an ice cold solution of 4 M NaOH in water into the mixture of benzaldehyde and acetophenone\textsuperscript{214}. Then the mixture was stirred at room temperature and the crude product was then purified and recrystallized with ethanol (Scheme 2.1). The physical data of all synthesized chalcone derivatives (1-7) is given in Table 2.9.

<table>
<thead>
<tr>
<th>No.</th>
<th>R\textsubscript{1}</th>
<th>R\textsubscript{2}</th>
<th>No.</th>
<th>R\textsubscript{1}</th>
<th>R\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>H</td>
<td>5</td>
<td>3-OH</td>
<td>3-NO\textsubscript{2}</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>4-F</td>
<td>6</td>
<td>3-OH</td>
<td>2-OH</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>4-Cl</td>
<td>7</td>
<td>4-NH\textsubscript{2}</td>
<td>3-Cl</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>4-Br</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Scheme 2.1: Synthesis of chalcones (1-7)

2.2.1.1. Characterization

FTIR spectral data of the chalcones (1-7) exhibited the characteristic C=O absorption at 1714-1695 cm\(^{-1}\). All of the synthesized compounds were characterized by \(^1\)H NMR, \(^{13}\)C NMR, and they gave satisfactory analytical and spectroscopic data. Physical data of chalcones is given in Table 2.10.

<table>
<thead>
<tr>
<th>No.</th>
<th>m.p(°C)</th>
<th>Yield (%)</th>
<th>(R_f)*</th>
<th>No.</th>
<th>m.p(°C)</th>
<th>Yield (%)</th>
<th>(R_f)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>71(^{215})</td>
<td>71</td>
<td>0.60</td>
<td>5</td>
<td>75(^{54})</td>
<td>75</td>
<td>0.65</td>
</tr>
<tr>
<td>2</td>
<td>76(^{215})</td>
<td>76</td>
<td>0.71</td>
<td>6</td>
<td>61(^{54})</td>
<td>61</td>
<td>0.68</td>
</tr>
<tr>
<td>3</td>
<td>69(^{215})</td>
<td>69</td>
<td>0.62</td>
<td>7</td>
<td>64(^{214})</td>
<td>64</td>
<td>0.59</td>
</tr>
<tr>
<td>4</td>
<td>73(^{214})</td>
<td>73</td>
<td>0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(*\)Solvent system. Ethyl acetate: Pet ether (1:4)

\(^1\)H NMR spectra showed the characteristic signal appearing in the range of \(\delta 7.41-7.96\) ppm assigned as doublet for CH protons with coupling constant of \(J=7.0\) Hz. In case of compounds 5 and 6, a characteristic singlet for one proton of OH appeared at \(\delta 9.12\) ppm and 9.23 ppm respectively, while in case of compound 7, a singlet for two protons at \(\delta 4.82\) ppm has been assigned to NH\(_2\). In \(^{13}\)C NMR spectra, a signal for carbonyl carbon appeared in the range of \(\delta 172.40-188.30\) ppm and a signal for CH carbon was observed ranging from \(\delta 135.20-147.07\) ppm in different chalcones.

2.2.2. Synthesis of dihydropyrimidines (8-13)

Dihydropyrimidines (H-10) was also found to be important hit in dry lab and some of its derivatives were synthesized in wet lab.

The synthesis of dihydropyrimidine derivatives (8-13) was achieved by reacting the synthesized chalcones (5-7) with urea or thiourea in the presence of NaOH and ethanol (Biginelli
reaction\textsuperscript{216}. The reaction was monitored by TLC and product was purified and recrystallized with ethanol (Scheme 2.2).

\begin{equation}
\begin{align*}
\text{R} & \quad \text{O} \\
\text{(5-7)} & \\
\text{+} & \\
\text{H}_2\text{N} & \quad \text{X} \\
\text{NH}_2 & \\
\text{NaOH, EtOH} & \\
\rightarrow & \\
\text{R} & \quad \text{N} & \quad \text{X} \\
\equiv & \\
\text{NH} & \quad \text{S} & \text{O} \\
\end{align*}
\end{equation}

<table>
<thead>
<tr>
<th>No.</th>
<th>R\textsubscript{1}</th>
<th>R\textsubscript{2}</th>
<th>X</th>
<th>No.</th>
<th>R\textsubscript{1}</th>
<th>R\textsubscript{2}</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>3-OH</td>
<td>3-NO\textsubscript{2}</td>
<td>S</td>
<td>11</td>
<td>3-OH</td>
<td>3-NO\textsubscript{2}</td>
<td>O</td>
</tr>
<tr>
<td>9</td>
<td>3-OH</td>
<td>2-OH</td>
<td>S</td>
<td>12</td>
<td>3-OH</td>
<td>2-OH</td>
<td>O</td>
</tr>
<tr>
<td>10</td>
<td>4-NH\textsubscript{2}</td>
<td>3-Cl</td>
<td>S</td>
<td>13</td>
<td>4-NH\textsubscript{2}</td>
<td>3-Cl</td>
<td>O</td>
</tr>
</tbody>
</table>

**Scheme 2.2:** Synthesis of dihydropyrimidines (8-13)

2.2.2.1. Characterization

All the synthesized dihydropyrimidine derivatives (8-13) were obtained in good yield in the range of 56-79 % and they were characterized by their physical constants and spectroscopic data. Melting points of all compounds were recorded and found in the range of 148-161 °C.

$^1$H NMR (DMSO-\textit{d}_6) showed characteristic broad signal for one proton of NH as singlet ranging from δ 8.42-10.85 ppm and another signal \textit{m} appearing at δ 1.21-2.82 ppm was assigned to CH\textsubscript{2} group. The dd for one proton of CH group appeared in the range of δ 3.71-3.96 ppm. In $^{13}$C NMR, the most deshielded carbon appeared at δ 184.94-185.83 ppm, which was assigned to carbon of C=S group and the deshielded carbon of C=O was observed at δ 166.98-168.48 ppm (Annexure 1 for $^1$H and $^{13}$CNMR spectra of compound 10). GCMS spectra of dihydropyrimidines (8-13) were recorded. Molecular ion peak was observed in all the compounds, but this peak did not correspond to the base peak. In compounds containing a Cl or Br atom, in addition to M$^{+}$ ion peak, M$^{+}$+2 peak was observed due to isotopic natural abundance of $^{37}$Cl and $^{81}$Br (Annexure 2 for GCMS of compound 12). Physiochemical and spectral data of all synthesized dihydropyrimidine derivatives (8-13) is given in Table 2.11.
Table 2.1: Yield, m.p and spectral data of synthesized dihydropyrimidine derivatives (8-13)

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield (%)</th>
<th>m.p (°C)</th>
<th>(^1)H NMR</th>
<th>(^13)C NMR</th>
<th>m/z (M^+ )</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>61</td>
<td>157-8</td>
<td>1.91 2H m</td>
<td>-CH_2</td>
<td>54.5 45.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.96 1H dd</td>
<td>-CH</td>
<td>165.2 185.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.42 1H s</td>
<td>-NH</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.22 1H s</td>
<td>-OH</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>66</td>
<td>150-1(^{217})</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>69</td>
<td>163-5</td>
<td>2.21 2H m</td>
<td>-CH_2</td>
<td>41.5 40.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.77 1H dd</td>
<td>-CH</td>
<td>166.4 315</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.53 2H s</td>
<td>-NH_2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.18 1H s</td>
<td>-NH</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>76</td>
<td>148-50(^{217})</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>74</td>
<td>136-8(^{217})</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>67</td>
<td>150-3(^{217})</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

2.2.3. Synthesis of benzoheteroazepines (14-19)

During pharmacophore identification of hits, some 7-member benzoazepine (H-8) was also identified and its derivatives benzodiazepines (14-16) and benzothiazepines (17-19) were synthesized by reacting the chalcones (1-7) with \(\alpha\)-phenylenediamine and 2-aminobenzene-thiol respectively in THF\(^{81}\).

![H-8](image)

The resulted filtrate was concentrated under reduced pressure the crude product was recrystallized with MeOH leading to aza heteroazepine derivatives (Scheme 2.3).

![Scheme 2.3](image)

X=NH_2 (14-16) Benzodiazepines
X=SH (17-19) Benzothiazepines
2.2.3.1. Characterization

Characterization of synthesized heteroazepines (14-19) was carried out by FTIR spectra with appearance of C=\( \text{N} \) stretching band at 1538 cm\(^{-1} \) and disappearance of carbonyl absorption bands. The characteristic strong absorption of the NH group appeared at 3412-3428 cm\(^{-1} \). In \(^1\text{H}\) NMR spectra, a singlet peak at \( \delta 8.18-8.92 \) ppm for NH proton in case of compounds (14-16). A dd for CH proton appeared at \( \delta 3.44-3.88 \) ppm and the aromatic protons appeared in the range of \( \delta 6.46-8.48 \) ppm. In \(^{13}\text{C}\) NMR the characteristic peak for CH and CH\(_2\) carbon appeared at \( \delta 40.9-62.6\)ppm and imino carbon (C=\( \text{N} \)) in range of \( \delta 168.70-187.77 \) ppm. LC-MS analysis of heteroazepines (14-19) was performed. All the synthesized compounds (14-19) were characterized by molecular ion peak (M-H)\(^{-} \) (Annexure 3 for LCMS of compound 14 and Annexure 4 for \(^1\text{H}\) and \(^{13}\text{C}\)NMR spectra of compound 15). The physiochemical data of all synthesized 2,3-dihydrobenzazepines (14-19) is given in Table 2.12.

Table 2.12: Yield, m.p and spectral data of synthesized heteroazepines (14-19)

| No. | Yield (%) | m.p(°C) | \( \delta \) (ppm) | Integration | Multiplicity | J (Hz) | Assignment | CH | CH\(_2\) | C=\( \text{N} \) | m/z (M-H) | m/z (M-H)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<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>14</td>
<td>59</td>
<td>97-98</td>
<td>2.49</td>
<td>2H</td>
<td>m</td>
<td>-</td>
<td>-CH(_2)</td>
<td>62.6</td>
<td>40.9</td>
<td>168.6</td>
<td>346</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.88</td>
<td>1H</td>
<td>dd</td>
<td>11.2, 5.4</td>
<td>-CH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.49</td>
<td>2H</td>
<td>s</td>
<td>-</td>
<td>-NH(_2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.80</td>
<td>1H</td>
<td>s</td>
<td>-</td>
<td>-NH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>83</td>
<td>89-92</td>
<td>3.18</td>
<td>2H</td>
<td>m</td>
<td>-</td>
<td>-CH(_2)</td>
<td>59.8</td>
<td>42.0</td>
<td>187.0</td>
<td>358</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.85</td>
<td>1H</td>
<td>dd</td>
<td>12.0, 6.0</td>
<td>-CH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.18</td>
<td>1H</td>
<td>s</td>
<td>-</td>
<td>-NH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.47</td>
<td>1H</td>
<td>s</td>
<td>-</td>
<td>-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>76</td>
<td>98-100(^\text{16})</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>330</td>
</tr>
<tr>
<td>17</td>
<td>79</td>
<td>84-86</td>
<td>2.92</td>
<td>2H</td>
<td>m</td>
<td>-</td>
<td>-CH(_2)</td>
<td>58.7</td>
<td>40.7</td>
<td>169.1</td>
<td>329</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.44</td>
<td>1H</td>
<td>t</td>
<td>7.2</td>
<td>-CH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.45</td>
<td>2H</td>
<td>s</td>
<td>-</td>
<td>-NH(_2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>75</td>
<td>96-98</td>
<td>3.40</td>
<td>2H</td>
<td>m</td>
<td>-</td>
<td>-CH(_2)</td>
<td>58.8</td>
<td>41.7</td>
<td>169.0</td>
<td>363</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.86</td>
<td>1H</td>
<td>t</td>
<td>8.6</td>
<td>-CH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.70</td>
<td>1H</td>
<td>s</td>
<td>-</td>
<td>-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>68</td>
<td>99-101(^\text{16})</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>346</td>
</tr>
</tbody>
</table>
2.2.4. Synthesis of pyrazole carbaldehydes (20-25)

Pyrazole carbaldehyde (H-7) was one of the hit identified in dry lab. Some of its derivatives were synthesized by reacting the corresponding chalcones (5-7) with formohydrazide while compound (23) was synthesized by using chalcone (4) and hydrazine hydrate\textsuperscript{218}.

![Pyrazole Carbaldehyde (H-7)](image)

The pyrazole ethanone derivatives (24-25) were synthesized by reacting the chalcones (1) and (4) with acetohydrazide at reflux temperature in ethanol (Scheme 2.4).

![Scheme 2.4: Synthesis of pyrazole derivatives (20-25)](image)

### Table 2.4: Synthesis of pyrazole derivatives (20-25)

<table>
<thead>
<tr>
<th>No.</th>
<th>R\textsubscript{1}</th>
<th>R\textsubscript{2}</th>
<th>R\textsubscript{3}</th>
<th>No.</th>
<th>R\textsubscript{1}</th>
<th>R\textsubscript{2}</th>
<th>R\textsubscript{3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>3-OH</td>
<td>3-NO\textsubscript{2}</td>
<td>H</td>
<td>23</td>
<td>H</td>
<td>4-Br</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>3-OH</td>
<td>2-OH</td>
<td>H</td>
<td>24</td>
<td>H</td>
<td>H</td>
<td>CH\textsubscript{3}</td>
</tr>
<tr>
<td>22</td>
<td>4-NH\textsubscript{2}</td>
<td>3-Cl</td>
<td>H</td>
<td>25</td>
<td>H</td>
<td>4-Br</td>
<td>CH\textsubscript{3}</td>
</tr>
</tbody>
</table>

2.2.4.1. Characterization

All the synthesized pyrazole derivatives (20-25) were characterized by their \textsuperscript{1}H NMR and \textsuperscript{13}C NMR data. The percentage yield of these compounds was generally good ranging from 59-72 \%.

The \textsuperscript{1}H NMR spectra of compounds (20-25) displayed the characteristic signal for CH proton as dd appearing at δ 3.88-5.54 ppm (J=12.9 and 3.3 ppm) and in case of compounds 24 and 25, a
singlet for three protons of CH₃ group appeared at δ 2.31 and 2.30 ppm respectively. In ¹³C NMR a characteristic peak for carbonyl carbon appeared in range of δ 165.72-167.87 ppm and a peak for imine carbon (C=N) at δ 150.98-154.14 ppm besides the signals for aromatic protons (Annexure 5 for ¹H and ¹³C NMR spectra of compound 25). In the GCMS and LCMS spectra of synthesized compounds (20-25), the molecular ion peak was observed in almost all cases although its intensity was low. The loss of aldehydic and ketonic fragments was a common observation. M+2 was observed in all chloro- and bromo-substituted compounds as a result of natural isotopic abundance (Annexure 6 for LCMS of compound 25). The physiochemical and spectral data of all synthesized pyrazole derivatives (20-25) is given in Table 2.13.

Table 2.13: Yield, m.p and spectral data of synthesized pyrazole derivatives (20-25)

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield (%)</th>
<th>m.p (°C)</th>
<th>¹H NMR</th>
<th>¹³C NMR</th>
<th>m/z (M⁺)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ (ppm)</td>
<td>Integration</td>
<td>Multiplicity</td>
<td>J (Hz)</td>
<td>Assignment</td>
</tr>
<tr>
<td>20</td>
<td>66</td>
<td>112-4</td>
<td>1.95</td>
<td>2H</td>
<td>m</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>3.92</td>
<td>1H</td>
<td>dd</td>
</tr>
<tr>
<td></td>
<td>5.94</td>
<td>1H</td>
<td>s</td>
<td>-</td>
<td>OH</td>
</tr>
<tr>
<td></td>
<td>8.08</td>
<td>1H</td>
<td>s</td>
<td>-</td>
<td>-CHO</td>
</tr>
<tr>
<td>21</td>
<td>65</td>
<td>106-8</td>
<td>2.14</td>
<td>2H</td>
<td>m</td>
</tr>
<tr>
<td></td>
<td>4.07</td>
<td>1H</td>
<td>dd</td>
<td>12.3, 6.3</td>
<td>-CH</td>
</tr>
<tr>
<td></td>
<td>4.86</td>
<td>2H</td>
<td>s</td>
<td>-</td>
<td>-2OH</td>
</tr>
<tr>
<td></td>
<td>8.93</td>
<td>1H</td>
<td>s</td>
<td>-</td>
<td>-CHO</td>
</tr>
<tr>
<td>22</td>
<td>69</td>
<td>109-11</td>
<td>2.01</td>
<td>2H</td>
<td>m</td>
</tr>
<tr>
<td></td>
<td>3.88</td>
<td>1H</td>
<td>dd</td>
<td>11.4, 5.1</td>
<td>-CH</td>
</tr>
<tr>
<td></td>
<td>5.49</td>
<td>2H</td>
<td>s</td>
<td>-</td>
<td>-NH₂</td>
</tr>
<tr>
<td></td>
<td>8.95</td>
<td>1H</td>
<td>s</td>
<td>-</td>
<td>-CHO</td>
</tr>
<tr>
<td>23</td>
<td>72</td>
<td>98-100</td>
<td>3.16</td>
<td>1H</td>
<td>dd</td>
</tr>
<tr>
<td></td>
<td>3.92</td>
<td>1H</td>
<td>dd</td>
<td>18.0, 11.4</td>
<td>H-3b</td>
</tr>
<tr>
<td></td>
<td>5.94</td>
<td>1H</td>
<td>dd</td>
<td>3.3, 11.4</td>
<td>H-2</td>
</tr>
<tr>
<td>24</td>
<td>70</td>
<td>102-3°H</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>69</td>
<td>106-8</td>
<td>2.30</td>
<td>-</td>
<td>s</td>
</tr>
<tr>
<td></td>
<td>3.17</td>
<td>-</td>
<td>dd</td>
<td>18.3, 4.8</td>
<td>H-3a</td>
</tr>
<tr>
<td></td>
<td>3.87</td>
<td>-</td>
<td>dd</td>
<td>18.0, 12.0</td>
<td>H-3b</td>
</tr>
<tr>
<td></td>
<td>5.54</td>
<td>-</td>
<td>dd</td>
<td>11.7, 4.5</td>
<td>H-2</td>
</tr>
<tr>
<td></td>
<td>8.95</td>
<td>1H</td>
<td>s</td>
<td>-</td>
<td>-CHO</td>
</tr>
</tbody>
</table>
2.2.5. Synthesis of pyrazole carbothioamides (26-28)

Pyrazole carbothioamides (26-28) were synthesized by reacting the corresponding chalcones with thiosemicarbazide and refluxing the mixture in ethanol\(^{218}\) as shown in Scheme 2.5. The product was then purified and recrystallized.

![Scheme 2.5: Synthesis of pyrazole carbothioamides (26-28)](image)

<table>
<thead>
<tr>
<th>No.</th>
<th>(R_1)</th>
<th>(R_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>27</td>
<td>H</td>
<td>4-F</td>
</tr>
<tr>
<td>28</td>
<td>H</td>
<td>4-Cl</td>
</tr>
</tbody>
</table>

2.2.5.1. Characterization

The synthesized pyrazole carbothioamides (26-28) were obtained in good yield ranging from 68-72%. \(^1\)H NMR spectra of compounds (26-28) showed the characteristic peak for CH proton as dd appeared at \(\delta \) 5.93 ppm. In \(^{13}\)C NMR a characteristic peak for thionyl carbon appeared in range of \(\delta \) 176.58-178.18 ppm and a peak for imine carbon (C=N) at \(\delta \) 154.02-155.85 ppm (Annexure 7 for \(^1\)H and \(^{13}\)CNMR spectra of compound 28). LC-MS analysis of synthesized compounds (26-28) was also performed. For all compounds [M+H]\(^+\) peak was observed. The physiochemical and spectral data of all synthesized pyrazole carbothioamides (26-28) is given in Table 2.14.
Table 2.14: Yield, m.p and spectral data of synthesized pyrazolecarbothioamides (26-28)

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield (%)</th>
<th>m.p (°C)</th>
<th>δ (ppm)</th>
<th>Integration</th>
<th>Multiplicity</th>
<th>J (Hz)</th>
<th>Assignment</th>
<th>CH</th>
<th>CH₂</th>
<th>C=N</th>
<th>C=S</th>
<th>m/z (M+H)⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>71</td>
<td>183-5219</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>H-3a</td>
<td>62.7</td>
<td>42.6</td>
<td>155.3</td>
<td>176.5</td>
<td>281</td>
</tr>
<tr>
<td>27</td>
<td>68</td>
<td>261-5219</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>H-3b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>299</td>
</tr>
<tr>
<td>28</td>
<td>70</td>
<td>183-9</td>
<td>3.18</td>
<td>1H</td>
<td>dd</td>
<td>18.3, 3.6</td>
<td>H-2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>299</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.91</td>
<td>1H</td>
<td>dd</td>
<td>18.3, 11.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>315</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.93</td>
<td>1H</td>
<td>dd</td>
<td>3.6, 11.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>315</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.10</td>
<td>2H</td>
<td>s</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>315</td>
</tr>
</tbody>
</table>

*Solvent system. Ethyl acetate: Pet ether (1:4)

2.2.6. Synthesis of benzimidazoles (29-32)

The benzimidazole (H-3) was one of the important hits found in dry lab.

![H-3]

Some benzimidazole derivatives (29-32) were synthesized according to literature procedure⁵ by reacting an aqueous solution of sodium metabisulfite, different substituted benzoic acid or aldehyde at 0-4 °C. In the next step o-phenylenediamine was added. The mixture was heated at 110 °C in DMF resulted in the formation of benzimidazoles Scheme 2.6).

![Scheme 2.6: Synthesis of benzimidazoles (29-32)]
The ability of carboxylic group in two commercially available drugs namely ibuprofen and cetirizine were also exploited for their conversion to their corresponding amides 31 and 32. Ibuprofen is a NSAID while cetirizine is anti-allergic drug. These drugs were used with an expected new indication as immunomodulating activity.

### 2.2.6.1. Characterization

In spectral analysis of compounds (29-32), $^1$H NMR showed characteristic broad signal for one proton of NH as singlet at δ 11.09-11.36 ppm. $^{13}$C NMR spectral data showed the characteristic peak of carbon (N-C-N) at δ 146.38-154.34 ppm. The physical data of all synthesized benzimidazole (29-32) is given in Table 2.15. Please see the Annexure 8 for GCMS of compound 31.

#### Table 2.15: Yield, m.p and spectral data of anthraquinone sulfonamide derivatives (29-32)

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield (%)</th>
<th>m.p (°C)</th>
<th>$^1$H NMR</th>
<th>$^{13}$C NMR</th>
<th>m/z (M$^+$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>δ (ppm)</td>
<td>J (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Integration</td>
<td>Multiplicity</td>
<td>Assignment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CH</td>
<td>CH$_2$</td>
<td>C=N</td>
</tr>
<tr>
<td>29</td>
<td>70</td>
<td>331-3</td>
<td>1.28</td>
<td>6H</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.57</td>
<td>3H</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.18</td>
<td>1H</td>
<td>m</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.88</td>
<td>1H</td>
<td>q</td>
</tr>
<tr>
<td>30</td>
<td>62</td>
<td>183-4</td>
<td>2.07</td>
<td>4H</td>
<td>m</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.33</td>
<td>4H</td>
<td>m</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.56</td>
<td>2H</td>
<td>m</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.01</td>
<td>2H</td>
<td>s</td>
</tr>
<tr>
<td>31</td>
<td>69</td>
<td>109-11</td>
<td>2.67</td>
<td>6H</td>
<td>m</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7.08</td>
<td>1H</td>
<td>m</td>
</tr>
<tr>
<td>32</td>
<td>68</td>
<td>278-80</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### 2.2.7. Synthesis of anthraquinone sulfonamide derivatives (33-36)

One important hit found in dry lab was (H-13) and its derivatives were synthesized by reacting benzimidazoles (29-32) with freshly prepared anthraquinonesulfonyl chloride yielded their corresponding anthraquinone sulfonamide derivatives (33-36) as shown in Scheme 2.7$^{220}$. 

![Scheme 2.7](image-url)
2.2.7.1. Characterization

The anthraquinone sulfonamide derivatives (33-36) were synthesized in good yield in the range of 60-78% and they were characterized by physical constants and spectral data. Melting points of all these compounds were recorded and found in the range of 251-297°C. $^1$H and $^{13}$CNMR spectra of synthesized compounds (33-36) were recorded and the data is given in Table 2.16 (Annexure 9 for $^1$H and $^{13}$CNMR spectra of compound 36). LCMS analysis of anthraquinone sulfonamide derivatives (33-36) was performed. All the synthesized compounds (33-36) were characterized by molecular ion peak (M+H)$^+$ while base peaks were same for all compounds. The physiochemical and spectral data of all synthesized anthraquinone sulfonamides (33-36) is given in Table 2.16.
Table 2.16: Yield, m.p and spectral data of anthraquinone sulfonamide derivatives (33-36)

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield (%)</th>
<th>m.p (°C)</th>
<th>δ (ppm)</th>
<th>Integration</th>
<th>Multiplicity</th>
<th>J (Hz)</th>
<th>Assignment</th>
<th>CH</th>
<th>CH₂</th>
<th>C=N</th>
<th>C=O</th>
<th>m/z (M+H)^+</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>76</td>
<td>281-2&lt;sup&gt;224&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>508</td>
</tr>
<tr>
<td>34</td>
<td>78</td>
<td>297-8</td>
<td>7.0-8</td>
<td>14H</td>
<td>m</td>
<td>-</td>
<td>Ar-H</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>553</td>
</tr>
<tr>
<td>35</td>
<td>74</td>
<td>289-91</td>
<td>1.28</td>
<td>6H</td>
<td>d</td>
<td>11.7</td>
<td>2CH₃</td>
<td>20.0</td>
<td>22.2</td>
<td>152.3</td>
<td>180.9</td>
<td>549</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.57</td>
<td>3H</td>
<td>d</td>
<td>8.7</td>
<td>-CH₃</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.18</td>
<td>1H</td>
<td>m</td>
<td>-</td>
<td>-CH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>64</td>
<td>273.5</td>
<td>2.07</td>
<td>4H</td>
<td>m</td>
<td>-</td>
<td>-C₄H₄</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>731</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.33</td>
<td>4H</td>
<td>m</td>
<td>-</td>
<td>C₄H₄</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.56</td>
<td>2H</td>
<td>m</td>
<td>-</td>
<td>-CH₂</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.01</td>
<td>2H</td>
<td>s</td>
<td>-</td>
<td>-OCH₂</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.25</td>
<td>1H</td>
<td>s</td>
<td>-</td>
<td>-CH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

2.2.8. Synthesis of Schiff base derivatives (37-41)

Schiff base (H-9) was found one of the important hits and its some derivatives were synthesized in wet lab.

The reaction of aromatic amines with substituted benzaldehydes in the presence of acetic acid and ethanol afforded Schiff base derivatives<sup>221</sup> (37-41) as shown in Scheme 2.8.
2.2.8.1. Characterization

$^1$H NMR showed the singlet for CH proton that appeared in the range of $\delta$ 8.26-8.36 ppm. All aryl protons appeared in the range of $\delta$ 6.34-7.81 ppm. In case of compounds 37 and 39, the signal for one proton of OH appeared at $\delta$ 9.40 and 9.02 ppm respectively. In $^{13}$C NMR the signal for carbonyl carbon was observed at $\delta$ 175.12-178.52 ppm and a signal for imines carbon ($C=\text{N}$) was observed at $\delta$ 162.89-167.12 ppm. LC-MS analysis of synthesized compounds (37-41) was also performed. For all compounds [M+H]$^+$ peak was observed which was also the base peak, data is presented in Table 2.17. LCMS of synthesized compound 39 is presented in Annexure 10. The physiochemical and spectral data of all synthesized Schiff base derivatives (37-41) is given in Table 2.17.

### Table 2.17: Yield, physiochemical and spectral data of synthesized Schiff base derivatives (37-41)

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield (%)</th>
<th>m.p (°C)</th>
<th>$^1$H NMR</th>
<th>$^{13}$C NMR</th>
<th>m/z (M+H)$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\delta$ (ppm)</td>
<td>Integration</td>
<td>Multiplicity</td>
</tr>
</tbody>
</table>
| 37  | 58        | 200-1$^{226}$ | -        | -            | -            | -    | -       | -   | -   | -   | 447
| 38  | 67        | 214-6$^{226}$ | -        | -            | -            | -    | -       | -   | -   | -   | 451
| 39  | 57        | 183-4     | 3.89     | 1H           | d            | 12.8 | -CH     | 119.9| 138.2| 163.8| 318
|     |           |           | 4.73     | 1H           | m            | -    | -CH     |      |      |      |      |
|     |           |           | 6.12     | 1H           | d            | 12.8 | -NCH    |      |      |      |      |
| 40  | 61        | 203-5     | 5.73     | 1H           | t            | 11.7 | -CH     | 113.2| 119.0| 163.7| 413
|     |           |           | 5.90     | 1H           | d            | 12.6 | -CH     |      |      |      |      |
| 41  | 67        | 204-5$^{226}$ | -        | -            | -            | -    | -       | -   | -   | -   | 499

2.2.9. Synthesis of pyrazole derivatives (42-43)

Pyrazole (H-10) was also an important hit found in dry lab and its derivatives were synthesized in wet lab.
The reaction of different amines with acetylacetone in the presence of sodium nitrite and acid yielded their corresponding intermediates, which were then reacted with separately synthesized 8-quinolinoxyacetic acid hydrazide in presence of acetic acid to afford pyrazole derivatives \(^{(42-43)}\) as given in Scheme 2.9.

![Scheme 2.9: Synthesis of pyrazole derivatives (42-43)](image)

### 2.2.9.1. Characterization

FTIR spectral analysis of pyrazoles \((42-43)\) showed the peaks for aliphatic sp\(^3\) hybridized carbons that appeared in the range of 2951-2969 cm\(^{-1}\) and 2839-2859 cm\(^{-1}\). \(^1\)H NMR showed the two singlets for two CH\(_3\) groups appeared at \(\delta\) 1.85-1.90 ppm and \(\delta\) 2.14-2.20 ppm. All aromatic protons appeared in the range of \(\delta\) 7.42-7.97 ppm. In \(^{13}\)C NMR the signals for two CH\(_3\) carbons were observed at \(\delta\) 19.49-19.56 ppm and \(\delta\) 21.31-21.47 ppm and a signal for carbonyl carbon (C=O) was observed at \(\delta\) 168.26-168.75 ppm (Annexure 11 for \(^1\)H and \(^{13}\)CNMR spectra of compound 43). LCMS spectra were recorded for the synthesized compounds \((42-43)\) and characterized by molecular ion peak [M+H]\(^+\), data is given in Table 2.18. The physiochemical data of all synthesized pyrazole derivatives \((42-43)\) is given in Table 2.18.
Table 2.18: Yield, m.p and spectral data of synthesized pyrazole derivatives (42-43)

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield (%)</th>
<th>m.p (°C)</th>
<th>δ (ppm)</th>
<th>Integration</th>
<th>Multiplicity</th>
<th>J (Hz)</th>
<th>Assignment</th>
<th>CH</th>
<th>CH₂</th>
<th>C=N</th>
<th>C=O</th>
<th>m/z (M+H)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>78</td>
<td>199-200</td>
<td>2.51</td>
<td>6H</td>
<td>s</td>
<td>-</td>
<td>2CH₃</td>
<td>19.4</td>
<td>70.9</td>
<td>150.4</td>
<td>168.2</td>
<td>431</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.36</td>
<td>2H</td>
<td>s</td>
<td>-</td>
<td>-CH₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>77</td>
<td>195-6</td>
<td>1.85</td>
<td>3H</td>
<td>s</td>
<td>-</td>
<td>-CH₃</td>
<td>18.2</td>
<td>70.4</td>
<td>158.2</td>
<td>168.1</td>
<td>402</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.35</td>
<td>3H</td>
<td>s</td>
<td>-</td>
<td>-CH₃</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.04</td>
<td>2H</td>
<td>s</td>
<td>-</td>
<td>-CH₂</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.02</td>
<td>1H</td>
<td>s</td>
<td>-</td>
<td>-OH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*Solvent system. Ethyl acetate: Pet ether (1:4)

2.2.10. Synthesis of benzamides (44-46)

One of the important hits found in dry lab was (H-15) benzamide.

![H-15](image)

The synthesis of amide derivatives (44-46) was carried out by reacting naphthyl amine or benzidine with carboxylic acids or a carbonyl group containing drugs ibuprofen and citerazine by using 5-methoxy-2-iodophenylboronic acid (MIBA) as catalyst under mild conditions at ambient temperature in the presence of molecular sieves²²³ (Scheme 2.10).

![Scheme 2.10](image)

<table>
<thead>
<tr>
<th>No.</th>
<th>R</th>
<th>No.</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td></td>
<td>46</td>
<td>[H₃C]COOH</td>
</tr>
<tr>
<td>45</td>
<td>Ph</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scheme 2.10: Synthesis of benzamides (44-46)
A possible catalytic cycle is based on the supposed formation of an acylborate intermediate. MIBA is used to promote direct amidations of carboxylic acids and amines in catalytic amounts and it avoids the requirement of pre-activation of the carboxylic acid or use of coupling reagents. The final products were recrystallized from ethanol.

### 2.2.10.1. Characterization

FTIR spectra of benzamides (44-46) showed the appearance of absorption band at 3256 and 3139 cm\(^{-1}\) for free and associated NH group along with the peak at 1697 cm\(^{-1}\) for carbonyl carbon. \(^1\)H NMR indicated the NH as broad singlet at 8.84-9.31 ppm, while aryl protons appeared in range of 6.82-7.94 ppm and alkyl protons observed in range of 1.20-4.69 ppm. In \(^{13}\)C NMR signal for carbonyl carbon appeared at 169.26 ppm. Aromatic carbons appeared at 113.64-151.38 ppm while alkyl carbons appeared in range of 77.42-61.52 ppm (Annexure 12 for \(^1\)H and \(^{13}\)CNMR spectra of compound 45). The physiochemical and spectral data of all synthesized amides (44-46) is given in Table 2.19.

#### Table 2.19: Yield, m.p and spectral data of synthesized benzamides (44-46)

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield (%)</th>
<th>m.p (°C)</th>
<th>(^1)H NMR</th>
<th>(^{13})C NMR m/z (M+H)+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>δ (ppm)</td>
<td>Integration</td>
</tr>
<tr>
<td>44</td>
<td>81</td>
<td>197-8(^{229})</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>45</td>
<td>79</td>
<td>129-30</td>
<td>2.07</td>
<td>4H</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.34</td>
<td>4H</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.56</td>
<td>2H</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.06</td>
<td>2H</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.54</td>
<td>2H</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.12</td>
<td>1H</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.94</td>
<td>1H</td>
</tr>
<tr>
<td>46</td>
<td>78</td>
<td>316-8(^{230})</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### 2.3. (STEP 3) IL-2 Inhibition assay

All the hits identified in dry lab were successfully synthesized in wet lab and they were characterized with different spectroscopic techniques. The members of the synthesized library (1-46) were subjected to in vitro IL-2 inhibitory activity following the protocol of Manger B. et al.\(^{224}\) IC\(_{50}\) values were calculated in μg/ml and data is reported in Table 2.9 as mean ± SD. All the compounds showed promising IL-2 inhibition activity. Positive and negative control means cultures were performed in parallel in the presence of cyclosporin A (+CsA) or absence of (-CsA) using a concentration of 1mg/ml. Cyclosporin has IC\(_{50}\) value of 0.06 μg/mL.
2.3.1. Chalcones (1-7)

All compounds from this group were found potently inhibiting IL-2 production. Their IC\textsubscript{50} values ranges from <2 to 5.30 µg/mL (Table 2.20), except for compounds 3 and 6, which showed low and moderate level of inhibition. Noteworthy, compound 1 was found to possess the most potent inhibitory activity of this group with IC\textsubscript{50} <2 µg/mL.

Table 2.20: IL-2 inhibitory activities of chalcones (1-7)

<table>
<thead>
<tr>
<th>No.</th>
<th>IL-2 Inhibition IC\textsubscript{50}(µg/mL)</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;2</td>
<td>high</td>
</tr>
<tr>
<td>2</td>
<td>3.31 ± 0.05</td>
<td>high</td>
</tr>
<tr>
<td>3</td>
<td>&gt;50</td>
<td>low</td>
</tr>
<tr>
<td>4</td>
<td>5.30 ± 0.14</td>
<td>high</td>
</tr>
<tr>
<td>5</td>
<td>5.06 ± 0.5</td>
<td>high</td>
</tr>
<tr>
<td>6</td>
<td>25.05 ± 2.6</td>
<td>moderate</td>
</tr>
<tr>
<td>7</td>
<td>2.26 ± 0.01</td>
<td>high</td>
</tr>
</tbody>
</table>

2.3.2. Compounds (Heterocyclic compounds derived from chalcones) 8-28

Among all tested compounds, of this group were found to have strong inhibition on IL-2 production (Table 2.21). Their IC\textsubscript{50} values range from <2 to 8.9 µg/mL. Compound 11 with an IC\textsubscript{50} value of <2 was found to be the most potent inhibitor in this group. Compounds 14, 19,
23 and 27 showed very weak to low level of inhibition their IC$_{50}$ ranges between 41.7 to >50 µg/mL. All other compounds from this group were found to possess moderate inhibition on IL-2 production (IC$_{50}$ ranges from 10.7 to 32.4 µg/mL).

### Table 2.21: IL-2 inhibitory activities of azaheterocycles (8-28)

<table>
<thead>
<tr>
<th>No.</th>
<th>IC$_{50}$ (µg/mL)</th>
<th>Activity</th>
<th>No.</th>
<th>IC$_{50}$ (µg/mL)</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>24.7 ± 0.8</td>
<td>moderate</td>
<td>19</td>
<td>&gt;50</td>
<td>low</td>
</tr>
<tr>
<td>9</td>
<td>14.79 ± 0.4</td>
<td>moderate</td>
<td>20</td>
<td>10.78 ± 4.2</td>
<td>moderate</td>
</tr>
<tr>
<td>10</td>
<td>19.8 ± 0.8</td>
<td>moderate</td>
<td>21</td>
<td>41.75 ± 1.06</td>
<td>moderate</td>
</tr>
<tr>
<td>11</td>
<td>&lt;2</td>
<td>high</td>
<td>22</td>
<td>3.12 ± 0.02</td>
<td>high</td>
</tr>
<tr>
<td>12</td>
<td>8.95 ± 1.2</td>
<td>high</td>
<td>23</td>
<td>&gt;50</td>
<td>low</td>
</tr>
<tr>
<td>13</td>
<td>14.95 ± 1.63</td>
<td>moderate</td>
<td>24</td>
<td>20.92 ± 4.08</td>
<td>moderate</td>
</tr>
<tr>
<td>14</td>
<td>&gt;50</td>
<td>low</td>
<td>25</td>
<td>5.83 ± 0.1</td>
<td>high</td>
</tr>
<tr>
<td>15</td>
<td>11.51 ± 2.4</td>
<td>moderate</td>
<td>26</td>
<td>8.36 ± 0.5</td>
<td>high</td>
</tr>
<tr>
<td>16</td>
<td>5.35 ± 0.21</td>
<td>high</td>
<td>27</td>
<td>&gt;50</td>
<td>low</td>
</tr>
<tr>
<td>17</td>
<td>4.30 ± 0.02</td>
<td>high</td>
<td>28</td>
<td>32.4 ± 10.1</td>
<td>moderate</td>
</tr>
<tr>
<td>18</td>
<td>5.08 ± 0.3</td>
<td>high</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2.14:** Bar graphs for *in vitro* IL-2 inhibition assay of dihydropyrimidines, benzimidazoles, pyrazoles (8-28)
2.3.3. Compounds (Benzimidazoles and anthraquinone sulfonamide derivatives) 29-36

Among all tested compounds of this group were found to exert potent inhibitory activity on IL-2 and their IC$_{50}$ was recorded as 6.1 and 8.9 µg/mL respectively (Table 2.22). Moderate level of inhibition was associated with compound 32 and 36 (IC$_{50}$ 11.5 and 15.4 µg/mL) and the remaining compounds of this group showed very weak inhibitory effect (IC$_{50}$ >50 µg/mL).

<table>
<thead>
<tr>
<th>No.</th>
<th>IC$_{50}$ (µg/mL)</th>
<th>Activity</th>
<th>No.</th>
<th>IC$_{50}$ (µg/mL)</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>&gt;50</td>
<td>low</td>
<td>33</td>
<td>&gt;50</td>
<td>low</td>
</tr>
<tr>
<td>30</td>
<td>&gt;50</td>
<td>low</td>
<td>34</td>
<td>&gt;50</td>
<td>low</td>
</tr>
<tr>
<td>31</td>
<td>6.13 ± 0.11</td>
<td>high</td>
<td>35</td>
<td>8.98 ± 0.39</td>
<td>High</td>
</tr>
<tr>
<td>32</td>
<td>11.55 ± 1.20</td>
<td>moderate</td>
<td>36</td>
<td>15.45 ± 0.92</td>
<td>moderate</td>
</tr>
</tbody>
</table>

Table 2.22: IL-2 inhibitory activities of benzimidazoles and anthraquinone sulfonamide derivatives (29-36)

Figure 2.15. Bar graphs for in vitro IL-2 inhibition assay of benzimidazoles and anthraquinone sulfonamide derivatives (29-36)

2.3.4. Compounds (Schiff base derivatives) 37-41

In this group compound 37 and 39 (IC$_{50}$ 7.5 and 3.6 µg/mL) showed potent inhibitory effect on IL-2. Remaining compounds showed very weak level of inhibition (IC$_{50}$ >50 µg/mL).
### Table 2.23: IL-2 inhibitory activities of Schiff bases (37-41)

<table>
<thead>
<tr>
<th>No.</th>
<th>IC(_{50}) (µg/mL)</th>
<th>Activity</th>
<th>No.</th>
<th>IC(_{50}) (µg/mL)</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>7.58 ± 0.86</td>
<td>high</td>
<td>40</td>
<td>&gt;50</td>
<td>low</td>
</tr>
<tr>
<td>38</td>
<td>&gt;50</td>
<td>low</td>
<td>41</td>
<td>&gt;50</td>
<td>low</td>
</tr>
<tr>
<td>39</td>
<td>3.63 ± 0.3</td>
<td>high</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Figure 2.16. Bar graphs for in vitro IL-2 inhibition assay of Schiff base derivatives (37-41)](image)

**2.3.5. Compounds (Pyrazole and benzamide derivatives) 42-46**

In this group, pyrazoles 42 and 43, (IC\(_{50}\) ranges from <2 to 3.65 µg/mL) showed very strong inhibitory effect on IL-2 (Table 2.24). Compound 44 showed moderate level of inhibition (IC\(_{50}\) 24.7 µg/mL). Remaining compounds showed very weak inhibitory effect on IL-2 cytokine (IC\(_{50}\) >50 µg/mL).

### Table 2.24: IL-2 inhibitory activities of optimized hitspyrazoles and benzamides (42-46)

<table>
<thead>
<tr>
<th>No.</th>
<th>IC(_{50}) (µg/mL)</th>
<th>Activity</th>
<th>No.</th>
<th>IC(_{50}) (µg/mL)</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>3.65 ± 0.01</td>
<td>high</td>
<td>45</td>
<td>&gt;50</td>
<td>low</td>
</tr>
<tr>
<td>43</td>
<td>&lt;2</td>
<td>high</td>
<td>46</td>
<td>&gt;50</td>
<td>low</td>
</tr>
<tr>
<td>44</td>
<td>24.7 ± 0.14</td>
<td>moderate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Current study of the computer aided identification of IL-2 inhibitors involves working in a number of rounds of experiments both in dry lab and wet lab as discussed in the following sections.

In vitro studies showed that all synthesized compounds showed IL-2 inhibitory potential to a larger or ion expected to be good. The IL-2 inhibition activity of all these forty six compounds was already predicted by using 3D pharmacophore mapping and molecular docking studies. Hence all in silico studies carried out for these compounds showed that they exhibit good binding energies and all necessary chemical features required for the binding in the active site. It was observed that that all the active compounds were deeply embedded in the active site of IL-2/IL-2Ra complex and all these compounds were stabilized by multiple hydrophobic and hydrophilic interactions.

The most promising candidates for IL-2 inhibition identified through this rational designing are described in Figure. 2.18.
Apart from this the calculated molecular descriptors indicated that these compounds also possess drug like properties hence these compounds may act as good inhibitors of IL-2. An inspection of data given in Table 2.9 reveals that all the compounds obey RO5 and their molecular descriptors lie in the range acceptable for drug-like properties. This study represents a successful identification of new IL-2 inhibitors following a rational drug design approach. The compounds synthesized in wet lab (1-46) were the synthetic analogs of hits (H1-H15) identified in dry lab. All the synthesized analogs belonging to different classes of heterocycles showed varied potential against IL-2 as drug target and can be considered as potential candidates for developing into novel immunomodulating agents.

Figure 2.18. Designed and synthesized potential candidates for developing into immunomodulators.
Chapter 3

Experimental
CHAPTER 3: EXPERIMENTAL

3.1. In silico guided identification of IL-2 inhibitors (Dry lab)

3.1.1. Hardware specifications

All the computational studies were performed using Molecular Operating Environment (MOE)\textsuperscript{80} version 2012.10, Chemical Computing Group. The program operated under ‘Windows Server 2003 R2’ operating system installed on an IBM System x3400 with 2048 Ram and four Intel (R) Xeon (TM) CPU 3.00 GHz processors.

3.1.2. Selection and preparation of protein protein complex

Special care was taken to select protein-protein complexes. To consider the flexibility of protein, human IL-2 protein-protein structures were downloaded from Protein Data Bank (PDB entry code 1Z92\textsuperscript{34} for pharmacophore designing. The target was selected on the basis of criteria below:

1) The complex should be of human origin.
2) The target should have high quality resolution (<3.0° A).
3) The protein-protein or protein-ligands interact with one another through non-covalent interactions and the experimental binding data should be available.

Initially the cofactors, non-interacting water molecules and counter-ions were removed from receptor-protein coordinate file. Hydrogen atoms were added to the receptor atoms by MOE. The energy of the retrieved protein molecule was minimized using the default parameters of MOE energy minimization algorithm (gradient: 0.05, Force Field: MMFF94X)\textsuperscript{80}. The co-crystallized protein IL-2 was extracted from its corresponding receptor and saved as the reference structure which was used for RMSD calculation.

3.1.3. Determination of active site of protein

The IL-2Ra was taken for further computational studies. For structure based pharmacophore modeling active site determination is a very crucial step and this was accomplished through Site Finder tool of MOE. It created alpha spheres at the active site. The cavity having key contributing amino acids is taken as the active site and is generally large in size.

3.1.4. Pharmacophore generation

When the 3D structure (X-ray crystal structure) of the molecular target is available, structure-based pharmacophore model generation can be performed. Pharmacophore model was built by
using pharmacophore Query Editor of MOE. The pharmacophore scheme of Polarity-Charge-Hydrophobicity (PCH) was applied throughout the study. Critical amino acids present in the active site of *IL-2Rα* involved in binding interactions with *IL-2* protein were a sufficient input to generate the structure-based pharmacophore.

Qualitative 60-70 hypotheses were generated based on the key contributing amino acids on *IL-2* binding surface. Multiple chemical features were detected and mapped. Alternative HBA and/or HBD, Hyd/Aro, Cat/Anionic sites are considered simultaneously on the receptor surface within the limits of geometric constraints. Structure based annotations were generated through Pharmacophore Query panel of MOE and pharmacophoric features were generated over the key amino acids. The features projecting into the binding pocket were selected while those that projected out of the pocket were deleted. The generated pharmacophore was complimentary to the receptor active site. Excluded volume spheres were also added to the structure-based model in order to characterize the inaccessible areas for any potential ligand.

Pharmacophore model included three features which were two hydrophobic (Hyd) and one cationic (Cat) while the tolerance radius of each feature was adjusted to achieve a balance between sensitivity and specificity. The distances and angles among the pharmacophoric features were calculated through the ‘Measure’ distances and angles panel of MOE.

### 3.1.5. Excluded volumes

To confine the search of compound conformations within a range, the concept of excluded volume was introduced. The excluded volume is defined as an area in a protein that cannot be occupied by drug\(^{225}\). The shape of the binding pocket was approximated by a set of excluded volume spheres added as spatial restraints to the proteins nearby the binding pocket. Specifically the generation of excluded volume was based on those amino acids which could not involve in drug binding and were rendered as excluded volume spheres. Moreover an excluded volume was added on the basis of all the atoms of the residues lining the binding pocket thus accounting its shape and size. The generation of excluded volumes helped in preventing the identification of leads that would fit the pharmacophore elements well but that would not overlap with the receptor atoms. So a three point pharmacophore model was built which including three excluded volumes.
3.1.6. Generation of training set (S-1 to S-30)

For the validation of the developed pharmacophore, a training set of thirty compounds of different classes of heterocycles were selected. The selected compounds were mostly amides and ketones along with standard drugs azathioprine, 6-mercaptopurine, tacrolimus, cyclosporine A etc. Selected compounds were reported in literature\textsuperscript{41, 44, 210-212} as IL-2 inhibitors and had good IC\textsubscript{50} values ranging from 0.06-80.0\mu M (Table 2.1).

3.1.7. Building of compounds

Structures of all training set compounds were built using the Builder Interface of MOE program. All the structures were then energy minimized using Force field MMFF94x and these energy minimized structures were then saved in the mdb file format.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Code</th>
<th>Structure</th>
<th>IC\textsubscript{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-1</td>
<td>CHEMBL104 594</td>
<td><img src="image" alt="Structure S-1" /></td>
<td>0.2</td>
</tr>
<tr>
<td>S-2</td>
<td>CHEMBL107 320</td>
<td><img src="image" alt="Structure S-2" /></td>
<td>2</td>
</tr>
<tr>
<td>S-3</td>
<td>CHEMBL108 123</td>
<td><img src="image" alt="Structure S-3" /></td>
<td>0.4</td>
</tr>
<tr>
<td>S-4</td>
<td>CHEMBL421 412</td>
<td><img src="image" alt="Structure S-4" /></td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2.1: The structure and IC\textsubscript{50} values of IL-2 inhibitors from literature
<table>
<thead>
<tr>
<th>S-5</th>
<th>CHEBI:47417 (SP-4206)</th>
<th><img src="image" alt="Chemical Structure" /></th>
<th>0.06</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-6</td>
<td>CHEMBL106 951</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>0.6</td>
</tr>
<tr>
<td>S-7</td>
<td>CHEMBL317 898</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>9</td>
</tr>
<tr>
<td>S-8</td>
<td>CHEMBL181 983</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>20</td>
</tr>
<tr>
<td>S-10</td>
<td>CHEMBL104 746</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>20</td>
</tr>
<tr>
<td>S-11</td>
<td>CHEMBL104 914</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>40</td>
</tr>
<tr>
<td>S-12</td>
<td>CHEMBL182 704</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>0.6</td>
</tr>
</tbody>
</table>

Continued
| S-13 | CHEMBL182 098 | ![Molecule S-13](image) | 48 |
| S-14 | CHEMBL350 354 | ![Molecule S-14](image) | 6.5 |
| S-15 | CHEMBL164 641 | ![Molecule S-15](image) | 0.8 |
| S-16 | CHEMBL166 149 | ![Molecule S-16](image) | 2 |
| S-17 | CHEMBL103 894 | ![Molecule S-17](image) | 0.26 |
| S-18 | CHEMBL100 390 | ![Molecule S-18](image) | 0.37 |
| S-19 | CHEMBL101 202 | ![Molecule S-19](image) | 0.30 |

Continued
<table>
<thead>
<tr>
<th>S-20</th>
<th>CHEMBL167 745</th>
<th><img src="image1" alt="Chemical Structure" /></th>
<th>0.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-21</td>
<td>CHEMBL350 780</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>0.6</td>
</tr>
<tr>
<td>S-22</td>
<td>CHEMBL164 498</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>0.8</td>
</tr>
<tr>
<td>S-23</td>
<td>CHEMBL419 362</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>3.0</td>
</tr>
<tr>
<td>S-24</td>
<td>CHEMBL106 262</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>40</td>
</tr>
<tr>
<td>S-25</td>
<td>CHEMBL106 518</td>
<td><img src="image6" alt="Chemical Structure" /></td>
<td>10</td>
</tr>
</tbody>
</table>

Continued
3.1.8. Validation of pharmacophore model

The quality of the pharmacophore model was tested using a literature active training set of thirty compounds from diverse chemical scaffolds and with different activities. This pharmacophore model was used to virtually filter the fitting hits from the training set in order to evaluate the model. The training set of molecules was screened against the pharmacophore model using the ‘Pharmacophore search’ function of MOE.

All settings were kept as default. If a conformation of any compound can properly bind with the binding site of the protein, the pharmacophore in this conformation should reasonably match the pharmacophoric feature of the active site. In addition, the conformation of the compound should
never overlap with the excluded volume around the binding site. To find out such conformations, the pharmacophore search was conducted exhaustively on the training set and the procedure was performed on all conformations in the database. The conformations that appropriately match all the pharmacophoric features of the binding site and free from steric clashes with the excluded volumes were stored in output database.

3.1.9. Virtual screening

Pharmacophore-based virtual screening can be efficiently used to find novel potential leads for further development from a virtual database. After 3D pharmacophore model generation and validation, the pharmacophore model was used as a 3D search query for retrieving potent molecules from the ZINC and MOE databases. Compounds were downloaded from ZINC database and filtered according to Lipinski rule of five\(^226\) by FILTER program\(^227-228\). ZINC database contains a total of 1,594,931 compounds out of which 1.5 million compounds passed the filtration criteria of Lipinski’s RO5 and 15,000 compounds were randomly selected for virtual screening in the next step. Enrichment studies were performed using 30 compounds including quite a few standard drugs selected from literature with IC\(_{50}\) 0.06-80 μg/ml against human IL-2. These active compounds were seeded into 15,000 decoy molecules selected from ZINC database. The resulting data set of 15,030 compounds was imported into MOE database containing 10,000 compounds leading to a new set of database containing 25,030 compounds having a variety of chemical scaffolds. The next step i.e. pharmacophore-based virtual screening, reduced the number of compounds from 25,030 to 500 compounds that mapped on the developed pharmacophore model. The output database of the resulting 500 compounds was sorted out on the basis of RMSD in ascending order. These initially identified 500 compounds from the output file were selected for further evaluation using molecular docking. The top ranked docked poses were re-scored and binding energies were calculated using LIGX option implemented in MOE to prioritize these compounds. Fig 3.1 summarizes the protocol used for virtual screening in this study.

3.1.10. Docking accuracy

The predictive ability of docking was assessed by RMSD of the top ranked solution. The following criteria were used.

1) **Good:** A top ranked docked solution with RMSD~2.0 Å.
2) Fair: A top ranked docked solution with RMSD>2.0 and ~3.0Å.
3) In accurate/wrong solution: top ranked docked solution with RMSD>3.0.

Figure 3.1: Schematic presentation of virtual screening protocol

3.1.11. Descriptor based filtering of the database

First filter applied was ‘Lipinski’s rule of five’ RO5 for all compounds of MOE database with the ‘Calculate descriptors’ function of MOE. RO5 states that an orally active drug could not violate the following criteria:\[226\].
- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
- A molecular mass less than 500 Daltons
- An octanol-water partition coefficient log P not greater than 5

Screening of whole database was carried out on the basis of this rule. The number of hydrogen bond donors, hydrogen bond acceptors, molecular weight and log P was calculated for each compound of the database. The compounds which did not comply with Ro5 cut off limits were eliminated.
3.1.12. 3D pharmacophore based filtering of database
A structure based 3D pharmacophore was used as search queries on the compounds, that cover all pharmacophoric features were retained. Among these hits only those compounds were selected which had RMSD up to 3.0. This filter further shortlisted the MOE database and reduced the number of compounds to be chosen for the next step of molecular docking.

3.1.13. Receptor based virtual screening of hits
The 3D in-house virtual collection was generated using the MOE. All compounds which passed through above mentioned filters were subjected further to molecular docking studies. The binding mode of all retrieved compounds in 3D structure of IL-2Ra/IL-2 was evaluated. The binding energy for each docked pose was calculated and the docked mdb files were rearranged on the basis of the binding energy in descending order and all compounds having high binding energies were selected. The top rank docked poses were rescored. Following docking and rescoring, the top 5% of the best-scored compounds were retrieved and their binding modes were visually analyzed using MOE lig plot. The selection of the hits compounds was based on ranking and molecular interactions. The receptor based virtual screening protocol is presented in Fig 3.2.

![Figure 3.2: Receptor based virtual screening protocol](image-url)
After identification of hits (Figure 2.6) in dry lab the next step was the optimization and synthesis of these hits in wet lab.

### 3.2. Hits optimization

In the next step, an in house library of 46 compounds was designed by using identified hits. Pharmacophore mapping and molecular docking studies were done in order to know their pharmacophoric behavior, binding mode and binding energies.

#### 3.2.1. Pharmacophore mapping

The generated 3D pharmacophore was mapped on these optimized hits and RMSD values of all the compounds were calculated which were in the range of 0.42 to 2.51.

#### 3.2.2. Molecular docking

All these optimized hits were docked into the active site of 1Z92 and binding energy for each docked conformation was calculated. To obtain the structural insight into the inhibitory mechanism of IL-2 inhibitors, their binding mode in the active site was investigated. The binding interactions were determined by docking of the inhibitors into the active site of the target and their binding energies were also calculated. In order to identify the type of interactions which were involved in binding the inhibitors in the active site of complex. Ligand-protein interaction diagrams of each docked conformation were generated which showed the key amino acids involved in binding.

### 3.3. Synthesis of hits identified (Wet lab)

#### 3.3.1. Chemicals used

Acetophenone acetone, acetonitrile, stannous chloride, anhydrous calcium chloride, ethyl acetate, sodium acetate, sodium hydroxide, anhydrous potassium carbonate, calcium oxide, ethanol, ethyl acetate, dichloromethane, methanol, chloroform, dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), calcium hydride were purchased from E. Merck. Benzaldehyde, 4-flourobenzaldehyde, 4-chlorobenzaldehyde, 4-bromobenz-aldehyde, 3-hydroxyacetophenone, 3-nitrobenzaldehyde, 2-hydroxybenz-aldehyde, 4-aminoacetophenone, 3-chlorobenzaldehyde, 4-N,N-dimethylbenzaldehyde, 3-hydroxy-benzaldehyde, 4-flourobenzaldehyde, 3-(3-hydroxy-5-nitrophenyl)acryl aldehyde, 2,4-dichlorobenzaldehyde, acetaldehyde, cinnamaldehyde, 3-
nitroaniline, 4-hydroxyaniline, phenylenediamine, 3-nitrophénynediamine, biphenylamine, 1-naphthylamine, 2,6-diamino anthraquinone, 8-quinolinoxyacetic acid hydrazide, hydrazine hydrate, formohydrazide, acetohydrazide, thiosemicarbazide, 2-aminobenzenethiol, urea, thiourea were purchased from BDH. Ibuprofen and cetirizine were purchased from Sigma Aldrich.

3.4. Purification of solvents
All the solvents were used after necessary purification and drying according to standard procedures. The dried solvents were stored over molecular sieves (4 Å). A brief account of the purification procedure follows.

3.4.1. Acetone
Anhydrous CaCl$_2$ (200 g) was introduced into round bottom flask containing one litre of acetone it was left for 4-5 hours. Pure acetone was distilled at 56 °C$^{231}$.

3.4.2. Absolute ethanol
Commercial ethanol was distilled first and then the distilled ethanol was refluxed over calcium oxide and distilled again. The 99 % ethanol obtained was refluxed after adding magnesium turnings and iodine. When the color of iodine disappeared, it was distilled again at 77-78 °C$^{231}$.

3.4.3. Ethyl acetate
Ethyl acetate was purified by shaking it with 5 % sodium carbonate, then with saturated brine solution. It was then dried by stirring it with calcium hydride and distilled$^{231}$.

3.4.4. Chloroform and dichloromethane
Chloroform and dichloromethane were dried over anhydrous calcium chloride for 3 hours and then distilled at 39-41 °C and 64-65 °C respectively$^{231}$.

3.4.5. Absolute methanol
Procedure for absolute methanol is the same as above for absolute ethanol which was distilled at 64-65 °C.
3.4.6. **Dimethyl formamide (DMF)**
Dimethyl formamide was purified and dried by keeping it over calcium hydride and then distilled over sodium. Fraction boiling at 95 °C was collected\(^\text{231}\).

3.4.7. **Dimethyl sulfoxide (DMSO)**
Calcium hydride (200 g) was added to dimethyl sulfoxide (1000 mL) and allowed to stand overnight. The solvent was filtered and fractionally distilled over calcium hydride\(^\text{231}\).

3.5. **Instruments used**

3.5.1. **Melting Point**
Melting points were determined in open capillaries using Gallenkamp melting point (MP-D).

3.5.2. **NMR-spectrometer**
\(^1\)H and \(^{13}\)C-NMR spectra were recorded on a Bruker spectrometer at 300 and 75 MHz respectively in DMSO-\(d_6\) solution. Solvent was used as internal reference. Chemical shifts are given in \(\delta\) scale (ppm). Abbreviations s, d, t, q, m were used for singlet, doublet, triplet, quartet, multiplet respectively. Coupling constants are presented in Hz.

3.5.3. **Mass spectrometer**
Mass spectra were recorded on Agilent technologies 6890N Gas chromatography (GC) and an inert mass selective detector 5973 GC-mass spectrometer.

3.6. **Chromatographic techniques**

3.6.1. **Thin layer chromatography (TLC)**
The progress of all the reactions was monitored by TLC on 2.0 x 5.0 cm aluminum sheets precoated silica gel 60F\(_{254}\) with a layer thickness of 0.25 mm (Merck).
The chromatograms were visualized under UV light (254-366 nm) or iodine vapors. They were developed by using different solvent systems. Following solvent systems were used for the development of chromatogram:

- A Petroleum ether: Ethyl acetate (3:2)
- B Petroleum ether: Ethyl acetate (7:3)
- C Chloroform: Methanol (5:1)
3.7. Synthesis of chalcones (1-7)

General procedure

Chalcones were synthesized to use as precursors for 2,3-dihydro-1,5-benzothiazepines. All chalcones were synthesized by literature procedure. An Erlenmeyer flask fitted with mechanical stir bar was loaded with solution of acetophenone (5 mmol) in rectified spirit (15 mL) the flask was cooled in ice bath and solution of benzaldehyde (5 mmol) in rectified spirit (15 mL) was added on stirring. To this mixture was added an ice cold solution of 4 M NaOH in water. The mixture was stirred vigorously at room temperature and monitored through TLC. The mixture was kept at 0-3 °C overnight, neutralized with ice cold aqueous HCl. The precipitates were filtered and washed with distilled water thoroughly; the product obtained was pure in many cases. In some cases the crude product was recrystallized using hot ethanol. Since chalcones were already synthesized and documented by our group therefore characterization was not reproduced here. Chalcones (1-7) were characterized through their physical constants, their melting points were found in agreement with literature.

3.7.1: 3-Phenyl-1-phenylprop-2-en-1-one (1)

Compound (1) was synthesized by general procedure using acetophenone and benzaldehyde. R<sub>f</sub> = 0.60. Yield: 71 %. m.p. 86-88 °C.

3.7.2: 3-(4-Fluorophenyl)-1-phenylprop-2-en-1-one (2)

Compound (2) was synthesized by general procedure using acetophenone and 4-fluorobenzaldehyde. R<sub>f</sub> = 0.71. Yield: 76 %. m.p. 84-86 °C.

3.7.3: 3-(4-Chlorophenyl)-1-phenylprop-2-en-1-one (3)

Compound (3) was synthesized by general procedure using acetophenone and 4-chlorobenzaldehyde. R<sub>f</sub> = 0.62. Yield: 69 %. m.p. 112-114 °C.
3.7.4: 3-(4-Bromophenyl)-1-phenylprop-2-en-1-one (4)

Compound (4) was synthesized by general procedure using acetophenone and 4-bromobenzaldehyde. Rf = 0.53. Yield: 73 %. m.p. 113-115 °C.

3.7.5: 1-(3-Hydroxyphenyl)-3-(3-nitrophenyl)prop-2-en-1-one (5)

Compound (5) was synthesized by general procedure using 3-hydroxyacetophenone and 3-nitrobenzaldehyde. Rf = 0.65. Yield: 75 %. m.p. 140-142 °C.

3.7.6: 3-(2-Hydroxyphenyl)-1-(3-hydroxyphenyl)prop-2-en-1-one (6)

Compound (6) was synthesized by general procedure using 3-hydroxyacetophenone and 2-hydroxybenzaldehyde. Rf = 0.68. Yield: 61 %. m.p. 148-149 °C.

3.7.7: 1-(4-Aminophenyl)-3-(3-chlorophenyl)prop-2-en-1-one (7)

Compound (7) was synthesized by general procedure using 4-aminoacetophenone and 3-chlorobenzaldehyde. Rf = 0.59. Yield: 64 %. m.p. 83-85 °C.

3.8. Synthesis of dihydropyrimidines (8-13)

**General procedure**

A mixture of chalcone (0.848 g, 0.002 mol), urea or thiourea (0.22 g, 0.002 mol) and 0.2 g NaOH in 25 mL of 80% dilute ethanol was refluxed for 7.5 h, then concentrated and cooled, the precipitates were filtered off and recrystallized from ethanol.

3.8.1: 4-(3-Hydroxyphenyl)-6-(3-nitrophenyl)-5,6-dihydropyrimidine-2(1H)-thione (8)

Compound (8) was synthesized by general procedure using corresponding chalcone (5) and thiourea. Rf = 0.52. Yield: 61 %. m.p. 157-158 °C. 1H NMR (300 MHz, DMSO-d6): δ
1.91 (m, 2H, CH₂), 3.96 (dd, J=11.9 Hz, J=5.2 Hz, 1H, CH), 7.22-7.35 (Ar-H), 8.42 (s, 1H, NH). ¹³C NMR (75 MHz, DMSO-d₆): δ 45.32 (CH), 54.50 (CH₂), 106.29-135.99 (aryl C), 150.33 (C-NO₂), 162.23 (C-OH), 165.26 (C=N), 185.43 (C=S). LCMS (m/z): 328 (M+H)⁺.

3.8.2: 6-(2-Hydroxyphenyl)-4-(3-hydroxyphenyl)-5,6-dihydropyrimidine-2(1H)thione (9)

Compound (9) was synthesized by general procedure using corresponding chalcone (6) and thiourea. Rₖ = 0.61. Yield: 66 %. m.p. 150-151 °C.¹H NMR (300 MHz, DMSO-d₆): δ 2.21 (m, 2H, CH₂), 3.85 (dd, J=12.9 Hz, J=6.9 Hz, 1H, CH), 6.76-7.33 (Ar-H), 9.22 (s, 1H, NH). ¹³C NMR (75 MHz, DMSO-d₆): δ 39.05 (CH₂), 48.90 (CH), 120.03-140.11 (aryl C), 158.89 (C-Cl), 164.89 (C=N), 185.83 (C=S). LCMS (m/z): 299 (M+H)⁺.

3.8.3: 4-(4-Aminophenyl)-6-(3-chlorophenyl)-5,6-dihydropyrimidine-2(1H)-thione (10)

Compound (10) was synthesized by general procedure using corresponding chalcone (7) and thiourea. Rₖ = 0.55. Yield: 69 %. m.p. 163-165 °C.¹H NMR (300 MHz, CDCl₃): δ 2.21 (m, 2H, CH₂), 3.77 (dd, J=12.3 Hz, J=5.4 Hz, 1H, CH), 5.52 (s, 2H, NH₂), 7.82-8.27 (Ar-H), 9.19 (s, 1H, NH). ¹³C NMR (75 MHz, CDCl₃): δ 40.94 (CH₂), 41.51 (CH), 109.11-137.96 (aryl C), 158.69 (C-Cl), 159.34 (C-NH₂), 166.46 (C=N), 189.74 (C=S). LCMS (m/z): 316 (M+H)⁺.

3.8.4: 4-(3-Hydroxyphenyl)-6-(3-nitrophenyl)-5,6-dihydropyrimidin-2(1H)-one (11)

Compound (12) was synthesized by general procedure using corresponding chalcone (5) and urea. Rₖ 0.67 (C). Yield: 76 %. m.p. 148-150 °C.¹H NMR (300 MHz, DMSO-d₆): δ 2.60 (m, 2H, CH₂), 3.71 (dd, J=12.2 Hz, J=7.4 Hz, 1H, CH), 6.32-7.55 (Ar-H), 9.32 (s, 1H, OH), 10.45 (s, 1H, NH). ¹³C NMR (75 MHz, DMSO-d₆): δ 41.75 (CH₂), 50.16 (CH), 108.93-139.56 (aryl C), 149.61 (C-NO₂), 157.35 (C-OH), 164.37 (C=N), 165.94 (C=O). LCMS (m/z): 312 (M+H)⁺.
3.8.5: 6-(2-Hydroxyphenyl)-4-(3-hydroxyphenyl)-5,6-dihydropyrimidin-2(1H)-one (12)\textsuperscript{215}

Compound (12) was synthesized by general procedure using corresponding chalcone (6) and urea. R\textsubscript{f} 0.58 (C). Yield: 74 %. m.p. 136-138 °C. \textsuperscript{1}H NMR (300 MHz, DMSO-\textit{d}_6): δ 2.82 (m, 2H, CH\textsubscript{2}), 3.78 (dd, J=13.1 Hz, J=6.2 Hz, 1H, CH), 6.74-7.48 (Ar-H), 9.30 (s, 1H, OH), 10.61 (s, 1H, NH). \textsuperscript{13}C NMR (75 MHz, DMSO-\textit{d}_6): δ 41.49 (CH\textsubscript{2}), 50.55 (CH), 112.73-139.44 (aryl C), 158.75 (C-OH), 164.85 (C=N), 166.44 (C=O). GCMS (m/z, %): 282 (M\textsuperscript{+}, 14), 265(M\textsuperscript{+}-17, 13), 249 (M\textsuperscript{+}-34, 100).

3.8.6: 4-(4-Aminophenyl)-6-(3-chlorophenyl)-5,6-dihydropyrimidin-2(1H)-one (13)\textsuperscript{215}

Compound (13) was synthesized by general procedure using corresponding chalcone (7) and urea. R\textsubscript{f} 0.64 (C). Yield: 67 %. m.p. 150-153 °C. \textsuperscript{1}H NMR (300 MHz, DMSO-\textit{d}_6): δ 2.79 (m 2H, CH\textsubscript{2}), 3.83 (dd, J=12.3 Hz, J=7.3 Hz, 1H, CH), 4.80 (s, 1H, NH\textsubscript{2}), 6.45-7.50 (Ar-H), 9.64 (s, 1H, OH), 10.78 (s, 1H, NH). \textsuperscript{13}C NMR (75 MHz, DMSO-\textit{d}_6): δ 41.57 (CH\textsubscript{2}), 51.34 (CH), 115.20-138.88 (aryl C), 143.75 (C-Cl), 153.22 (C-NH\textsubscript{2}), 164.48 (C=N), 165.29 (C=O). LCMS (m/z): 300 (M+H\textsuperscript{+}).

3.9. Synthesis of 2,3-dihydro-1,5-heteroazepines (14-19)

General procedure\textsuperscript{81}

A solution of chalcone (4 mmol) in dry THF (10 mL) was taken in 100 mL Erlenmeyer flask. Silica (8 g) was added and stirred for 5 minutes. Solvent was removed under reduced pressure, 1.2 equivalent of o-phenylenediamine was added to this mixture. The flask was fitted with a three way stopcock, evacuated and stirred for 4 hours at 85 °C under nitrogen atmosphere. After completion of reaction, the mixture was stirred with ethyl acetate (15 mL) and filtered through cotton plug. The filtrate was concentrated under reduced pressure the crude product was recrystallized with MeOH to afford crystalline solid. Silica was recycled by sequential washing, stirring ethyl acetate (30 mL) and subsequent filtration and dried in oven for 2 hours.
3.9.1: 4-(4-(3-Chlorophenyl)-4,5-dihydro-3H-benzo(b)(1,4)diazepin-2-yl)-benzenamine (14)

Compound (14) was synthesized by general procedure using corresponding chalcone (7) and o-phenylenediamine. Rf = 0.59. Yield: 59 %. m.p. 97-98 °C. \(^1\)H NMR (300 MHz, DMSO-d6): δ 2.49 (m, 2H, CH\(_2\)), 3.86 (dd, \(J_{2,3a}=11.2 \text{ Hz, } J_{2,3b}=5.4 \text{ Hz, } 1\text{H, C}\_H\)), 5.49 (s, 2H, NH\(_2\)), 7.21-7.35 (Ar-\(H\)), 9.80 (s, 1H, NH). \(^{13}\)C NMR (75 MHz, DMSO-d6): δ 40.92 (C\_H\_2), 62.67 (CH), 120.12-139.72 (aryl C), 150.02 (C-Cl), 158.27 (C-OH). LCMS: 346.43 (M-H)\(^-\).

3.9.2: 3-(4-(3-Nitrophenyl)-4,5-dihydro-3H-benzo(b)(1,4)diazepin-2-yl)phenol (15)

Compound (15) was synthesized by general procedure using corresponding chalcone (5) and o-phenylenediamine. Rf = 0.63. Yield: 83 %. m.p. 89-92 °C. \(^1\)H NMR (300 MHz, DMSO-d6): δ 3.18 (m, 2H, CH\(_2\)), 3.97 (dd, \(J_{2,3a}=12 \text{ Hz, } J_{2,3b}=6 \text{ Hz, } 1\text{H, C}\_H\)), 7.17-7.80 (Ar-\(H\)), 8.81 (s, 1H, NH). 9.47 (s, 1H, OH). \(^{13}\)C NMR (75 MHz, DMSO-d6): δ 42.60 (CH\(_2\)), 59.89 (CH), 125.89-131.55 (aryl C), 154.61 (C-NO\(_2\)), 167.86 (C-OH). LCMS (m/z): 358 (M-H)\(^-\).

3.9.3: 3-(4-(2-Hydroxyphenyl)-4,5-dihydro-3H-benzo(b)(1,4)diazepin-2-yl)phenol (16)

Compound (16) was synthesized by general procedure using corresponding chalcone (6) and o-phenylenediamine. Rf = 0.66. Yield: 76 %. m.p. 98-100 °C. \(^1\)H NMR (300 MHz, DMSO-d6): δ 2.78 (m, 2H, CH\(_2\)), 3.20 (dd, \(J_{2,3a}=12.3 \text{ Hz, } J_{2,3b}=6.2 \text{ Hz, } 1\text{H, CH}\)), 6.53-7.26 (Ar-\(H\)), 8.10 (s, 1H, NH). 8.30 (s, 2H, OH). \(^{13}\)C NMR (75 MHz, DMSO-d6): δ 38.90 (CH\(_2\)), 118.01-152.10 (aryl C), 158.33 (C-OH), 161.80 (C=N). LCMS (m/z, %): 329(M-H)\(^-\).

3.9.4: 4-(2-(3-Chlorophenyl)-2,3-dihydrobenzo(1,4)thiazepin-4-yl)benzenamine (17)

Compound (17) was synthesized by general procedure using corresponding chalcone (7) and 2-aminobenzenethiol. Rf = 0.52. N Yield: 79 %. m.p. 84-86 °C. \(^1\)H NMR (300 MHz, DMSO): δ 2.86
(m, 2H, CH₂), 3.38 (t, J=7.2 Hz, 1H, CH), 5.45 (s, 2H, NH₂), 6.96-8.20 (m, 12H, Ar-H). ¹³C NMR (75 MHz, DMSO): δ 37.0(CH₂), 58.0(CH), 114.1-158.1(Ar-CS), 169.1(C-NH₂). LCMS (m/z): 363(M-H)⁻.

3.9.5: 3-(2-(3-Nitrophenyl)-2,3-dihydrobenzo(b)(1,4)thiazepin-4-yl)phenol (18)

Compound (18) was synthesized by general procedure using corresponding chalcone (5) and 2-aminobenzenethiol. Rf = 0.60. Yield: 75 %. m.p. 81 °C. ¹H NMR (300 MHz, DMSO-d₆): δ 3.40 (m, 2H, CH₂), 3.40 (t, J=8.6 Hz, 1H, CH), 66.9-8.22 (m, 12H, Ar-Hs), 9.70 (s, 1H, OH). ¹³C NMR (75 MHz, DMSO-d₆): δ 58.7 (CH), 40.7 (C-H₂), 114.1-158.1 (Ar-CS), 152.6 (C-NO₂). LCMS (m/z): 375(M-H)⁻.

3.9.6: 3-(2-(2-hydroxyphenyl)-2,3-dihydrobenzo(b)(1,4)thiazepin-4-yl)phenol (19)²¹⁶

Compound (19) was synthesized by general procedure using corresponding chalcone (6) and 2-aminobenzenethiol. Rf = 0.58. Yield: 68%. m.p. 99-101 °C. ¹H NMR (300 MHz, acetone-d₆): δ 3.27 (m, J=12.0 Hz, J=3.4 Hz, 2H, CH₂), 3.83 (t, J=6.0 Hz, 1H, CH), 7.3-8.20 (m, 12H, Ar-Hs), 8.81 (s, 2H, OH). ¹³C NMR (75 MHz, acetone-d₆): δ 29.5 (C-3), 48.0 (C-2), 115.7-159.8 (Ar-CS), 168.0 (C-4). LCMS: 346.2(M-H)⁻.

3.10. Synthesis of pyrazolecarbaldehydes (20-25) and pyrazolecarbothioamides (26-28)

General procedure²¹⁸

A mixture of chalcone (0.84 g, 0.001 mol) and hydrazine hydrate (0.20 mL, 0.002 mol) was heated under reflux for 10 h, in 25 mL absolute ethanol then cooled and the residual material was filtered off and recrystallized from ethanol.

3.10.1: 3-(3-Hydroxyphenyl)-5-(3-nitrophenyl)-4,5-dihydropyrazole-1-carbaldehyde (20)

Compound (20) was synthesized by general procedure using corresponding chalcone (5) and formohydrazide. Rf 0.43 (C). Yield: 66 %. m.p. 112-114 °C. ¹H NMR (300 MHz, DMSO-d₆):
\[ \delta \ 1.95 \ (dd, \ J=12.9 \ Hz, \ J=5.7 \ Hz, \ 2H, \ CH_2), \ 3.93 \ (dd, \ J_{2,3a}=12.9 \ Hz, \ J_{2,3b}=5.7 \ Hz, \ 1H, \ CH), \ 5.94 \ (s, \ 1H, \ OH), \ 7.10-7.92 \ (Ar-H), \ 8.08 \ (CHO). \]  \[ ^{13}C \ \text{NMR} \ (75 \ MHz, \ DMSO-d_6): \ \delta \ 42.75 \ (CH_2), 62.87 \ (CH), 115.83-131.06 \ (aryl \ C), 131.31 \ (C-NO_2), 139.63 \ (C=N), 155.39 \ (C-OH), 163.17 \ (C=O). \]  LCMS: 312 \ (M+H)^+.

### 3.10.2: 5-(2-Hydroxyphenyl)-3-(3-hydroxyphenyl)-4,5-dihydropyrazole-1-carbaldehyde (21)

![Diagram](image)

Compound (21) was synthesized by general procedure using corresponding chalcone (6) and formohydrazide. \( R_f \ 0.46 \) (C). Yield: 65 %. m.p. 106-108 °C. \(^1H\) NMR (300 MHz, DMSO-d_6): \( \delta \ 2.15 \ (dd, \ J=12.3 \ Hz, \ J=6.3 \ Hz, \ 2H, \ CH_2), 4.07 \ (dd, \ J_{2,3a}=12.3 \ Hz, \ J_{2,3b}=6.3 \ Hz, \ 1H, \ CH), 4.86 \ (s, \ 1H, \ OH), 5.07 \ (s, \ 1H, \ OH), 6.84-7.01 \ (Ar-H), 8.93 \ (CH) \).  \[ ^{13}C \ \text{NMR} \ (75 \ MHz, \ DMSO-d_6): \ \delta \ 28.20 \ (CH_2), 41.13 \ (CH), 121.02-145.87 \ (aryl \ C), 151.39 \ (C=N), 205.11 \ (C-OH), 205.38 \ (C=O). \]  LCMS: 283 \ (M+H)^+.

### 3.10.3: 3-(4-Amiphenyl)-5-(3-chlorophenyl)-4,5-dihydropyrazole-1-carbaldehyde (22)

![Diagram](image)

Compound (22) was synthesized by general procedure using corresponding chalcone (7) and formohydrazide. \( R_f \ 0.44 \) (C). Yield: 69 %. m.p. 109-111 °C. \(^1H\) NMR (300 MHz, DMSO-Cl d_6): \( \delta \ 2.01 \ (dd, \ J=11.4 \ Hz, \ J=5.1 \ Hz, \ 1H, \ CH_2), 3.88 \ (s, \ 2H, \ NH_2), 5.49 \ (s, \ 1H, \ CH), 7.21-7.68 \ (Ar-H), 8.95 \ (CHO). \]  \[ ^{13}C \ \text{NMR} \ (75 \ MHz, \ DMSO-d_6): \ \delta \ 40.95 \ (CH_2), 60.98 \ (CH), 116.04-131.85 \ (aryl \ C), 132.16 \ (C-Cl), 160.95 \ (C=O). \]  LCMS: 300 \ (M+H)^+.

### 3.10.4: 5-(4-Bromophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole (23)

![Diagram](image)

Compound (23) was synthesized by general procedure using corresponding chalcone (4) and hydrazine hydrate. \( R_f \ 0.48 \) (C). Yield: 72 %. m.p. 98-100 °C. \(^1H\) NMR (300 MHz, DMSO-d_6): \( \delta \ 3.16 \ (dd, \ J_{3a,3b}=18.0 \ Hz, \ J_{3a,2}=3.3 \ Hz, \ 1H, \ H_{3a}, 3.92 \ (dd, \ J_{3b,3a}=18.0 \ Hz, \ J_{3b,2}= 11.4 \ Hz, \ 1H, \ H_{3b}), 5.94 \ (dd, \ J_{2,3a}=3.3 \ Hz, \ J_{2,3b}=11.4 \ Hz, \ 1H, \ H_2), 8.05 \ (s, \ 1H, \ NH), 7.14-7.79 \ (Ar-H). \]  \[ ^{13}C \ \text{NMR} \ (75 \ MHz, \ DMSO-d_6): \ \delta \ 42.86 \ (CH_2), 63.30 \ (CH), 120.66-142.24 \ (aryl \ C), 155.38 \ (C-Br), 155.39 \ (C=N). \]  LCMS: 302 \ (M+H)^+. 

110
3.10.5: 1-(3,5-Diphenyl-4,5-dihydropyrazol-1-yl)ethanone (24)

Compound (24) was synthesized by general procedure using corresponding chalcone (1) and acetohydrazine. Rf 0.47 (C). Yield: 70 %. m.p. 102-103 °C. \(^1\)H NMR (300 MHz, DMSO-d\(_6\)) \(\delta\) 1.89 (s, 3H, CH\(_3\)), 3.15 (dd, \(J_{3a,3b}=18.0\) Hz, \(J_{3a,2}=4.5\) Hz, 1H, H\(_{3a}\)), 3.85 (dd, \(J_{3a,3b}=18\) Hz, \(J_{3b,2}=12\) Hz, 1H, H\(_{3b}\)), 5.58 (dd, \(J_{2,3a}=4.5\) Hz, \(J_{2,3b}=12.0\) Hz, 1H, H\(_2\)), 6.56-7.80 (Ar-H). \(^{13}\)C NMR (75 MHz, DMSO-d\(_6\)) \(\delta\) 22.19 (CH\(_3\)), 52.60 (CH\(_2\)), 59.89 (CH), 121.62-136.85 (aryl C), 154.61 (C=N), 176.86 (C=O). LCMS: 300 (M+H)

3.10.6: 1-(5-(4-Bromophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)ethanone (25)

Compound (25) was synthesized by general procedure using corresponding chalcone (4) and acetohydrazide. Rf 0.39 (C). Yield: 69 %. m.p. 106-108 °C. \(^1\)H NMR (300 MHz, DMSO-d\(_6\)) \(\delta\) 1.91 (s, 3H, CH\(_3\)), 3.17 (dd, \(J_{3a,3b}=18.3\) Hz, \(J_{3a,2}=4.8\) Hz, 1H, H\(_{3a}\)), 3.87 (dd, \(J_{3a,3b}=18\) Hz, \(J_{3b,2}=12\) Hz, 1H, H\(_{3b}\)), 5.54 (dd, \(J_{2,3a}=4.8\) Hz, \(J_{2,3b}=12\) Hz, 1H, H\(_2\)), 6.86-8.05 (m, Ar-H). \(^{13}\)C NMR (75 MHz, DMSO-d\(_6\)) \(\delta\) 21.16 (CH\(_3\)), 42.32 (CH\(_2\)), 59.37 (CH), 120.52-131.06 (aryl C), 142.24 (C-Br), 154.67 (C=N), 167.94 (C=O). GCMS (m/z, %): 344(M\(^+\)+2, 45), 342 (M\(^+\), 45), 300 (M\(^+\)-42, 100), 259 (M\(^+\)-93, 25).

3.10.7: 3,5-Diphenyl-4,5-dihydropyrazole-1-carbothioamide (26)

Compound (26) was synthesized by general procedure using corresponding chalcone (1) and thiosemicarbazide. Rf = 0.40. Yield: 71 %. m.p. 105-107 °C. \(^1\)H NMR (300 MHz, DMSO-d\(_6\)) \(\delta\) 2.78 (dd, \(J_{3a,3b}=12.3\) Hz, \(J_{3a,2}=3.9\) Hz, 1H, H\(_{3a}\)), 3.20 (dd, \(J_{3a,3b}=12.3\) Hz, \(J_{3b,2}=6.9\) Hz, 1H, H\(_{3b}\)), 4.63 (dd, \(J_{2,3a}=3.9\) Hz, \(J_{2,3b}=6.9\) Hz, 1H, H\(_2\)), 5.23 (s, 2H, NH\(_2\)), 6.96-8.12 (Ar-H). \(^{13}\)C NMR (75 MHz, DMSO-d\(_6\)) \(\delta\) 40.38 (CH\(_2\)), 54.09 (CH), 120.42-140.15 (aryl C), 154.02 (C=N), 176.58 (C=S). LCMS: 281 (M+H)

111
3.10.8: 5-(4-Fluorophenyl)-3-phenyl-4,5-dihydropyrazole-1-carbothioamide (27)

Compound (27) was synthesized by general procedure using corresponding chalcone (2) and thiosemicarbazide. $R_f$ 0.48 (C). Yield: 68 %. m.p. 110-112 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 3.34 (m, 2H, $H_3$), 3.91 (dd, $J_{2,3a}=18.1$ Hz, $J_{2,3b}=5.7$ Hz, 1H, $H_2$), 5.94 (s, 2H, $NH_2$), 7.05-8.33 (Ar-H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 42.75 (CH$_2$), 62.68 (CH), 115.42-138.19 (aryl C), 155.39 (C-F), 163.17 (C=N), 176.54 (C=S). LCMS: 299 (M+H)$^+$. 

3.10.9: 5-(4-Chlorophenyl)-3-phenyl-4,5-dihydropyrazole-1-carbothioamide (28)

Compound (28) was prepared by general procedure using corresponding chalcone (3) and thiosemicarbazide. $R_f$ 0.47 (C). Yield: 70 %. m.p. 116-118 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 3.19 (dd, $J_{3a,3b}=18.5$ Hz, $J_{3a,2}=3.6$ Hz, 1H, $H_3$), 4.63 (dd, $J_{2,3a}=3.6$ Hz, $J_{2,3b}=11.7$ Hz, 1H, $H_3$), 7.14 (s, 2H, NH$_2$), 7.09-8.13 (Ar-H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 42.63 (CH$_2$), 62.77 (CH), 127.62-131.78 (aryl C), 142.46 (C-Cl), 155.38 (C=N), 176.53 (C=S). LCMS: 315 (M+H)$^+$. 

3.11. Synthesis of benzimidazoles (29-32)

**General procedure**

All benzimidazole derivatives were synthesized according to following literature procedure. To an aldehyde (15mmol/50 ml ethanol), an aqueous solution of sodium metabisulfite (1.6 g) was added, then the reaction mixture was stirred vigorously maintaining the temperature at 0-4 °C and the precipitate formed were filtered, dried. A mixture of adduct (2 mmol) and o-phenylenediamine (2 mmol) was heated at 110 °C for 4 hours in DMF (5 ml), the reaction mixture was cooled, poured into ice cold water and precipitates formed were filtered, washed and dried.

3.11.1: 2-(1-(4-Isobutylphenyl)ethyl)-1H-benzo(d)imidazole (29)

Compound (29) was synthesized by general procedure using ibuprofen and o-phenylenediamine. $R_f$ = 0.48. Yield: 62 %. m.p.
183-184°C. 1H NMR (300 MHz, DMSO-d6): δ 1.09 (d, J=9.7 Hz, 6H, (CH3)2), 1.73 (d, J=7.8 Hz, 3H, CH3), 2.10 (m, 1H, CHCH2), 2.83 (d, 2H, J=8.6 Hz, CHCH2), 4.42 (q, 1H, CHCH3), 7.02-7.66 (Ar-H), 11.36 (s, 1H, NH). 13C NMR (75MHz, DMSO-d6): 19.21 (CH3), 23.21 (CH3)2, 29.12 (CHCH2), 40.34 (CHCH3), 48.16 (CHCH2), 112.24-137.27 (aryl C), 146.38 (N-C-N). GCMS (m/z, %): 278 (M+, 44), 263 (M+-15, 18), 235(M+-43, 17), 221(M+-57, 100).

3.11.2: 2-(2-(4-(4-Chlorophenyl)(phenyl)methyl)piperazin-1-yl)ethoxy)methyl)-1H-benzo-(d)imidazole (30)

Compound (30) was synthesized by general procedure using a commercial sample of cetirizine and o-phenylenediamine. Rf = 0.50. Yield: 70 %. m.p. 331-333 °C. 1H NMR (300 MHz, acetone-d6): δ 2.24 (t, J=12.8 Hz, 2H, C=H2piperazine), 2.72 (t, J=9.5 Hz, 2H, N-C=H2), 3.67 (t, J=12.1 Hz, 2H, N-CH2C=H2), 4.78 (s, 2H, O-C=H2), 5.21 (N-C=H), 6.98-7.81 (Ar-H), 11.24 (s, 1H, NH). 13C NMR (75 MHz, acetone-d6): δ 48.98 (CHN-C=H2piperazine), 52.76 (C=H2piperazine), 55.09 (N-C=H2), 64.87 (O-C=H2), 69.05 (N-C=H2C=H2), 78.43 (N=CH), 118.65-140.54 (aryl C). LCMS: 460 (M+H)+.

3.11.3: N,N-dimethyl-4-(6-nitro-1H-benzo(d)imidazol-2-yl)benzenamine (31)

Compound (31) was synthesized by general procedure using 4-N,N-dimethylbenzaldehyde and 3-nitro-o-phenylenediamine. Rf = 0.68. Yield: 74 %. m.p. 289-291 °C. 1H NMR (300 MHz, DMSO-d6): δ 2.86 (s, 6H, N(CH3)2), 6.87-8.20 (Ar-H), 11.09 (s, 1H, NH). 13C NMR (75 MHz, DMSO-d6): δ 39.54 (N(CH3)2), 110.43-137.80 (aryl C), 145.12 (C-NO2), 150.35 (N-C), 154.06 (N-C-N). GCMS (m/z, %): 282(M+, 73), 267 (M+-15, 31), 252 (M+-30).

3.11.4: 4-(1H-benzo(d)imidazol-2-yl)-N,N-dimethylbenzenamine (32)

Compound (32) was synthesized by general procedure using 4-N,N-dimethylbenzaldehyde and o-phenylenediamine. Rf = 0.63. Yield: 68 %. m.p. 278-280 °C. 1H NMR (300 MHz, DMSO-d6): δ 2.79 (s, 6H, N(CH3)2), 7.06-8.35 (Ar-H), 11.24 (s, 1H, NH). 13C NMR (75 MHz, DMSO-d6): δ 39.75 (N(CH3)2), 120.24-138.12 (aryl C), 151.22 (N-C), 154.34 (N-C-N). LCMS: 238 (M+H)+.

**General procedure**

The anthraquinonesulfonyl chloride was synthesized according to literature procedure. In a 500 mL, two neck round bottom flask equipped with an are flux condenser under nitrogen condition, mixed anthraquinone-2-sulfonic acid sodium salt (0.15 mol, 46.539 g) and PCl₅ (0.15 mol, 31.236 g). The temperature of reaction mixture was maintained 175-180 °C. After four hours, reaction mixture was cooled to room temperature and mixed with spatula and again reaction temperature was raised to 175-180 °C. Following exercise of cooling to room temperature and mixing after intervals of four hours was repeated for sixteen hours. After completion of reaction, reaction mixture was cooled to room temperature and poured into 2 L beaker containing 1Kg crushed ice. Resulting precipitates were filtered and solubilize in chloroform to remove insoluble impurities by filtration. To remove water from chloroform solution, anhydrous sodium sulfate was added and left overnight. Solution was then filtered and concentrated on a rotary evaporator leading to formation of yellow crystals of anthraquinone-2-sulfonyl chloride (m.p.196 °C).

Sulfonamides were prepared by the general procedure. The benzimidazole (0.04 mol) was condensed with anthraquinonesulfonyl chloride (0.03 mol) in 50 ml 4N hydrochloric acid. The reaction mixture was stirred for about 4 h at 80 °C. The compound was precipitated by adding ammonia filtered through suction and washed with cold water. Compound was recrystallized from water and ethanol.

### 3.12.1: 2-(2-(4-(Dimethylamino)phenyl)-1H-benzo(d)imidazol-1-ylsulfonyl)-anthracene-9,10-dione (33)

![Chemical Structure](image)

Compound (33) was synthesized by general procedure using anthraquinone sulfonyl chloride and corresponding benzimidazole (32). Rf = 0.55. Yield: 76 %, m.p. 281-282 °C. ¹H NMR (300 MHz, DMSO-d₆): δ 3.35 (s, 6H, N(CH₃)₂), 7.04-8.45 (Ar-H). ¹³C NMR (75 MHz, DMSO-d₆): δ 40.09 (N(CH₃)₂), 110.67-139.70 (aryl C), 150.02 (N-C-N). 182.67 (C=O). LCMS: 508 (M+H)⁺.
3.12.2: 2-(2-(4-(Dimethylamino)phenyl)-6-nitro-1H-benzo(d)imidazol-1-ylsulfanyl)-anthracene-9,10-dione (34)

Compound (34) was synthesized by general procedure using anthraquinone sulfonyle chloride and corresponding benzimidazole (31). Rf = 0.62. Yield: 78 %. m.p. 297-298 °C. \(^1\)H NMR (300 MHz, DMSO-d\(_6\)): δ 2.67 (s, 6H, N(CH\(_3\))\(_2\)), 6.99-7.97 (Ar-H). \(^1\)C NMR (75 MHz, DMSO-d\(_6\)): δ 39.13 (N(CH\(_3\))\(_2\)), 1116.38-132.42 (aryl C), 139.95 (C-NO\(_2\)), 150.09 (N-C-N), 180.95 (C=O). LCMS: 553 (M+H)\(^+\).

3.12.3: 2-(2-(1-(4-Isobutylphenyl)ethyl)-1H-benzo(d)imidazol-1-ylsulfanyl)-anthracene-9,10-dione (35)

Compound (35) was synthesized by general procedure using anthraquinone sulfonyle chloride and corresponding benzimidazole (29). Rf = 0.66. Yield: 60 %. m.p. 251-252 °C. \(^1\)H NMR (300 MHz, DMSO-d\(_6\)): δ 1.28 (d, J=11.7 Hz, 6H, (CH\(_3\))\(_2\)), 1.57 (d, J=8.7 Hz, 3H, CH\(_3\)), 2.18 (m, 1H, CHCH\(_2\)), 3.88 (q, J=8.1 Hz, 1H, CHCH\(_3\)), 7.22-7.68 (Ar-H). \(^1\)C NMR (75MHz, DMSO-d\(_6\)): 39.13 (CH\(_3\)), 39.14 (CH\(_3\))\(_2\)), 40.80 (CH\(_2\)), 40.95 (CH\(_3\)), 116.04-132.16 (aryl C), 150.09 (N-C-N), 180.95 (C=O). LCMS: 549 (M+H)\(^+\).

3.12.4: 2-(2-(2-(4-Chlorophenyl)(phenyl)methyl)piperazin-1-ylmethoxy)methyl)-1H-benzo-(d)imidazol-1-ylsulfonylethoxy)methyl)-1H-benzo-(d)imidazol-1-ylsulfonylantracene-9,10-dione (36)

Compound (36) was synthesized by general procedure using anthraquinonesulfonyle chloride and corresponding benzimidazole (30). Rf = 0.63. Yield: 64 %. m.p. 273-275 °C. \(^1\)H NMR (300 MHz, acetone): δ 2.07 (m, 4H, CH\(_2\))
piperazine), 2.33 (m, 4H, CH$_2$ piperazine), 2.56 (m, 2H, CH$_2$), 4.01 (s, 2H, O-CH$_2$), 5.25 (s, 1H, N-CH), 6.84-7.04 (Ar-H). $^{13}$C NMR (75 MHz, acetone-d$_6$): δ 41.13 (CH$_2$ piperazine), 41.19 (N-CH$_2$), 54.21 (O-CH$_2$), 73.51 (N-CH), 121.02-138.30 (aryl C), 142.29 (C-Cl), 144.34 (C=N), 181.51 (C=O). LCMS: 732 (M+H)$^+$. 


**General procedure**

The Schiff base derivatives were synthesized by the following method. A solution of aromatic amine (0.007 mol) was dissolved in absolute ethanol and then it was slowly added to a solution of substituted benzaldehyde (0.014 mol) in ethanol. After stirring the reaction mixture for two hours at 40-50$^\circ$C, then on cooling the precipitates formed were collected by filtration. The product was washed several times with cold water and then recrystallized from ethanol.

3.13.1: 1,5-bis-(3-Hydroxybenzylideneamino)anthracene-9,10-dione (37)

![Structure of 1,5-bis-(3-Hydroxybenzylideneamino)anthracene-9,10-dione (37)](image)

Compound (37) was synthesized by general procedure using 2,6-diamino anthraquinonone (7 mmol) and 3-hydroxybenzaldehyde (0.014 mol). R$_f$ = 0.52. Yield: 58 %. m.p. 200-201 $^\circ$C. $^1$H NMR (300 MHz, DMSO-d$_6$): δ 6.78-7.81 (Ar-H), 8.36 (s, 1H, CH), 9.40 (s, 1H, OH). $^{13}$C NMR (75 MHz, DMSO-d$_6$): δ 112.6-145.70 (aryl C), 155.67 (C-OH), 163.4 (C=N), 175.12 (C=O). LCMS (m/z): 447 (M+H)$^+$. 

3.13.2: 1,5-bis-(4-Fluorobenzylideneamino)anthracene-9,10-dione (38)

![Structure of 1,5-bis-(4-Fluorobenzylideneamino)anthracene-9,10-dione (38)](image)

Compound (38) was synthesized by general procedure using 2,6-diamino anthraquinonoe (7 mmol) and 4-fluorobenzaldehyde (0.014 mol). R$_f$ = 0.32. Yield: 67 %. m.p. 214-216 $^\circ$C. $^1$H NMR (300 MHz, DMSO-d$_6$): δ 6.34-7.60 (Ar-H), 8.30 (s, 1H, CH). $^{13}$C NMR (75 MHz, DMSO-d$_6$): δ 112.6-145.70 (aryl C), 163.45 (C-F), 162.89 (C=N), 178.52 (C=O). LCMS: 451 (M+H)$^+$. 

116
3.13.3: 3-(3-(Naphthalen-1-ylimino)prop-1-enyl)-5-nitrophenol (39)

Compound (39) was synthesized by general procedure using 1-naphthylamine (0.01 mol), and 3-(3-hydroxy-5-nitrophenyl) acryaldehyde (0.01 mol). R_f = 0.43. Yield: 57 %.m.p. 183-184°C. ^1H NMR (300 MHz, DMSO-d_6): δ 3.89 (d, J=12.8 Hz, 1H, CH), 4.73 (m, J=12.7 Hz, 1H, CH), 6.12 (d, J=12.8 Hz, 1H, N-CH), 7.16-7.81 (Ar-H). 9.02 (s, 1H, OH), 13C NMR (75 MHz, DMSO-d_6): δ 116.12 (NCH-C), 147.12 (C-NO_2), 148.33 (C-N), 158.99 (C-OH), 166.70 (C=N). LCMS: 319 ((M+H)^+).

3.13.4: bis-N-3-phenylallylidene)biphenylamine (40)

Compound (40) was synthesized by general procedure using biphenylamine (0.01 mol), and cinnamaldehyde (0.02 mol). R_f = 0.49. Yield: 61 %.m.p. 70°C. ^1H NMR (300 MHz, CDCl_3): δ 5.27 (d, J=12.8 Hz, 1H, CH), 6.34 (t, J=12.8 Hz, 1H, CH), 7.10-7.79 (Ar-H). 13C NMR (75 MHz, CDCl_3): δ 112.29 (NCH-C), 120.75-138.50 (aryl C), 147.91 (C-N), 167.12 (C=N). LCMS (m/z): 413 ((M+H)^+).

3.13.5: bis-N-(2,4-dichlorobenzylidene)biphenylamine (41)

Compound (41) was synthesized by general procedure using biphenylamine (0.01 mol), and 2,4-dichlorobenzaldehyde (0.02 mol). R_f = 0.59. Yield: 67 %.m.p. 204-205°C. ^1H NMR (300 MHz, DMSO-d_6): δ 7.28-7.80 (Ar-H), 8.25 (s, 1H, CH). 13C NMR (75 MHz, DMSO-d_6): δ 121.01-155.68 (aryl C), 169.85 (CH). LCMS (m/z): 499 (M+H)^+.


General procedure

8-Quinolinoxyacetic acid hydrazide was prepared by the reaction of 0.001 mol of 8-hydroxyquinoline with ethyl chloroacetate (0.001 mol) in the presence of methanol and H_2SO_4. The resulting intermediate was then treated with hydrazine hydrate at rt.
A mixture of aniline (0.01 mol), conc HCl and water were stirred for 15 mints at 0°C. NaNO$_2$ was added in reaction mixture maintaining the temperature at 0°C. The mixture of ethyl acetate (0.01 mol) and sodium acetate was then added in reaction mixture with stirring. The precipitates of ethyl 3-oxo-2-(2-phenylhydrazono)butanoate (A) was collected. (Scheme 2.9)

8-Quinolinoxyacetic acid hydrazide (0.001 mol) was added to solution of ethyl 3-oxo-2-(2-phenylhydrazono)butanoate (0.001 mol) in acetic acid and it was refluxed for 6-7 hours. Precipitates were collected and recrystallized with ethanol.

3.14.1: 1-(4-((3-nitrophenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl-)2-(quinolin-8-yloxy)ethanone (42)

Compound (42) was synthesized by general procedure using 3-nitroaniline8-quinolinoxyacetic acid hydrazide. R$_f$ = 0.40. Yield: 78 %. m.p. 199-200°C.$^1$H NMR (300 MHz, DMSO-$d_6$): δ 3.36 (s, 6H, CH$_3$), 5.82 (s, 2H, O-C$_2$H$_2$), 6.93-7.90(Ar-H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ 18.35 (CH$_3$), 19.43 (CH$_3$), 70.92 (OCH$_2$), 113.17-146.93 (Ar-C), 168.26 (C-NO$_2$). LCMS: 431 (M+H)$^+$. 

3.14.2: 1-(4-((4-hydroxyphenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl-)2-(quinolin-8-yloxy)ethanone (43)

Compound (43) was synthesized by general procedure using 4-hydroxyaniline8-quinolinoxyacetic acid hydrazide. R$_f$ = 0.51. Yield: 77 %. m.p. 195-196°C.$^1$H NMR (300 MHz, DMSO-$d_6$): δ 1.85 (s, 3H, CH$_3$), 3.35 (s, 3H, CH$_3$), 4.04 (O-CH$_2$), 7.49-7.85(Ar-H), 9.02 (s, IH, OH).$^{13}$C NMR (75 MHz, DMSO-$d_6$): δ 19.56 (CH$_3$), 21.47 (CH$_3$), 70.42 (OCH$_2$), 126.84-134.19 (aryl C), 158.86 (C-OH). LCMS: 402(M+H)$^+$. 

3.15. Synthesis of benzamides (44-46)

General procedure$^{223}$

Direct synthesis of amide derivatives was carried out by reacting the anilines with acids by using 5-methoxy-2-iodophenylboronic acid (MIBA) as catalyst under mild conditions at ambient
temperature in the presence of molecular sieves. A possible catalytic cycle was based on the supposed formation of an acylborate intermediate. The product was recrystallized from ethanol.

3.15.1: 2-(4-Isobutylphenyl)-N-(naphthalen-2-yl)propanamide (44)

Compound (44) was synthesized by general procedure using a commercial sample of ibuprofen and 1-naphthylamine. $R_f = 0.58$. Yield: 81 %. m.p. 197-198 °C. $^1$H NMR (300 MHz, DMSO-d$_6$): $\delta$ 1.20 (d, $J$=9.7 Hz, 6H, (CH$_3$)$_2$), 1.67 (d, $J$=7.8 Hz, 3H, CH$_3$), 2.34 (m, 1H, CHCH$_2$), 2.72 (d, 2H, $J$=8.6 Hz, CHCH$_2$), 4.05 (q, $J$=7.8 Hz, 1H, CH$_2$CH$_3$), 6.82-7.46 (Ar-H), 11.15 (s, 1H, NH). $^{13}$C NMR (75MHz, DMSO-d$_6$): 18.78 (CH$_3$), 23.86 (CH$_3$)$_2$, 30.27 (CHCH$_2$), 41.45 (CHCH$_3$), 47.55 (CHCH$_2$), 104.64-140.38 (aryl C), 167.83 (C=O). LCMS: 332 ((M+H)$^+$).

3.15.2: 2-(2-(4-Chlorophenyl)(phenyl)methyl)piperazin-1-yloxy)-N-(naphthalen-2-yl)acetamide (45)

Compound (45) was synthesized by general procedure using a commercial sample of cetirizine and 1-naphthylamine. $R_f = 0.67$. Yield: 79 %. m.p. 129-131°C. $^1$H NMR (300 MHz, acetone-d$_6$): $\delta$ 2.07 (m, 4H, N-CH$_2$CH$_2$), 2.34 (m, 4H, N-CH$_2$CH$_2$), 2.56 (t, $J$=13.8 Hz, 2H, N-CH$_2$CH$_2$), 4.06 (s, 2H, O-CH$_2$), 5.12 (s, 1H, N-CH), 6.84-7.46 (Ar-H), 8.94 (s, 1H, NH). $^{13}$C NMR (75 MHz, acetone-d$_6$): $\delta$ 58.40 (CH$_2$piperazine), 59.22 (N-CH$_2$), 61.52 (O-CH$_2$), 77.42 (N-CH), 113.17-135.42 (aryl C), 150.41 (C-Cl), 168.26 (C=O). LCMS (m/z): 515 (M+H)$^+$.

3.15.3: bis-N-biphenylacetamide (46)

Compound (46) was synthesized by general procedure using biphenylamine and acetaldehyde. $R_f = 0.70$. Yield: 78 %. m.p. 316-318°C. $^1$H NMR (300 MHz, DMSO-d$_6$): $\delta$ 2.51 (s, 3H, CH$_3$), 7.38-8.42 (m, Ar-H), 11.18 (s, 1H, NH). $^{13}$C NMR (75 MHz, DMSO-d$_6$): $\delta$ 21.50 (CH$_3$), 128.06-136.67 (aryl C), 169.67 (C=O). LCMS: 269 (M$^+$), 253 (M+H)$^+$.
3.16. *IL*-2 inhibitory Studies

3.16.1 *IL*-2 Production and quantification

Jurkat (human T lymphocyte leukemia) cells were kindly provided by Prof. Daniel Hoessli from University of Geneva, Switzerland. The cells were maintained in RPMI-1640 (BioM Laboratories, Chemical Division, Malaysia) supplemented with 5% FBS and 1% penicillin / streptomycin (GIBCO New York U.S). Upon 70% confluency cells were plated in 96-well flat bottom plates (Costar, NY, USA) at a concentration of $2 \times 10^6$ cells/mL. The cells were activated by using 20 ng/mL of phorbolmyristate acetate (PMA) and 7.5 µg/mL of phytohemagglutinin (PHA) (SERVA, Heidelberg, Germany). Cells were then treated with three different concentrations (2, 10, 50 g/mL) of compounds and plate was incubated for 18 hours at 37 ºC in 5% CO$_2$. Supernatants were collected and *IL*-2 quantification was performed using human *IL*-2 Kits Duo Set (R&D systems, Minneapolis, USA) followed manufacturer’s instructions$^{224}$. 
4.1 CONCLUSION

Current study aims at establishing an effective CADD protocol for identification of IL-2 inhibitors as immunomodulating agents following structure based pharmacophore protocol using MOE software. Pharmacophore based virtual screening and molecular docking studies were used for hits identification. The binding mode analysis of these hits revealed that these compounds were able to block binding site of IL-2/IL-2Ra. In the next step these hits were optimized and forty six synthetic analogs of these hits were synthesized in wet lab using different experimental procedures.

Synthesis of chalcones (1-7) was carried out by Claisen Schmidt condensation. These synthesized chalcones were used as precursor for the synthesis of dihydropyrimidines (8-13), benzodiazepines (14-16), benzothiazepines (17-19), pyrazole carbaldehydes (20-25) and pyrazole carbothioamides (26-28). A series of benzimidazole derivatives (29-32) was synthesized by reacting an aldehyde with sodium metabisulfite, followed by the reaction of adduct with phenylene diamine. Anthraquinone derivatives of benzimidazoles (33-36) were prepared by condensation reaction of benzimidazoles with anthraquinone sulfonyl chloride. Schiff base derivatives (37-41) were synthesized by reacting aromatic amines with different substituted benzaldehydes. Multistep synthesis of pyrazole derivatives (42-43) was carried out by the reaction of anilines and ethylacetoacetate followed by the reaction of product with 8-quinolinoxy acetic acid hydrazide in acetic acid solution. Benzamide derivatives (44-46) were synthesized by reacting ibuprofen and cetirizine with aromatic amines. The newly synthesized compounds were characterized by their IR, MS, $^1$H and $^{13}$C NMR spectral data.

All the synthesized compounds were subsequently assayed for their ability to inhibit IL-2 production and they were found to be potent inhibitors with IC_{50} values ranging from < 2 to >50 μg/ml. These compounds may function as a starting point for the discovery of promising candidates as immunomodulatory agents. Moreover the synthesized derivatives of cetirizine (30, 36, 45) and ibuprofen (29, 35, 44) may serve as potential candidates for the development of repurpose drugs.

Current study resulted in a successful designing and synthesis of new IL-2 inhibitors in different rounds of dry and wet labs. Since all the compounds obeyed RO5 cut off limits, therefore, they may prove to be safe for human consumptions after their ADMET prediction.
4.2 FUTURE RECOMMENDATION

A variety of novel IL-2 inhibitors having different chemical scaffolds were designed through pharmacophore based designing protocol in dry lab subsequently synthesized in wet lab. All the compounds were found to inhibit IL-2 with moderate to high activity. To date no data is reported on the exact mechanism of action of these compounds, which deserves further investigation. Moreover, pharmacological, toxicological and clinical studies would be essentially required to elucidate the exact mechanism of IL-2 inhibition and to establish their efficacy and safety for their use as new immunomodulators.
References
REFERENCES


17. Ghaffar, A. Immunology Chapter Seventeen: Hypersensitivity Reactions. Microbiology and Immunology USC School of Medicine.: 2006.


39. Emerson, S. D.; Palermo, R.; Liu, C. M.; Tilley, J. W.; Chen, L.; Danho, W.; Madison, V. S.; Greeley, D. N.; Ju, G.; Fry, D. C. NMR characterization of interleukin-2 in complexes with the IL-2Rα receptor component, and with low molecular weight compounds that inhibit the IL-2/IL-Rα interaction. Protein science. 2003, 12, 811-822.


80. Molecular Operating Environment (MOE) [2012.10]. Chemical Computing Group, Inc.: Montreal, Quebec, Canada: 2012.


REFERENCES


REFERENCES


REFERENCES


188. Insuasty, B.; Tigreros, A.; Orozco, F.; Quiroga, J.; Abonía, R.; Nogueras, M.; Sanchez, A.; Cobo, J. Synthesis of novel pyrazolic analogues of chalcones and their 3-aryl-4-(3-aryl-4, 5-dihydro-1H-


Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*. 2012, 64, 4-17.


Annexure 1

$^1$H NMR & $^{13}$C NMR Spectra of compound 10
Annexure 2

GCMS of compound 12
Annexure 3

LCMS of compound 14
Annexure 4

$^1$H NMR & $^{13}$C NMR Spectra of compound 15
Annexure 5

GCMS of compound 25
Annexure 6

$^1$H NMR & $^{13}$C NMR Spectra of compound 25
Annexure 7

$^1$H NMR & $^{13}$C NMR Spectra of compound 28
Annexure 8

GCMS of compound 31
Annexure 9

$^1$H NMR & $^{13}$C NMR Spectra of compound 36
Annexure 10

LCMS of compound 39
Annexure 11

$^1$H NMR & $^{13}$C NMR Spectra of compound 43
Annexure 12

$^1$H NMR & $^{13}$C NMR Spectra of compound 45


15. *In silico* Molecular Docking and Design of Anti-Hepatitis B Drugs, Mahira Arooj, Madiha Sattar, Muniba Safdar, Saima Kalsoom, Shaheen Shahzad. *IOSR Journal of Pharmacy and Biological Sciences*, 2012, 3(6), 41-45.


19. In vitro and in silico exploration of IL-2 inhibition by small drug-like molecules, Saima Kalsoom, Umer Rashid, Awais Shaukat, Omer Mohamed Abdalla, Khalida Hussain,
LIST OF INTERNATIONAL PUBLICATIONS


155