Synthesis, characterization and biological activities of heterocycles: Peptides, O, N and S based small molecules

This work is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry, in the Faculty of Applied Sciences, at Durban University of Technology

Mr. Muthu Thangaraj

2018

Supervisor: Prof. Robert Moonsamy Gengan
DECLARATION

This thesis is being submitted to the Durban University of Technology for the degree of Doctor of Philosophy in Chemistry. I declare that this work is my own and has not been submitted before for any degree or examination to this or any other university or institution for this or any other degree or award.

Student Number: 21356991

Student: [Signature]  
Mr. Muthu Thangaraj  
Date: 30/04/2018

Supervisor: [Signature]  
Prof. Robert Moonsamy Gengan  
Date: 30/07/2018
Acknowledgement

I still remember the occasion, when I was only three years old, my mother took me in front of Goddess with my father, gave me a slate and chalk and taught me to write the letters அ, ஆ, இ, ஈ… etc. Today, I have finished writing my Ph.D. thesis. Since then it has been a long journey, and my father is always beside me keeping his hand on my shoulder to give me all the support that a father can give to his dearest son to see him on top of the world. My mother’s unconditional love, care and prayers have always been with me. She wanted to see me achieve the apex of education; and I am proud that I am still climbing the apex because of her love and wishes. My parents were the first and last to believe that I can achieve my dreams. I don’t like to thank my mother and father by means of just a few words. Today, I bow down in front of them for everything.

I am grateful to the lord almighty for his abundant blessings that have lifted me up to this level. I feel immense pleasure in expressing my deep sense of gratitude and indebtedness to my esteemed Gurunathar, Professor Robert Moonsamy Gengan, Department of Chemistry, Durban University of Technology, Durban, for his outstanding guidance, constructive criticism, motivation, valuable advice, untiring support and constant encouragement throughout the study holding me strong in all the places I faltered. I express my heartfelt thanks to Dr. D.H. Pienaar, Dr. Charlette Tiloke, Professor K.G. Moodley, Professor G.G. Redhi, Professor K. Bisetty and Dr. K. Ramluckan, Head of Department, Department of Chemistry, for their timely help and support.

I extend my thanks to Professor P. S. Mohan, Head of Department of Chemistry, Bharathiar University, for his motivation and encouragement for my higher studies. It is my pride and pleasure to seize the opportunity to record my deep sense of gratitude to Dr. K. J. Rajendra Prasad, former Professor and Head (UGC-Emeritus Professor), Dr. S. Govindarajan (UGC-Emeritus Professor), Dr. S. P. Rajendran, Professor (Retd), Dr. M. Ilanchelian, Asst. Professor and Dr. R. Prabhakaran, Asst. Professor, Department of Chemistry, Bharathiyar University. I sincerely thank Dr. K. Natarajan, the CSIR-Emeritus Scientist for his kind encouragement.

I express my profound thanks to Professor, Reji Varghese, Scientist, Indian Institute of Science Education and Research, Thiruvananthapuram, Kerala, India.

I also express my sincere thanks to Mr. Dilip Jagjivan, University of KwaZulu-Natal, Westville campus. Without him, a major part of my thesis would have remained blank. Thanks for filling the gap and training me in NMR spectral data acquisition. Words seem to be inadequate to express my heartfelt thanks to my colleagues Mr. M. Arul, Mr. A. Vasanthakumar, Mr. A. Nandhakumar, Dr. Deepak Gussain, Dr. Abishek Guldhe, Dr. G. Yathirajan and Mr. Ajay.
Vasudeo Rane for providing food, spending money all the time, their limitless care and support during the stay and their countless help to compile this dissertation successfully.

I am greatly indebted to Mr. G. Sateesh Kumar, Govt. Arts College, Nandanam, Dr. P. Govindaraj, Associate Professor and Head, Mrs. S. Radha, Mr. P. Ramasamy and Mr. C. Sathya Kumar, Department of Chemistry. I also thank Mr. Venkata Subbian, Department of Physics for his guidance. I wish to extend my thanks to Dr. D. Jacqueline Periyayakam, Associate Professor and Head, Dr. P. Rajendran, former Head and Professor, Mrs. A. Kamaleswari, Department of English, Dr. K. Sellathai and Mrs. Kaliyanantham, Department of Tamil, SBK College for their support and motivation.

I wish to thank my senior Dr. K. Anand for introducing Durban University of Technology in my life. I extend my thanks to Research seniors Dr. R. Selvakumar, Dr. A. Selvasharma, Dr. K. Shanmugaraj and Dr. P. Manivel, Dr. S. Packiyaraj, Bharathiar University, for their advice and motivation. I wish to extend my thanks to Mr. Jimmy Chetty, Dr. Thishana Singh and Mr. Rajein, other non-teaching staff members of the department for their encouragement and support.

I express my sincere thanks to Mr. Talent Makhanya for letting me to go forward and his motivation in all the time, Mr. M. Sureshkumar for supporting and motivating me in all the time and his mother for her wishes, Dr. Sivanandhan, Ms. Nikisha Rajkoomar and Ms. Kaunda Thabisile for their care, discussion and support. I also thank Dr. M. Shree Ramesh, Dr. B. Ravindran, Mr. Faiz Ahmad, Dr. Sanjay Kumar Gupta, Mrs. Poonam Singh, Dr. Gulshan Singh, Dr. Venkat, Dr. Mithil Kumar, Mr. Bibhuti Ranjan, Ms. Deepthi and Mr. Srinivasan for their support in tough times.

Acknowledgement will be unsound if I fail to thank my friends Mr. K. Neppoliyan and his family, Mr. N. Hariharan and his family, Mr. G. Naveen Vasu, Mr. M. Manikandan (Remo) and his mother, Mr. S. Suman, Mr. Senthilkumar, Mr. Kashi Viswanath and his family, Mr. B. Tamilarasan, Mr. S. Samraj, Mr. B. Alagarsamy, Dr. Deepak Raja (MBBS, Russia), Ms. A. Gnanasundari and her family, Mrs. Gnanadeepam and her family, Mrs. Karthigai Priya, Ms. Jeyadevi, Mrs. K. Suganya, Mrs. Bavani, Mrs. Revathy, Mrs. K. Suryakala, Mrs. R. Muthu Priya and her family, Ms. Chitra, Ms. Ajantha, Mrs. K. Suriya Praba, undergraduate and post-graduate friends.

In addition, several people have knowingly and unknowingly helped me in the successful completion of this project. My sincere gratitude goes to Professor Sibusiso Moyo, Deputy Vice-Chancellor and Dr. Bloodless Dzwario, Grants Administrator, for their help and my fellowship arrangements for research project. I acknowledge the Durban University of Technology and National Research Foundation for the financial support. My sincere gratitude goes to Professor Suren Singh, Executive Dean, Ms. Gill Shackleford, Faculty officer of the Faculty of Applied
Science, Durban University of Technology. I take this golden opportunity to express my heartfelt thanks to mother Mrs. Shirley Gengan and sisters Dr. Kerena and Ms. Trinisha for their affection and help.

I take this golden opportunity to express my heartfelt thanks to my parents Mr. V. Thangaraj and Mrs. T. Pandiyammal @ Parimala (late), my brothers Mr. T. Thirumurugan, Mr. T. Elangeswaran and his wife Mrs. E. Kanaga, my sisters Ms. T. Sivanandhini, Mrs. S. Krishnaveni and her husband Mr. S. Saravanan for their everlasting love, support, motivation and endless help.

I also widen my thanks to my uncle Mr. Mariyappan, Police Inspector Mr. Chinnathambi @ Selvaraj (late), Mrs. Chinnathayee, Mrs. Parvati (late), Mr. K.P. Karuppu and his family, Mr. Ravikumar and his wife, Mr. Jegan, Mr. Suresh, Mr. Ganesh and his family, Thandiyampatti Mr. Prabhu and his family, brother Mr. Muthupandi and his family, uncle Mr. Bose and his family, Mr. Ilaiyaraja and other relatives for their care and support.

I am deeply indebted to many, who helped me in different ways throughout this dissertation and without whom it could not have been achieved.

Muthu Thangaraj.
தான் கஷட்டுபட்டாலும் தான் பட்ட கஷட்டுகளை ஒன்றியாலும் பசங்க படக்கூடாது
மளையிலும் வெயில்லலயும் கஷட்டுபட்டுக்கூலின் பரவல் தை வெளிவந்து
புஷ்கரக் பட்ட வெளிவந்து கஷட்டுபட்டால் அப்பாவுக்கு. தன் பெருமாள் கஷட்டு
இருக்கின்றனர். ஒன்றிய ஒன்றிய ஓர் செயலானது கஷட்டுபட்டுக்கூலின் பரவல்
தை வெளிவந்து கஷட்டுபட்டால் அப்பாவுக்கு அப்பாவுக்கு அப்பாவுக்கு
நின்று செயல்கூடாது கஷட்டு பழந்தல்லூர் கஷட்டுக்கிறத்து....

அன்புடன், த. முத்து.
General remarks

The numbers representing the structure are for the particular chapter only. Each chapter contains a separate experimental section. The following abbreviations are used in text:

- **MDR**: multiple drug resistance
- **SAR**: structure-activity relationship
- **SBDD**: structure-based drug design
- **TOF**: time of flight
- **MS**: mass spectra
- **m.p**: melting point
- **℃**: Centigrade
- **mmol**: Milli mole
- **HASIL**: humic acid supported ionic liquid
- **nm**: Nano meter
- **BN**: boron nitride
- **Fe/BN**: iron loaded boron nitride
- **I/BN**: iodine loaded boron nitride
- **Ca/BN**: calcium loaded boron nitride
- **min**: minutes
- **TLC**: thin layer chromatography
- **XRD**: X-ray diffraction
- **SEM**: scanning electron microscopy
- **EDX**: Energy Dispersive X-ray
- **BET**: Brunauer-Emmett-Teller
- **TEM**: transmission electron microscopy
- **DSC**: differential scanning calorimetry
- **TGA**: thermogravimetric analysis
- **FTIR**: Fourier transform infrared spectroscopy
- **NMR**: nuclear magnetic resonance
- **POCl₃**: phosphoryl chloride
- **EtOAc**: ethyl acetate
- **PE**: petroleum ether
- **CHCl₃**: chloroform
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>US</td>
<td>United States of America</td>
</tr>
<tr>
<td>CHF</td>
<td>congestive heart failure</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>Et&lt;sub&gt;3&lt;/sub&gt;N</td>
<td>triethylamine</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>half maximal inhibitory concentration</td>
</tr>
<tr>
<td>THAM</td>
<td>tris-hydroxymethylaminomethane</td>
</tr>
<tr>
<td>TB</td>
<td>tuberculosis</td>
</tr>
<tr>
<td>Topo-I</td>
<td>topoisomerase-I</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>1,4-DHPs</td>
<td>1,4-Dihydropyridines</td>
</tr>
<tr>
<td>mGlu 1</td>
<td>metabotropic glutamate receptor 1</td>
</tr>
<tr>
<td>DLA</td>
<td>dihydrolipoic acid</td>
</tr>
<tr>
<td>AChE</td>
<td>acetylcholinesterase</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>AIDS</td>
<td>acquired immune deficiency syndrome</td>
</tr>
<tr>
<td>MCRs</td>
<td>multicomponent reactions</td>
</tr>
<tr>
<td>Ugi-4CR</td>
<td>Ugi four component reaction</td>
</tr>
<tr>
<td>UDC</td>
<td>Ugi-Deprotection-Cyclization</td>
</tr>
<tr>
<td>PTSA</td>
<td>p-Toluenesulfonic acid</td>
</tr>
<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
</tr>
<tr>
<td>DMAP</td>
<td>dimethylamino pyridine</td>
</tr>
<tr>
<td>HMTA</td>
<td>hexamethylenetetramine</td>
</tr>
<tr>
<td>CTABr</td>
<td>cetyl trimethyl ammonium bromide</td>
</tr>
<tr>
<td>MW</td>
<td>microwave</td>
</tr>
<tr>
<td>Cs&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Cesium carbonate</td>
</tr>
<tr>
<td>Pd/BN</td>
<td>palladium loaded on boron nitride</td>
</tr>
<tr>
<td>DETA</td>
<td>diethylenetriamine</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>ILs</td>
<td>ionic liquids</td>
</tr>
</tbody>
</table>
S. aureus: *Staphylococcus aureus*
B. cereus: *Bacillus cereus*
E. faecalis: *Enterococcus faecalis*
P. aeruginosa: *Pseudomonas aeruginosa*
E. coli: *Escherichia coli*
C. albicans: *Candida albicans*
UTI: urinary tract infections
DPPH: 2,2-diphenyl-1-picrylhydrazyl-hydrate
QLPs: quinolinyl-lipoyl peptides
QOLP: quinolonyl-lipoyl peptide
rt: room temperature
[bmim]BF₄: 1-butyl-3-methylimidazolium tetrafluoroborate
LB: Luria Bertani
PD: potato dextrose
MIC: minimal inhibitory concentration
QPs: quinolinyl-4H-pyrans
QOPs: quinolonyl-4H-pyrans
HASIL: humic acid supported ionic liquid
IPs: indolyl-4H-pyrans
HEAA: 2-hydroxyethyl ammonium acetate
BAIL: Brønsted acid ionic liquid
HA: humic acid
[bmim]SCN: 1-butyl-3-methylimidazolium thiocyanate
CFQ: 2-chloroquinoline-3-carbaldehyde
CMFQ: 2-chloro-6-methylquinoline-3-carbaldehyde
CFFQ: 2-chloro-7-fluoroquinoline-3-carbaldehyde
MQC: 2-methoxyquinoline-3-carbaldehyde
IOPN: 3-(3H-indol-3-yl)-3-oxopropanenitrile
BTQPs: α-aminobenzylthioquinolinyl phosphonates
APs: α-aminophosphonates
h-BN: hexagonal boron nitride
B₂O₃: boron trioxide
BTQC: 2-(benzylthio)quinoline-3-carbaldehyde
BTQ-DHPs: benzylthioquinolinyl-1,4-dihydropyridines
A Labcon Ultrasonic 5019U was used to generate ultrasonic irradiation and homogenize the reaction mixture. CEM discover microwave reactor was used for the reaction.

All chemicals were purchased from Sigma-Aldrich and used without further purification. Solvents used were of synthesis grade. The X-Ray diffraction analysis was conducted with a Philips PW 1050 diffractometer set at 1 minute with a scanning step size of 0.02° from 40° to 100° 2θ using monochromated CoKα irradiation. Data was captured with a sietronics 122D automated microprocessor linked to a diffractometer. A Carl Zeiss Ultra Plus scanning electron microscope with EDX detector was also used. The TGA and DSC analysis were conducted with TA instruments. TEM analysis was conducted with a JEOL 1010 TEM (Korea, Japan) and images were captured with iTEM Images capture software (v5.0). Melting points were determined by Stuart SMP10 and were uncorrected. The IR spectra were recorded on Perkin Elmer 537 spectrophotometer instrument, using ATR disc and the absorption frequencies were expressed as \( \nu_{\text{max}} \) cm\(^{-1}\). \(^1\)H-NMR and \(^{13}\)C-NMR spectra were recorded on BRUKER 400 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. The chemical shifts were quoted in parts per million (ppm). The following abbreviations are used:

- \( s \) : singlet
- \( \text{brs} \) : broad singlet
- \( d \) : doublet
- \( \text{dd} \) : doublet of doublet
- \( \text{dt} \) : doublet of triplet
- \( t \) : triplet
td : triplet of doublet
q  : quartet
m  : multiplet
\(J\) : coupling constant in Hertz (Hz)

The chemical shift values were recorded on \(\delta\) scale and the coupling constants \((J)\) were expressed in hertz. The progress of the reaction was monitored by TLC using aluminium plates with silica gel (Sigma-Aldrich and Fluka). Columns packed with activated silica gel (60-120 mesh) were used to purify the crude products. Petroleum ether used was of boiling range 60-80°C.

For some of the compounds especially for \(^1\)H-NMR values, splitting patterns, integral values and the intensity of peaks were ascertained from its expanded version of the spectrum and the copies are not produced in the thesis. \(J\) values were averaged. The elemental analyses (C, H and N) were obtained from a Perkin Elmer precisely 2400 analyzer.
# CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Declaration</td>
<td></td>
<td>i</td>
</tr>
<tr>
<td>Acknowledgement</td>
<td></td>
<td>ii</td>
</tr>
<tr>
<td>Dedication</td>
<td></td>
<td>v</td>
</tr>
<tr>
<td>General remarks</td>
<td></td>
<td>vi</td>
</tr>
<tr>
<td>Abstract</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Chapter-I</td>
<td>Introduction</td>
<td>3</td>
</tr>
<tr>
<td>Chapter-II</td>
<td>Literature survey</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2.1. Heterocyclic chemistry: oxygen, nitrogen and sulfur heterocycles</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2.1.1. The synthesis and importance of oxygen heterocycles</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2.1.2. The synthesis and importance of nitrogen heterocycles</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>2.1.3. The synthesis and importance of sulfur based heterocycles</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>2.2. Multi-component reactions</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>2.3. Ultrasonic and microwave irradiations</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>2.4. Heterogeneous catalysis</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>2.5. Biological applications</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>2.5.1. Antibacterial and antifungal activity against human diseases</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5.1.1. Antibacterial activity against <em>Staphylococcus aureus</em></td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>2.5.1.2 Antimicrobial activity against <em>Bacillus cereus</em></td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>2.5.1.3 Antibacterial activity against <em>Enterococcus faecalis</em></td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>2.5.1.4 Antibacterial activity against <em>Escherichia coli</em></td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>2.5.1.5. Antibacterial activity against <em>Pseudomonas aeruginosa</em></td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>2.5.1.6 Antifungal activity against <em>Candida albicans</em></td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>2.5.1.7 Antifungal activity against <em>Candida utilis</em></td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>2.5.2. Antioxidant activities</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>2.5.3. Toxicity assessment</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>2.5.4. Molecular Docking</td>
<td>53</td>
</tr>
<tr>
<td>Chapter-III</td>
<td>Synthesis of quinolinyl and quinolonyl-lipoyl peptides under microwave irradiation and antimicrobial, antioxidant, toxicity and molecular docking studies</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.1. Abstract</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>3.2. Introduction</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>3.3. Results and discussion</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>3.4. Conclusion</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>3.5. Experimental</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>Appendix-III</td>
<td>111</td>
</tr>
<tr>
<td>Chapter-IV</td>
<td>Microwave synthesis of quinolinyl, quinolonyl and indolyl pyrans by humic acid supported ionic liquid catalyst and antimicrobial, antioxidant, toxicity and molecular docking studies</td>
<td></td>
</tr>
<tr>
<td>Chapter</td>
<td>Title</td>
<td>Pages</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>V</td>
<td>Synthesis of α-aminobenzylthioquinolinyl phosphonates catalysed by an iron-loaded boron nitride material and their antimicrobial, antioxidant, toxicity assessment and molecular docking studies</td>
<td>221-259</td>
</tr>
<tr>
<td>VI</td>
<td>Ultrasonicated synthesis of benzylthioquinolinyl-1,4-dihydropyridines by iodine-loaded boron nitride catalyst and their antimicrobial, antioxidant, toxicity assessment and molecular docking studies</td>
<td>299-336</td>
</tr>
<tr>
<td>VII</td>
<td>Calcium loaded boron nitride material as a new catalyst for the synthesis of 2-amino-4H-pyran-3-carbonitriles and antibacterial studies</td>
<td>368-425</td>
</tr>
</tbody>
</table>

**Conclusion and recommendation for future studies**

List of conferences attended 428
List of publications 429
Abstract

This study is based on the synthesis and characterization of quinoline based peptides and heterocycles containing oxygen, nitrogen and sulfur atoms by using new catalysts. In addition, the biological activities of the novel small molecules is evaluated. A total of 71 small molecules were prepared by using multi-component reactions including Ugi and Kabachnik-Fields reaction. The Ugi four-component reaction was implemented for the synthesis of medicinally important 13 new quinolinyl-lipoyl peptides (QLPs) and one quinolonyl-lipoyl peptide (QOLP) by microwave irradiation using methanol as medium. A series of 12 new quinolinyl-4H-pyrans (QPs), two quinolonyl-4H-pyrans (QOPs) and one indolyl-4H-pyran (IP) were successfully synthesized via a three-component reaction using ethanol as solvent in the presence of a new catalyst: humic acid supported 1-butyl-3-methylimidazolium thiocyanate ionic liquid catalyst (HASIL) under microwave irradiation. By using Kabachnik-Fields reaction, a total of 14 novel α-aminobenzylthio-quinolinyl phosphonates (BTQPs) were synthesized in the presence of a catalytic amount of iron-loaded boron nitride (Fe/BN) catalyst by using water as medium. A series of 14 novel benzylthioquinolinyl-1,4-dihydropyridines (BTQ-DHPs) were synthesized with high yields in short reaction time by a four-component reaction in the presence of iodine-loaded boron nitride (I/BN) catalyst by using water as solvent. A total of 14 derivatives of 2-amino-4H-pyran-3-carbonitrile derivatives (APCs) were prepared by using calcium loaded boron nitride (Ca/BN) in ethanol as solvent. This transformation transpired via a Knoevenagel condensation, Michael addition and intra-molecular cyclization. The prepared catalysts: HASIL, Fe/BN, I/BN and Ca/BN were characterized by XRD, SEM with EDX, TEM, DSC, TGA, BET, Raman spectra and FTIR analysis. All the synthesized molecules (QLPs, QOLP, QPs, QOPs, IP, BTQPs, BTQ-DHPs and APCs) were confirmed by FTIR, 1H-NMR, 13C-NMR and elemental analysis. Moreover, 19F-NMR, 31P-NMR and TOF-MS analysis were included for some selected compounds. In every chapter, one model compound was selected and discussed with two-dimensional spectra such as HSQC, DEPT 90°, DEPT 135° (selected), COSY, NOESY and HMBC. Among the synthesized compounds, a total of 48 compounds (8 QLPs, 15 QPs, QOPs and IP), 10 BTQPs, 10 BTQ-DHPs and 5 APCs) were subjected to antimicrobial activities with Bacillus cereus, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, Candida albicans and Candida utilis and antioxidant studies were observed by the radical scavenging assay. The toxicity studies were
evaluated using the brine shrimp assay and the mortality rate was noted. Among them, 4 peptides, 7 pyrans, 8 aminophosphonates, 7 dihydropyridines and 5 carbonitriles showed good antimicrobial activity whilst 3 peptides, 9 pyrans, 6 aminophosphonates and 4 dihydropyridines showed antioxidant potential. Also, 4 peptides, 5 pyrans, 8 aminophosphonates and 5 dihydropyridines showed mortality rate less than 50% up to 48 h. The molecular docking studies were performed by Libdock score with DNA gyrase, Mtb gyrase and Staphylococcus aureus gyrase. A docking score of 183.24 kcal/mol and 165.01 kcal/mol were recorded for 2 peptides compared to ciprofloxacin. Among quinolinyl pyrans, one QP showed higher binding affinity of 96.96 kcal/mol with Mtb DNA gyrase. One BTQP showed more potency towards Staphylococcus aureus gyrase with 149.97 kcal/mol and one BTQ-DHP showed a strong ligand-protein interaction toward Staphylococcus aureus gyrase with Libdock score of 125.27 kcal/mol. The advantages of the synthetic methodology of this project are its green approach, easy work up, mild reaction conditions, the use of an inexpensive solvent, short reaction times with higher yields and recyclability of the catalyst.
Chapter-I

Introduction
Heterocyclic chemistry is an interesting branch of organic chemistry with practical and theoretical importance. It is a vast and expanding area of chemistry due to heterocyclic compounds being used in pharmacy, medicine, agriculture, plastic, polymer and several other fields. Many heterocyclic compounds are either synthesized or derived from natural sources which display therapeutic properties as they are employed in the treatment of diseases such as infection by multidrug-resistant bacteria, viral influenza, leishmaniasis, coccidiosis and infectious septic shock. There are millions of heterocycles already available however studies on heterocycles are in-exhaustive. More efficient and effective synthesis of new heterocycles is an on-going aim of synthetic chemists. Furthermore, their biological properties require investigation so that new drugs are developed and made available to improve human health.

An important structural feature of heterocycles is their ability to manifest substituents around a core scaffold in defined three dimensional representations. This is an advantage that can enable skilled researchers to strategically manipulate the molecule thereby leading to a variety of new compounds with multi-functional groups which can even be further modified. To accomplish this task, new synthetic strategies need to be implemented including the use of new catalysts, non-conventional heating protocols, new substrates, multi-component reactions, new reagents and reaction conditions.

The aim of this study is to prepare quinoline based peptides and synthesis of novel heterocycles containing oxygen, nitrogen and sulfur atoms by using new catalysts. In addition, the biological activities of the novel small molecules will also be evaluated.

The objectives were to synthesize and characterize:

1. Quinolinyl-lipoyl and quinolonyl-lipoyl peptides and determine their antimicrobial and antioxidant potential, evaluate toxicity and assess their binding with DNA-gyrase by molecular docking.

2. Humic acid-supported ionic liquid catalyst and quinolinyl-4H-pyrans, quinolonyl-4H-pyrans and indolyl-4H-pyran and determine their antimicrobial and antioxidant potential, evaluate toxicity and assess their binding with Mycobacterium tuberculosis (Mtb) gyrase by molecular docking.
3. Iron-loaded boron nitride catalyst and α-aminobenzylthioquinolinyl phosphonates and determine their antimicrobial and antioxidant potential, evaluate toxicity and assess their binding with *Staphylococcus aureus* gyrase by molecular docking.

4. Iodine-loaded boron nitride catalyst and benzylthioquinolinyl-1,4-dihydropyridines and determine their antimicrobial and antioxidant potential, evaluate toxicity and assess their binding with *Staphylococcus aureus* gyrase by molecular docking.

5. Calcium-loaded boron nitride catalyst and 2-amino-4H-pyran-3-carbonitriles and assess their anti-bacterial potential.

The **second chapter** of the thesis discusses and presents the literature on the heterocyclic system, in particular, the synthesis and utilization of oxygen, nitrogen and sulfur heterocyclic molecules. The advantages of multi-component synthesis, multidisciplinary of organic synthesis, green and sustainable methods via microwave and ultrasonic irradiation are also described. The necessity of a green catalyst in one-pot multi-component reactions is proposed. The importance of synthesizing drugs which can be used against infections from pathogenic bacteria and fungi such as *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Candida albicans* and *Candida utilis* is also discussed. Relevant literature on antioxidants, toxicity and molecular docking studies are described and discussed.

The **third chapter** reports a simple, cost effective, catalyst-free and eco-friendly synthesis of quinolinyl-lipoyl peptides and quinolonyl-lipoyl peptide through an Ugi four-component condensation reaction under microwave irradiation.
Lipoic acid, 2-methoxyquinoline-3-carbaldehyde derivatives, aniline derivatives and cyclohexyl isocyanide were employed to synthesize quinolinyl-lipoyl peptides. Quinolonyl-lipoyl peptide was synthesized from 2-oxo-1,2-dihydroquinoline-3-carbaldehyde, o-anisidine and cyclohexyl isocyanide. A total of 14 peptides were synthesized within 15-18 minutes. All synthesized peptides were characterized by FTIR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis. A total of eight peptides were subjected to antimicrobial, antioxidant and toxicity evaluation. In addition, toxicity of all peptides was evaluated using the brine shrimp test. Molecular docking studies were also conducted to determine the binding with DNA gyrase based on Libdock score.

The **fourth chapter** discusses the synthesis and applications of quinolinyl-$4H$-pyrans, quinolonyl-$4H$-pyrans and indolyl-$4H$-pyran. A series of 2-methoxyquinoline bearing $4H$-pyrans were synthesized under microwave irradiation using 2-methoxyquinoline-3-carbaldehyde derivatives, malononitrile and 1,3-diketones.

Two quinolonyl-$4H$-pyrans were synthesized from substrates 2-oxo-1,2-dihydroquinoline-3-carbaldehyde derivatives, malononitrile and 1,3-diketone and one indolyl-$4H$-pyran was synthesized from substrates 4-methylbenzaldehyde, malononitrile and 3-((3H-indol-3-yl)-3-oxopropanenitrile. A new humic acid-supported 1-butyl-3-methyl-imidazolium thiocyanate ionic liquid (HASIL) was used. The catalyst was characterized by XRD, SEM with EDX, TEM, TGA, DSC and FTIR analysis. All the synthesized $4H$-pyrans were characterized by FTIR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis. A total of 15 compounds were subjected to antimicrobial, antioxidant and toxicity evaluation. Also, the toxicity of all pyrans was evaluated using the brine shrimp test. Molecular docking studies were undertaken to determine binding with Mtb DNA gyrase based on Libdock score.
The **fifth chapter** discloses the synthesis of $\alpha$-aminobenzylthioquinolinyl phosphonates (BTQPs) and their applications. A series of $\alpha$-aminobenzylthioquinolinyl phosphonates were synthesized within 5-10 min by using a new iron-loaded boron nitride catalyst under microwave irradiation in water. The catalyst was characterized by XRD, SEM with EDX, TEM, TGA, DSC and FTIR. 2-(benzylthio)quinoline-3-carbaldehyde, various aniline derivatives and diethylphosphite were used to synthesize the phosphonates.

All synthesized $\alpha$-aminobenzylthioquinolinyl phosphonates were characterized by FTIR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis. A total of 10 compounds were subjected to antimicrobial, antioxidant testing and toxicity was assessed using the brine shrimp test. Molecular docking studies were conducted to determine binding with *Staphylococcus aureus* gyrase based on Libdock score.

The **sixth chapter** discusses the synthesis of benzylthioquinolinyl-1,4-dihydropyridines (BTQ-DHPs) and their applications.
A novel iodine-loaded boron nitride heterogeneous catalyst was used. The catalyst was characterized by XRD, SEM with EDX, TEM, TGA, DSC and FTIR. 2-(benzylthio)quinoline-3-carbaldehyde, dimethyl acetylenedicarboxylate, malononitrile and aniline derivatives were used as substrates. All synthesized benzylthioquinolinyl-1,4-dihydropyridines were characterized by FTIR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis. A total of 10 compounds were subjected to antimicrobial, antioxidant and toxicity evaluation. Also, the toxicity of all pyrans was evaluated using the brine shrimp test. Molecular docking studies were used to determine the binding with *Staphylococcus aureus* gyrase based on Libdock score.

The **seventh chapter** of the thesis presents the synthesis of 2-amino-4H-pyran-3-carbonitriles by a cheap alkaline earth metal calcium-loaded boron nitride catalyst. This transformation proceeds via a Knoevenagel condensation, Michael addition and intramolecular cyclization. An alkaline earth metal-based green catalyst was successfully prepared and characterized by XRD, SEM with EDX, Raman spectroscopy, BET, DSC-TGA and FTIR. All synthesized 2-amino-4H-pyran-3-carbonitriles were characterized by FTIR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis. A total of 5 compounds were subjected to antimicrobial evaluation against pathogenic bacteria *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* and the results are discussed.
Chapter-II

Literature survey
Chapter Two

Literature survey

Every living organism is made up of organic molecules. The proteins that constitute our hair, skin and muscles; the DNA that controls our genetic heritage; the foods that nourish us; the clothes that keep us warm; and the medicines that heal us are all made up of organic molecules (McMurry, 1999). Chemistry has played an important role in our understanding of the universe. It is the science of molecules. However, organic chemistry is more complexed. It evolves and re-creates itself as it grows. It is imperative that we study the various molecules in nature because they are interesting in their own right and their functions are important to our lives.

Organic chemistry often studies life by developing new and novel molecules that provide information which is not readily available from existing molecules that are present in living organisms. The creation of new molecules has led to new materials and products such as plastics, dyes to colour clothes, perfumes and drugs to treat and cure various diseases. Early Asian civilization (2500-3000 BC) extensively used natural materials from herbal extracts (Ayurveda) for treating illnesses (Patwardhan et al., 2005). One such extract was “ma huang” (Ephedra sinica) which reduced elevated blood pressure (Chan et al., 2016). It was found that it contained ephedrine, an organic compound similar in structure and physiological activity to adrenaline, a hormone secreted by the adrenal gland. Currently, almost all drugs that are prescribed for treatment of diseases are organic compounds. However, some are derived from natural sources; but many are the products of synthetic organic chemistry.

Morphine was isolated from opium produced from seed pods of the poppy plant (Papaver somniferum) (Da Cheng et al., 2015). Inspired by the discovery of penicillin, pharmaceutical research was expanded to include a massive screening of microorganisms for new antibiotics. Over the centuries there has been a long list of drugs discovered, however the ones obtained from natural sources which have undoubtedly revolutionized medicine include antibiotics (Fig. 1) such as penicillin (1), tetracycline, and erythromycin, antiparasitics such as avermectin (4), antimalarials such as quinine and artemisinin (2), lipid control agents such as lovastatin (3) and its analogs,
immunosuppressants such as cyclosporine and rapamycin (6) and anticancer drugs such as paclitaxel (7) and irinotecan (5) (Harvey, 2008).

Fig. 1. Important drugs extracted from natural sources

Modern synthetic organic chemists can synthesize some of the most intriguing natural molecules in the laboratory by new synthetic strategies and technologies. These
molecules improve various fields of study including biology and medicine due to their potent biological activity and can serve as new drug candidates for clinical development. In addition, by employing sophisticated catalytic reactions and appropriately designed synthetic processes, organic chemists can synthesize a myriad of organic molecules for potential applications in many areas of science, technology and in everyday life (Nicolaou, 2014).

The field of total synthesis continues to serve as the ultimate testing ground for new methodologies and strategies. Organic synthesis has experienced a remarkable increase in quality thereby empowering synthetic chemists to construct molecules of any degree of structural complexity. In 1975, Hendrickson defined the “ideal synthesis” as one which: “…creates a complex molecule...in a sequence of only construction reactions involving no intermediary re-functionalizations, and leading directly to the target, not only its skeleton but also its correctly placed functionality” (Hendrickson, 1975). This prescient statement encompassed and epitomized the “economies” of synthesis design (Newhouse et al., 2009). The field of total synthesis has a rich history and a vibrant future. Landmark advances and revolutionary strides in the approach to synthesis of compounds have placed the synthetic chemist in an enviable position of being able to create almost any molecule given sufficient time and resources. The stage is now set for organic chemists to aim for “ideality” in the way molecules are synthesized (Fig. 2).

![Today's Total Synthesis](image1.png)

**Fig. 2.** A schematic diagram showing a footpath of ideal synthesis

![Palauámine (8)](image2.png)
2.1. Heterocyclic chemistry: oxygen, nitrogen and sulfur heterocycles

Heterocyclic chemistry deals exclusively with the synthesis, properties and applications of heterocycles. The synthesis of heterocycles is arguably one of the oldest and most promising disciplines of organic chemistry (Cabrele and Reiser, 2016). Nitrogen, oxygen and sulfur are the most common heteroatoms but heterocyclic rings containing other heteroatoms are also widely known (Franzén, 2000). An enormous number of heterocyclic compounds are known and this number is increasing rapidly. The heterocycle scaffold, present in most medicinal drugs, reflects its central role in modern drug design. These scaffolds can be functionalised with appropriate groups to influence the lipophilicity, polarity and hydrogen bonding capacity of organic compounds, which may lead to improved pharmacological, pharmacokinetic, toxicological and physicochemical properties of new and existing medicinal drugs. Over 80% of the important drugs that were retailed by US (Lindsley, 2015) in 2014, were heterocycles (Fig. 3).

![Chemical structures](image-url)

**Esomerprazol (9)**
Treats: dyspepsia, peptic ulcer disease, gastroesophageal reflux disease

**Budesonide (10)**
Treats: asthma, chronic obstructive pulmonary disease

**Sitagliptin (11)**
Treats: diabetes mellitus type 2

**Rivaroxaban (12)**
Treats: venous thromboembolism
Fig. 3. Important drugs containing the heterocyclic scaffold.

The heterocyclic architecture allows chemists to devise innovative ways for the synthesis of complex structures. Such targets often require difficult transformations that appear impossible to achieve. This challenge is the major driving force for the development of
new and better synthetic methodologies that can subsequently lead to great value-based organic compounds. Moreover, the aim of the synthesis should include sustainable and green principles such as atom and step economy, convergent and cascade strategies, fragment coupling with high functional group tolerance, selective C, H-bond activations with minimal involvement of protecting groups, or catalytic transformations with environmentally benign and cost economic reagents.

2.1.1. The synthesis and importance of oxygen heterocycles

Oxygen heterocycles are an important class of heterocyclic compounds mainly due to their natural abundance and diverse biological functions. Many naturally occurring oxygen heterocycles such as sugars, vitamins, hormones, antibiotics and pigments are biologically active compounds (Xu et al., 2006). Saturated five-membered oxygen heterocycles occur widely in nature, especially as simple sugars. Most naturally occurring sugars are monosaccharides but they also serve as building blocks for other molecules. The anti-arrhythmic drug, amidarone, contains benzene fused furan nucleus (Anderson and Prystowsky, 1999). Six-membered fused pyran nuclei are found in essential natural products such as Vitamin E (α-tocopherol). Generally, heterocyclic compounds are either aliphatic or aromatic in nature. The aliphatic heterocycles are cyclic analogs of amines, ethers, amides etc. while the aromatic heterocyclic compounds behave like benzene. Another sub-class of heterocycles is the benzene fused heterocycles in which the heterocyclic N-ring is fused to one or more benzene rings (Chan, 1974).

Natural products also serve as lead compounds in the development of new drugs. Many natural and semi-synthetic oxygen heterocyclic compounds such as Taxol (Willenbring and Tantillo) as an anti-cancer drug, Digoxin for Congestive Heart Failure (CHF) treatment, Cyclosporine A as an immunosuppressant and Lovastatin as a hypolipidemic agent, are well known for their therapeutic effects (Koehn and Carter, 2005). Several studies have shown that 2-amino-4H-pyrans with amino and nitrile functions at the 2\textsuperscript{nd} and 3\textsuperscript{rd} positions (Fig. 4) are known to possess diverse pharmaceutical properties such as cytotoxic, myorelaxant, antioxidant, anti-proliferative, anti-microbial, anti-HIV, anti-rheumatic and anti-cancer activities (Raj et al., 2010; Saundane et al., 2013; Paliwal et al., 2013; Venkatesham et al., 2012; Makawana et al., 2012; Patil et al., 1993; Smith et al., 1995; Patil et al., 2012).
Fig. 4. The medicinal applications of 4\(H\)-pyrans

Many compounds containing pyran, as the structural unit, have an application as a cognitive enhancer in the treatment of neuro-degenerative diseases like Alzheimer’s and Parkinson’s (Bonsignore et al., 1993; Konkoy et al., 2001). Apart from their medicinal applications, compounds belonging to this class are customarily used in the field of cosmetics (Schweizer and Meeder-Nycz, 1977; Abd El-Rahman et al., 2007; Darbarwar and Sundaramurthy, 1982), agrochemicals (Sofan et al., 1989; Abdel-Galil et al., 1982) as well as dyes (Ellis, 1977; Armesto et al., 1989). Due to their wide range of applications, the development of efficient protocols for the synthesis of compounds containing 2-amino-3-cyano-4\(H\)-pyrans as the structural motif is highly desirable.

The synthesis of 4\(H\)-pyrans involves a one-pot, three-component condensation between an aldehyde, malononitrile and an enolizable C-H acid. The commonly used acids are dimedone, barbituric acid, naphthol (\(\alpha\) and \(\beta\)), 4-hydroxycoumarin, resorcinol, 2-
hydroxy-1,4-naphthoquinone and kojic acid (Han and Xia, 2010). Furthermore, this reaction is known to proceed via a Knoevenagel-Carba-Michael-Throbe-Ziegler type cascade pathway (Han and Xia, 2010).

Shestopalov et al., 2012 reported the synthesis of a series of 4H-pyran (32, 33 and 34), with poly alkoxy substituents (29), in the presence of triethylamine (Et$_3$N) as a catalyst by a Knoevenagel-Michael-hetero-Thore-Ziegler three-component domino reaction. The overall yield of the series of compounds was in the range of 45-82%. The targeted molecules were evaluated in a phenotypic sea urchin embryo assay for anti-mitotic and microtubule destabilizing activity (Tsyganov et al., 2014). The selected compounds of this investigation exhibited strong cytotoxicity in the NC160 human tumor cell line. The results suggested that poly alkoxy substituted 2-amino-4-phenyl-4H-chromene-3-carbonitriles could be feasible as an anti-cancer agent (Shestopalov et al., 2012).

Mahmoodi et al., 2010 synthesized a new series of 2-amino-4H-chromene-3-carbonitriles (37) bearing a 2-aryl thiazole-4-yl (35) moiety and their in-vitro activity was investigated in comparison with etoposide, a well-known anti-cancer drug, using 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) colorimetric assay.
Among them, 2-(2-chlorophenyl)thiazole-4-yl-4H-chromene-3-carbonitrile showed the most potent activity against nasopharyngeal epidermoid carcinoma KB, medulloblastoma DAOY and astrocytoma 1321N1. A 4H-chromene viz. 2-(4-chlorophenyl)thiazole-4-yl moiety exhibited the best growth inhibitory activity against breast cancer cells MCF-7, lung cancer cells A549, colon and adenocarcinoma cells SW480 with IC\textsubscript{50} values less than 5\textmu M (Lee et al., 2011).

Salama et al., 2017 reported an efficient and eco-friendly procedure for the synthesis of bis(4H-chromene-3-carbonitrile) derivatives (40) using chitosan with piperidine as a catalyst. The reaction was carried out under microwave irradiation conditions. The maximum yield of 88% was obtained within ten minutes under reflux.
Gong et al., 2009 designed an efficient and convenient method for the synthesis of polyfunctionalized 4H-pyrans (43, 45 and 46) through the condensation of aldehydes (41), malononitrile (30), 4-hydroxycoumarin (42), phenols (44) or active methylene carbonyl compounds such as 1, 3-cyclohexanedione and dimesdone (39) in the presence of 1-butyl-3-methylimidazolium hydroxide ([bmim]OH) as a catalyst in aqueous media. In this reaction, 10 mol % of the catalyst was used to attain maximum yield. Also, the synthesized compounds were compared with previous studies. Although the procedure was simple and higher product yields were obtained, recyclability of the catalyst was not determined.

Pandit et al., 2015 developed an efficient protocol for the synthesis of medicinally important tetrahydro[b]pyrans (46, 49, 51 and 53) and pyran-annulated heterocycles using a commercially available and non-toxic tris-hydroxymethylaminomethane (THAM) as an organocatalyst. A 30 mol % of the catalyst was used in the reaction to attain the best yield. The avoidance of conventional isolation, the recyclability of the catalyst for five consecutive runs were advantages which improved the practical utility of this reaction protocol.
2.1.2. The synthesis and importance of nitrogen heterocycles

Nitrogen heterocycles are ubiquitous in natural products (Joule, 2016), pharmaceuticals (Joule and Mills, 2010) and materials science (Anton and Baird, 2006; Baxter and Dali-Youcef, 2005; Hab and Mlostoń, 2017). Their preparation has always been an important aspect of organic synthesis. Over the past few decades, great efforts have been made to develop novel and efficient methods for the construction of nitrogen-containing heterocycles (Li and Gribble, 2000; Husson and Royer, 2009; Orru et al., 2010; Eicher et al., 2013; Wolfe, 2013; Vo and Bode, 2014; Allais et al., 2014; Yamamoto, 2014).

Quinoline, a well-known nitrogen heterocyclic compound, and its derivatives are widely used as “parental” compounds to synthesize molecules with medical benefits including anti-malarial and antimicrobial activities. The quinoline scaffold has been reported to possess a diverse range of pharmacological activities (Orhan et al., 2013; Solomon and Lee, 2011; Lavrado et al., 2010; Kumar et al., 2009; Michael, 2002; Michael, 1999; Kharwar et al., 2011; Singh and Bodiwala, 2010; Williams et al., 2011; Chauhan and
Srivastava, 2001; Chen et al., 2001; Roma et al., 2000) including anti-protozoal (Bawa et al., 2010; Gryzlo and Kulig, 2014; Kaur et al., 2010; Gorka et al., 2013; Bongarzone and Bolognesi, 2011; Reynolds et al., 2013), anti-tubercular (Keri and Patil, 2014; Singh et al., 2015), anti-cancer (Solomon and Lee, 2011; Afzal, 2015; Vlahopoulos et al., 2014), anti-psychotics (Zajdel et al., 2014), anti-inflammatory (Mukherjee and Pal, 2013; Mukherjee and Pal, 2013), antioxidant (Vo et al., 2014; Allais et al., 2014; Yamamoto, 2014), anti-HIV (Musiol, 2013), anti-fungal (Musiol, 2010), efflux pump inhibitors (Mahamoud et al., 2006), treatment of neuro-degenerative diseases (Bongarzone and Bolognesi, 2011) and lupus (Costedoat-Chalumeau et al., 2014).

The quinoline moiety also contributes to the effect of drugs used for several other diseases. This includes the fluoroquinoline antibiotic ciprofloxacin (57), pitavastatin (58) as a cholesterol lowering agent, lenvatanib (59) as a kinase inhibitor for cancer and its structural analogs such as cabozantinib and bosutinib, tipifarnib (60) as a farnesyl transferase inhibitor for leukaemia, saquinavir (61) as an antiretroviral and bedaquiline (62) as an anti-tuberculosis drug (Mahajan, 2013).

The 2-(2-fluorophenyl)-6,7-methylene-dioxy quinoline-4-one monosodium phosphate (CHM-1-P-Na) (63) is a pre-clinical anti-cancer agent, showing anti-tumor activity in a SKOV-3 xenograft nude mice model (Chou et al., 2010; Chou et al., 2010). Several quinoline-based compounds (64-69) also showed the inhibition of kinases which are involved in cancer progression (Solomon and Lee, 2011). The chemical structures of various quinoline-based drugs (a) and kinase inhibitors (b) are shown in Fig. 5.

Quinoline and its analogs have recently been examined for their mode of function in the inhibition of tyrosine kinases, proteasome, tubulin polymerization, topoisomerase and DNA repair. Substitution of a group in a suitable position of a bioactive molecule is found to exert a profound pharmacological effect (Gasparotto et al., 2006). The quinoline nucleus is present in many naturally occurring alkaloids with anti-tumor activity such as camptothecin (Wall et al., 1966). Buta et al., 1978 isolated camptothecin, a quinoline analogous from Camptotheca acuminata. It was the first compound identified which directly blocks the topoisomerase (Topo-I), a DNA replication enzyme, thus stopping cell division.
Aravinda et al., 2009 presented a simple approach for the accession of quinoline peptide (72) analogs: 1,4-disubstituted 1,2,3-triazoles using 3-(azidomethyl)-2-chloroquinoline (70) as a building block. Their synthesis and applications including DNA binding and photonuclease activity were reported. DNA binding studies have shown a good interaction with quinoline peptides and peptidomimetics when it binds with base pairs of calf thymus-DNA.

Ghandi et al., 2017 described a successful synthesis of novel tetrazole based quinoline (76) derivatives via a one-pot reaction in moderate to good yields. These reactions proceeded presumably through Ugi-azole or Ugi-azole/Pictet-Spengler processes.
El-Gamal, 2016 reported the synthesis and antimicrobial activity of polyfunctionalized heterocyclic systems especially quinoline-based 2-cyano-N-(3-cyano quinoline-2-yl) acetamide moiety (81).

The synthetic methods depended on region-selective attack and/or cyclization by the cyanoacetamide moiety of quinoline. All synthesized compounds were characterized by...
spectral data and elemental analysis. It was further screened for their antimicrobial activity.

1,4-Dihydropyridines (1,4-DHPs) as privileged pharmacophores, have gained a vital place in the field of pharmaceuticals. Valuable commercial drugs such as nifedipine (91) (Murphy et al., 1983; Masotti et al., 1985; Janis et al., 1983; Morad et al., 1983; Loev et al., 1974), cilnidipine (92) (Pajouhesh et al., 2010; Yamamoto and Takahara, 2009; Oike et al., 1990; Takahara et al., 2003), nicardipine (93) (Nobili et al., 2006; Sorkin and Clissold, 1987), and nimodipine (95) (Lavilla et al., 2002; Varache-Lembège et al., 1996; Janis et al., 1987) are calcium channel blockers with useful effects on cardiovascular disorders including hypertension or cardiac arrhythmias (Edraki et al., 2009; Safak and Simsek, 2006; Shan et al., 2004; Velázquez and Knaus, 2004; Gaudio et al., 1994; Goldmann et al., 1991; Triggle et al., 1989; Bossert and Vater, 1989; Bossert et al., 1981; Janis and Triggle, 1984).

In addition, functionalized 1,4-DHPs are common to numerous bioactive compounds, which exhibit a broad range of biological activities such as antidiabetic (Briede et al., 2008; Kharkar et al., 2002), antitumor (Abbas et al., 2010; Swarnalatha et al., 2011), antitubercular (Sriram et al., 2010; Nayyar et al., 2006), radioprotective (Ivanov et al., 1990), neuroprotective (Klimaviciusa et al., 2007; Klusa et al., 1995), bronchodilator (Suresh et al., 2007) and anti-ischemic for the treatment of Alzheimer’s disease (Khadilkar and Borkar, 1998; Priego et al., 1992; Cooper et al., 1992) amongst several others (Trivedi et al., 2011; Hilgeroth, 2002; Vijesh et al., 2011; Mai et al., 2009; Hilgeroth, 2002; Kawase et al., 2002; Gullapalli and Ramaraoo, 2002; Tasaka et al., 2001; Zhu et al., 2001; Zhao and Cheng, 2000).

Replacement of the aryl group of the DHPs with the bioactive heterocyclic compounds leads to the formation of scaffolds with various biological properties. Isoxazolyl dihydropyridine (95) inhibits the multidrug resistance transporter (Hulubei et al., 2012). Moreover, coumarin and pyrazole-based 1,4-DHPs such as (97) and (98) are known as bone anabolic (Sashidhara et al., 2012) and potential antitubercular (Trivedi et al., 2011) agents, respectively (Fig. 6).
Fig. 6. Examples of 1,4-DHPs and quinoline derivative as potential antitubercular agents.

Niraj et al., 2011 demonstrated a microwave-assisted Hantzsch reaction for the synthesis of tetrazolo[1,5-\(a\)]quinoline-based 1,4-dihydropyridines (101), acridine-1,8-diones (104) and polyhydroquinolines via a three-component coupling reaction of tetrazolo[1,5-\(a\)]quinoline-4-carbaldehyde (99, 102 and 105), dimedone (103) and ethyl/methyl...
acetoacetate (107) in the presence of ammonium acetate as a catalyst. All synthesized compounds were screened for their antimicrobial activity against bacteria and fungi.

Gandhi and Zarezadeh, 2015 reported quinolinyl 1,4-dihydropyridines (112) in a facile and efficient protocol via a one-pot four-component condensation of 2-chloroquinoline-3-carbaldehydes (109), aryl amines (110), acetylenedicarboxylates (111) and malononitrile (30) in moderate to excellent yields. Triethylamine was used as a catalyst, ethanol as the solvent under reflux condition for 10 h: a maximum of 92 % yield was obtained.
Kathrotiya and Patel, 2013 described a one-pot three-component cyclocondensation of β-aryloxyquinoline-3-carbaldehydes (113) and beta enaminones (114). Piperidine was used as a catalyst in ethanol. Anti-tuberculosis activity were evaluated against *Mycobacterium tuberculosis* H37Rv.

\[ R_1 = H & CH_3; R_2 = CH_3 & CH_2CH_3; R_3 = H & CH_3; R_4 = 4-Cl & 4-OCH_3. \]

**2.1.3. The synthesis and importance of sulfur based heterocycles**

The synthesis of sulfur-based molecules has attracted significant attention due to their application in industry, medicine and organic chemistry (Metzner and Thuillier, 1994; Nagao, 1977; Nudelman, 1984; Chatgilialoglu and Asmus, 2013). Thiolic precursors are fundamental substrates for the synthesis of many organosulfur compounds (Koval, 2007). Alicyclic thiols (benzyl mercaptans) are considered as masked thiols as they can be readily transformed by hydrolysis or acyl group transfer reactions under mild conditions (Wuts and Greene, 2006; Koval, 1994; Mukaiyama *et al*., 1973; McGarvey *et al*., 1986; Conrow and Portoghese, 1986). The presence of sulfur atoms result in
significant changes to the cyclic molecular structure due to the availability of unshared pairs of electrons and the difference in electronegativity between heteroatoms and carbon. As a result, these heterocycles are considered as promising materials in different areas of pharmaceutical and agrochemical research, and also more recently as compounds with interesting physical properties for magnetism and conductivity. Sulfur-based heterocycles are common constituents of petroleum and liquids derived from coal, and they are found in various secondary metabolites of micro-organisms and plants. For example, penicillins and cephalosporins have sulfur-containing rings.

Simple thiol favoring agents are the alkyl, alicyclic, and aromatic thiols which may be metabolized along several pathways. Simple aliphatic and aromatic thiols undergo S-methylation in mammals to produce the corresponding methyl thioether or sulfide. Methylation is catalyzed by thiopurine methyltransferase in the cytoplasm and thiol methyltransferase in microsomes (Glauser et al., 1993). Quinoline containing fused sulfur heterocycles, such as 3,4-dihydro-2H-thiopyrano[2,3-b]quinoline (116) showed metabotropic glutamate receptor antagonistic activity, in particular, mGlu 1 receptor activity (Xiao et al., 2006), and 2H-thiopyrano[2,3-b]quiunoline-2-carboxylic acid (117) could be used as a strong antioxidant to protect against oxidative DNA damage from harmful free radicals and inhibitors of protein kinase casein kinase II (Fig. 7) (Syniugin et al., 2016).

![mGlu 1 receptor antagonist (116)](image1)

![antioxidant (117)](image2)

**Fig. 7.** Biologically active molecules containing 2H-thiopyrano[2,3-b] quinoline ring systems.

Wu et al., 2013 reported an efficient procedure for the stereo controlled construction of 2H-thiopyran[2,3-b]quinoline (120) starting from simple compounds. The domino Michael/Aldol reaction between 2-mercapto benzaldehydes (118) and 3-phenylprop-2-enal (119), promoted by chiral diphenylprolinol TMS ether, proceeded with excellent chemo- and enantioselectivity. Synthetically useful and pharmaceutically valuable 2H-thiopyrano-[2,3-b]quinolines (120), high yields of 90-99 %, were obtained.
Hagrs et al., 2015 presented a series of new 6-methoxy quinoline-3-carbonitrile derivatives (125, 127 and 129) and evaluated their antimicrobial activity against Gram-positive bacteria, *Streptococcus pneumoniae*, *Bacillus subtilis* and fungi *Aspergillus fumigatus*, *Syncephalastum racemosum*, *Geotrichum candidum* and *Candida albicans*.

Amongst the sulfur heterocycles, interestingly lipoic acid is known as a universal antioxidant (Biewenga et al., 1997; Packer, 1998; Packer et al., 1995; Tirosh et al., 1999). Its antioxidant activity is attributed to the capacity to scavenge free radicals in both membrane and aqueous domains. By chelating transition metals in biological systems, lipoic acid prevents membrane lipid peroxidation and protein damage through the redox regeneration of endogenous antioxidants such as vitamin E (α-tocopherol (132)), vitamin
C (ascorbic acid (131)) and glutathione (130) (Fig. 8), thus maintaining an intracellular antioxidant balance (Harnett et al., 2002). They have also been extensively studied as useful neuroprotective agents (Boldyrev et al., 2004; Roghani and Behzadi, 2001; Stamford et al., 1999). Lipoic acid (133) is readily absorbed by diet, transported, taken up by cells, and reduced to dihydrolipoic acid (DLA) (134) in various tissues, including the brain.

Fig. 8. Chemical structure of natural antioxidants glutathione, ascorbic acid (vitamin C) and α-tocopherol (vitamin E), lipoic acid and its reduced metabolite dihydrolipoic acid

Recently, focus has been placed on pharmacological tools that are able to act as far upstream as possible in the neurodegenerative cascade which are able to reach different selected targets. Therefore the structure of lipoic acid (133) was combined with a pharmacophore with well-established biological properties such as the ability to inhibit acetylcholinesterase (AChE) activity. The design strategy leading to these compounds is outlined by linking the structure of lipoic acid to that of an appropriate pharmacophore. Lipoic acid represents a privileged structure on which the selected pharmacophore is inserted to achieve selectivity for given targets.
Fig. 9. Examples of multi-target directed compounds.

Lipocrine (136) emerged as a candidate for drug development, displaying multiple biological properties such as inhibition of AChE activity, inhibition of AChE induced Aβ aggregation, ability to protect cells against reactive oxygen species (ROS) (Rosini et al., 2005) and as promising lead compounds for treatment of Alzheimer (Fig. 9).

Benchekroun et al., 2016 reported the synthesis of novel multifunctional tacrines (114) for the treatment of Alzheimer’s disease, by Ugi reaction of a lipoic acid (133), melatonin like isocyanide (143), formaldehyde (142) and tacrine derivatives (141). One of the synthesized compounds was identified as a promising permeable agent which showed excellent antioxidant properties, strong cholinesterase inhibitory activity, less hepatotoxicity and best neuroprotective capacity.
There is a rise in the number of publications confirming the beneficial effect of lipoic acid in the therapy of many diseases, including diabetes mellitus, atherosclerosis, degenerative processes in neurons, diseases of joints, or acquired immune deficiency syndrome (AIDS). An interest in contemporary medicine of this compound results from the unique reductive power of lipoic acid (Roy and Packer, 1998).

2.2. Multi-component reactions

Multi-component reactions (MCRs) are generally defined as one-pot reactions where more than two starting materials react to form a product, incorporating essentially all of the atoms of the educts. MCRs have gained considerable popularity in the field of contemporary organic synthesis. They represent ideal synthetic tools to generate structures of great complexity and diversity from simple starting materials and therefore are extensively applied by medicinal chemists to construct diverse chemical libraries of “drug-likeness” (Domling et al., 2012; Akritopoulou-Zanze, 2008; Weber, 2002; Hulme and Gore, 2003). The rapid, easy access to biologically relevant compounds and the scaffold diversity of MCRs has been recognized by the “synthetic” community and academia as a preferred method to design and discover biologically active compounds. MCRs chemistry has been reviewed multiple times in the past, focusing mostly on diverse synthetic and structural aspects (Dömling, 2006; Simon et al., 2004; Ugi, 1962; Ugi, 1982; Armstrong et al., 1996; Montgomery, 2000; Dömling and Ugi, 2000;
Ulaczyk-Lesanko and Hall, 2005; Mironov, 2006; Tempest, 2005; Ramón and Yus, 2005; Kappe, 2003; Orru, and de Greef, 2003; Ugi, 2003; Balme et al., 2003; Zhu, 2003; Hulme and Gore, 2003; Masciadri et al., 2003; Dömling, 2002; Lockhoff and Frappa, 2002; Orru and Ruijter, 2010; Toure and Hall, 2009; Kalinski et al., 2010). The biological chemistry of MCRs is very rich and provides great opportunities for investigation of small molecular weight compounds with biological activity. Generally, there are different classification schemes of MCRs according to the reaction mechanisms, the components involved, or their intrinsic variability (Zhu and Bienayme, 2005). Many basic MCRs are named reactions, for example, Ugi (Ugi, 1959), Passerini (Passerini, 1921), Van Leusen (Van Leusen et al., 1977), Strecker (Strecker, 1854), Hantzsch (Hantzsch, 1881), Bignelli (Biginelli, 1891) or one of their many variations.

**Fig. 10.** UDC strategy allows for great scaffold diversification of an initial Ugi reaction by using orthogonal protected bifunctional starting materials.
The four-component Ugi reaction (Ugi-4CR) has attracted enduring attention owing to its broad input scope, high diversity of products, unmatched versatility and simple operation (Ugi et al., 1965). It is noteworthy that the Ugi-4CR provides a linear dipeptide-like skeleton rather than a more valuable heterocyclic ring. A versatile example for the libraries of drug-like advanced compounds is the Ugi-Deprotection-Cyclization (UDC) procedures leading to great scaffold diversity, e.g., benzimidazoles (145, 146 and 152), benzodiazepindione (147 and 148), tetrazolodiazepinone (151), quinoxalinones (149), γ-lactams and piperazines (150) (Fig. 10) (Keating and Armstrong, 1996; Szardenings et al., 1997; Hulme et al., 2000; Hulme et al., 1999; Golebiowski et al., 2000; Tempest et al., 2001; Nixey et al., 2002; Faggi et al., 2002; Zhang and Tempest, 2004; Hulme and Dietrich, 2009; Tejedor and Garcia-Tellado, 2007; Isambert and Lavilla, 2008; Willy and Mueller, 2008; Simon et al., 2004).
Recently, Chandgude and Dömling, 2017 reported Ugi-4CR with N-hydroxyimides (154) as a novel carboxylic acid isostere (153) towards one-pot synthesis of α-hydrazino amides (157). The reaction required 30 mol % of ZnCl₂ and toluene at room temperature for 12 h. The readily available N-hydroxyimides, which replaced the toxic and unstable hydrazines/oxaziridine is used for the synthesis of α-hydrazino amides.

Venkata Prasad et al., 2017 described the novel synthesis of pyrrole[2,3-c]pyridone (168) by Ugi-4CR. At 50℃, 50 mol % of PTSA was used as a catalyst in methanol for one h and the Ugi adducts were formed with a maximum yield of 91 %. The methodology was successfully employed to the electron deficient anilines (167) and phenyl glyoxylic acids (166) having a CF₃ group.

\[
\begin{align*}
\text{164} & \rightarrow \text{165} + \text{166} \rightarrow \text{167} \\
\text{R} = 4\text{-Br, 4-Cl, 4-CH₃, 4-OCH₃, 2,4-Cl} & \quad \text{168}
\end{align*}
\]

The Kabachnik-Fields reaction (Kabachnik and Medved, 1952) is an important multi-component reaction using aldehydes, amines and dialkyl phosphites in the presence of a Brønsted or Lewis acid, to produce α-amino phosphonates. In recent years, much attention has been made on the α-amino phosphonates. They can be considered as structural analogs of the corresponding α-amino acids and transition state mimics of peptide hydrolysis. α-Amino phosphonates have several biological activities, such as an antibiotic, an enzyme inhibitor, HIV protease and anti-tumor agents (Atherton et al., 1986; Paige et al., 1989; Peyman et al., 1994; Jin et al., 2006).

Sambath et al., 2013 described a facile method for the synthesis of novel α-amino phosphonates (172) by Kabachnik-Fields reaction. The reaction was carried out with equimolar quantities of 4-amino-N-2-thiazoyl-benzenesulfonamide (171) (Sulfathiazole), dimethyl phosphite (169) and aldehydes in dry toluene at reflux conditions. 70-80 % product yield was obtained at 50℃ between 5-6 h. Antibacterial and antifungal applications were further investigated for the synthesized compounds.
Amulrao et al., 2012 presented a convenient, one-pot three-component Kabachnik-Fields reaction of novel N-phenyl isoquinolone-1-phosphonates (176) from ethyl 2-(2-formyl-4,5-dimethoxyphenyl) acetate (173) and anilines (174). The reaction was successfully carried out by using trifluoroacetic acid as a catalyst in acetonitrile: 64-74 % yield was obtained. Antibacterial studies were investigated for the synthesized compounds.

Badadhe et al., 2011 reported the synthesis and antibacterial activity of novel α-amino phosphonates (179). The reaction was achieved by reacting 1-phenyl-3-(pyridine-4-yl)-1H-pyrazole-4-carbaldehyde (177) with various anilines (178), in ethanol, in the presence of glacial acetic acid. Further treatment with diethyl phosphite (175) in the presence of conc. HCl at room temperature resulted in the product.
Hantzsch reaction is a well-known three-component MCR, which affords 1,4-dihydropyridine derivatives (91) using β-ketoesters (180), aldehydes (181) and ammonia. A calcium channel blocker, “Nifedipine” was synthesized by this reaction (Khadilkar and Chitnavis, 1995; Khadilkar et al., 1995).

Rao and Parthiban, 2014 reported the simple, convenient one-pot synthesis of a library of highly functionalized hexa-substituted 1,4-dihydropyridines (185, 186 and 187) (1,4-DHPs) from 2-aminopyridine (188) catalyzed pseudo three-component reaction of nitroketene-N, S-acetals (183) and aldehydes (184).
Also, an analog of neonicotinoid (189), for example, nitenpyram was designed by the treatment of a solution of 1,4-DHPs with the synthesized amines in ethanol under reflux for 5 h.

Chao et al., 2014 reported a Hantzsch type four-component synthesis of spiro-oxindole derivatives (194) bearing dihydropyridines. This one-pot reaction was successfully achieved by malononitrile (30), aromatic amines (191), N-pyrrolidine-2,4-dione (190), and isatin (192) where 4-dimethylamino pyridine (4-DMAP) was used as a catalyst in ethanol: the recorded yield was 80-95 %.

Recently, Habibi et al., 2017 presented a three-component efficient synthesis of 4H-pyrans (198) from aromatic aldehydes (196), malononitrile (30) and β-keto esters (197) by using hexamethylenetetramine (HMTA) as a catalyst. These molecular building blocks are well known for their biological activity and were synthesized by using 25 mol % of HMTA and water: 81-95 % yield was obtained.
Magar et al., 2013 reported a three-component condensation of the 2-aminochromene derivative (204) by a one-pot fashion from aromatic aldehydes (203), phenol (202) and active methylene compounds (30), in the presence of silica gel supported polyamine heterogeneous catalyst (201). IR, SEM and TGA studies were used for the catalyst characterization. A maximum of 95% yield was afforded after 2 to 5.30 h under reflux conditions.
Recently, Romdhane and Jannet, 2013 reported a one-pot three-component condensation reaction of aldehydes (205), malononitrile (30) with methyl acetoacetate (180) and 8-hydroxyquinoline (207), separately. The reaction was carried out by using sodium carbonate as a catalyst in ethanol for 3 h: maximum yield of 81% was obtained.

![Chemical structure of the reaction](image)

El-Agrody and Al-Ghamdi, 2011 published the synthesis of 4H-pyrano[3,2-\(h\)]quinoline-3-carbonitrile derivatives (211) from 8-hydroxy-2-methylquinoline (209) with \(\alpha\)-cyano-\(p\)-chloro/bromocinnamionitriles (210). A maximum of 91% yield was reported when piperidine in ethanol was used as a catalyst under refluxing condition for 60 minutes.

![Chemical structure of the reaction](image)

R= H, CH₃
\(\text{Ar} = p\)-ClC₆H₄, \(p\)-BrC₆H₄, \(p\)-FC₆H₄
X= CN, COOEt

These classical methods for the synthesis of biologically active scaffold molecules such as peptides, \(\alpha\)-aminophosphonates, 1,4-dihydropyridines, 4\(H\)-chromenes and 4\(H\)-pyrans have several drawbacks such as long reaction time, lower product yields and harsh refluxing conditions. Thus, it is evident that the development of more flexible and effective protocols, such as ultrasonication and microwave irradiation are required.
2.3. Ultrasonic and Microwave irradiations

Ultrasound in chemistry, also referred to as sonochemistry, offers a method of chemical activation which has broad applications. It uses equipment which is relatively inexpensive. The driving force for sonochemistry is cavitation therefore the general requirement is that at least one of the phases should be a liquid. The use of ultrasound has wide applicability but presents a significant scientific challenge in understanding its underlying physical phenomenon i.e. acoustic cavitation. The use of sonochemistry fits into the theme of sustainable chemistry because it aims to use less/non-hazardous chemicals and solvents, reduce energy consumption, and increase product selectivity (Mason, 2003).

Recently, Guo and Yuan, 2010 reported a L-proline catalyzed efficient multi-component Hantzsch reaction, in ethanol, at 60°C under ultrasound irradiation to afford the corresponding 1,4-dihydropyridine (214) derivatives in high yields. A maximum of 92% yield was obtained within 30 minutes whilst the classical synthesis was completed in 6-8 h.

![Chemical structure](image)

R = H, 4-F, 3-F, 4-CH₃, 4-OCH₃, 2-Cl, 6-CH₃

Multi-component synthesis of 4H-benzo[h]pyrans (216) was achieved by Jin et al., 2004 by reacting aromatic aldehydes (215), malononitrile (30) and naphthols (207), in one-
pot, using cetyl trimethyl ammonium bromide (CTABr) as a catalyst under ultrasonic irradiation. The reaction was carried out at room temperature for 2.5 h to afford 95% yield.

Due to the ability of solids and liquids being able to transform electromagnetic energy into heat, microwave (MW) radiation has been widely employed as an energy source. Microwave irradiation has several advantages over conventional heating and these include homogeneous, rapid heating (deep internal heating), acceleration in reactions as a result of the heating rate (which cannot be reproduced by classical heating) and selective heating.

Recently, Lei Zhang et al., 2013 reported a novel and efficient methodology for the synthesis of three distinct sets of indole-based heterocyclic compounds from 1H-indole-2-carbaldehyde (217) via the Ugi condensation reaction. Cesium carbonate (Cs$_2$CO$_3$) was used as a base catalyst and the reaction was carried out using DMSO as a solvent at 80°C under MW for 40 min.
Keglevich et al., 2011 described an efficient MW synthesis of α-hydroxyphosphonates (226) via Kabachnik-Fields reaction. Sodium carbonate was used as a catalyst under MW condition for 20 minutes.

Al-Awadi et al., 2012 presented a Hantzsch-type pseudo four-component reaction for the synthesis of 2-unsubstituted dihydropyridines (230) from enaminones (228). The reaction offered 84-95 % yield under MW irradiation. Moderated yields were obtained under conventional route.

These investigations indicate that ultrasonic and MW-assisted organic reactions produce high yields of the target compound whilst lower quantities of side products are obtained. Hence the purification of the products via column chromatography is easier and faster. Indeed, new reactions and conditions that cannot be achieved by conventional heating can be performed using these techniques.

2.4. Heterogeneous catalysis

A heterogeneous catalyst is a material that continually creates active sites with its reactants under optimal reaction conditions. These active sites change the rates of the chemical reactions of the reactants localized on them without changing the thermodynamic equilibrium between the materials. In catalysis, a catalyst support plays
an important role and these materials are usually solid with high surface area. The activity of heterogeneous catalysts occurs at the surface area atoms. Consequently, great effort is made to maximize the surface area of a catalyst by distributing it over the support. The most frequently used catalyst supports are silica, graphene, zeolites and alumina (Ma and Zaera, 2006).

Due to its remarkable properties, boron nitride (BN) has shown promise as a novel support in catalysis and as a replacement for traditional oxide supports in different processes (Sun et al., 2016). Hexagonal BN has not been studied extensively as a support; there are recent reports in the literature about the good performance of BN based catalysts in some catalytic processes. Several studies describe the use of BN supported noble metals for the deep oxidation of volatile organic compounds. Yabe et al., 2012 reported a novel palladium-supported-on-BN catalyst (Pd/BN) (233) which is applicable for the partial-hydrogenation of mono and disubstituted alkynes to furnish the corresponding alkenes in the presence of diethylenetriamine (DETA), which exhibited an unprecedented acceleration effect for hydrogenation.

\[ \text{Pb(OAc)}_2 + \text{BN} \xrightarrow{\text{MeOH}} \text{Pd/BN} \]

Preparation of Pd/BN catalyst

\[ \text{MeO} - \text{H}_2 - \text{MeO} \]

Wu et al., 2001 have shown that a Pt/BN catalyst can remain active for 80 h at 185°C, while the activity of traditional Pt/γ-Al₂O₃ at the same temperature was found to decrease continuously with time. Lin et al., 2002 and Wu et al., 2003 have studied Pt/BN as a
catalyst for the deep oxidation of methanol and benzene. Jacobsen, 2001 studied a Ba-Ru/BN catalyst for ammonia synthesis and found that the activity of this catalyst is significantly higher than that of traditional catalysts. The selective hydrogenation of α,β-unsaturated aldehydes into unsaturated alcohols was achieved by BN supported Pt-Sn catalysts (Wu and Chen, 2005).

Boron nitride can be considered as a good candidate support. The choice of BN as the support was also directed by the search for new hydrophobic supports with high thermal conductivity, adapted to high-energy oxidation reactions under severe conditions. BN is one of the most interesting non-oxide ceramic materials because of its low density, excellent resistance to chemical attacks, high melting point, good thermal conductivity and high stability with respect to oxidation (Postole et al., 2005; Perdigón-Melón et al., 2002). Prior to the discovery of BN, materials traditionally used as supports of active phases were γ-Al₂O₃ and SiO₂. These materials possess low thermal conductivity and many acidic and basic sites, which causes sintering of the supported metal on hot spots and coverage of the catalyst by water (Yao et al., 1979; Trimm and Önsan, 2001). Moreover, the metal support interaction present in most oxide-supported metal catalysts has a negative influence on the catalytic activity (Carstens et al., 1998).

Ionic liquids (ILs) are gaining considerable attention as catalysts and support for multi-phasic catalysis because they can be tuned for specific applications. In comparison with the traditional catalysts, a main advantage of ILs is their structural diversity, which allows tuning of their properties. The tunable structures of the ILs by a combination of different cations with anions make them highly promising candidates for tailored catalysts (Rogers, 2007). ILs based imidazolium cations have been extensively studied with a variety of structural modifications leading to differences in the physical and chemical properties of the liquid. The use of ILs has broadened its scope to its role as a catalyst and reagent.

Recently, Siddiqui et al., 2014 reported a novel three-component one-pot methodology for rapid access to pyrido[1,2-c][1,3,5]thiadiazin-4-ones (239). A task-specific ionic liquid [bmim]SCN was used which could be recycled. The usage of water and easy recyclability of the ionic liquid were the green chemistry aspects of the study which was further explained.
Shaterian and Kangani, 2013 reported the synthesis of pyrazolo[1,2-a][1,2,4]triazole-1,3-dione derivatives (242) via a three-component reaction of aryl aldehydes (240), 4-phenylurazole (241) and malononitrile (30) in the presence of catalytic amount of 2-hydroxyethyl ammonium formate and 2-hydroxyethyl ammonium acetate as effective mild basic ILs at room temperature.

2.5. Biological applications

2.5.1. Antibacterial and antifungal activity against human diseases

Antibiotics are one of the most important weapons in fighting against bacterial infections and have greatly benefited the health-related quality of human life since their introduction. Over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less effective against certain illnesses due to the bacteria developing resistance to antibiotics. Thus, it is essential to investigate alternate
drugs with reduced resistance. Novel drugs which are synthesized are playing a significant role in the prevention and treatment of human diseases.

The discovery and development of antimicrobial agents that have met with enormous success over the past 50 years provided many classes of natural products and semi-synthetic or synthetic compounds (marketing of over 100 antibacterial agents). Among them, quinoline and its derivatives, oxygen, nitrogen and sulfur-based molecules are still an important class of therapeutically useful antibacterial drugs (Hafez et al., 2015). The control of deadly infectious diseases including tuberculosis, caused by *Mycobacterium tuberculosis* is seriously threatened as multi-drug resistance is emerging and dissemination of resistant pathogenic microbes is the major concern. Additionally, patients with HIV/AIDS are immuno-compromised and very susceptible to opportunistic microbial infections including tuberculosis which necessitate continuous research into novel classes of antimicrobial agents. This requires active investigation with the goal of overcoming the phenomenon of multiple drug resistance (MDR). It is well-known that the quinoline nucleus and its derivatives, oxygen and sulfur-based moieties play a vital role in the search for a wide antibacterial activity spectrum. Structure-activity relationship (SAR) studies revealed that the antimicrobial activity of these heterocyclic molecules depends on the nature of the peripheral substituents and their spatial relationship within the nitrogen, oxygen and sulfur ring skeleton. Thus, these are promising new chemical targets for bacteria and fungi as well as host-based, immunological approaches and as evolving strategies for antimicrobial therapy.

2.5.1.1. Antibacterial activity against *Staphylococcus aureus*

![SEM images of Staphylococcus aureus](Hess et al., 2012)

*Staphylococcus aureus* (*S. aureus*) (Fig. 11) is a Gram-positive and a major human pathogen that causes a wide range of infections after injury or surgery. It affects
approximately 500,000 patients in hospitals annually (Awasthi, 2009). In animals and humans that are immune compromised or immune deficient, the bacteria may be life-threatening. Approximately 30% of individuals carry *S. aureus* in their nose, pharynx or back of the throat and on their skin. *S. aureus* causes numerous infections at various sites of the body (Hess *et al.*, 2012). Some of these include, skin infections (causes boils, furuncles, styles, impetigo and other superficial skin infections in humans), infections of surgical and trauma wounds, urinary tract infections, food poisoning and gastro-intestinal tract infections, infections of organs including pneumonia (lung), osteomyelitis (bone), endocarditis (heart), phlebitis (veins and blood vessels), mastitis (breast and formation of abscesses) and meningitis (brain) (Tong *et al.*, 2015).

### 2.5.1.2 Antibacterial activity against *Bacillus cereus*

![Fig. 12. SEM images of Bacillus cereus (Nishimura *et al.*, 2003)](image)

*Bacillus cereus* (*B. cereus*) (*Fig. 12*) is a Gram-positive bacteria, rod-shaped, aerobic, motile, a betahemolytic bacterium that produces toxins. These toxins can cause diarrhea, abdominal cramps, nausea and vomiting. The bacteria are present in foods and can multiply quickly at room temperature. Some strains are harmful to humans and can cause foodborne illness. *B. cereus* is also known to cause difficult-to-eradicate chronic skin infections, though less aggressive than necrotizing fasciitis, it can cause keratitis (Pinna *et al.*, 2001).

### 2.5.1.3 Antibacterial activity against *Enterococcus faecalis*

*Enterococcus faecalis* (*E. faecalis*) (*Fig. 13*) normally a gut commensal, is a Gram-positive and a frequent cause of many serious human infections, including urinary tract infections, endocarditis, bacteraemia, and wound infections (Pinheiro and Mayer, 2014).
Among the diseases that *E. faecalis* causes, urinary tract infections are the most common, responsible for approximately 110,000 cases yearly, many of which are nosocomial.

**Fig. 13.** SEM images *Enterococcus faecalis* (Bulacio *et al*., 2015)

Infections with *E. faecalis* can be difficult to treat because of their frequent resistance to multiple antibiotics, including vancomycin (Zhanel *et al*., 2001), a drug of last resort for many Gram-positive infections.

### 2.5.1.4 Antibacterial activity against *Escherichia coli*

*Escherichia coli* (*E. coli*) (**Fig. 14**) is a Gram-negative bacterium that lives in the digestive tracts of humans and animals. There are many types of *E. coli* and some can cause bloody diarrhea. Various strains of *E. coli* such as strain O157: H7, may also cause severe anaemia or kidney failure, which can lead to death. Other strains of *E. coli* can cause urinary tract infections amongst other infections (Vincent *et al*., 2010). Normally, *E. coli* infection occurs by coming into contact with faeces of humans or animals. This can happen when you drink water or eat food that has been contaminated by faeces.

**Fig. 14.** *Escherichia coli* (Leung *et al*., 2016)

### 2.5.1.5. Antibacterial activity against *Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* (*P. aeruginosa*) is Gram-negative and an important pathogen which causes 10% to 20% of infections in most hospitals. *P. aeruginosa* infection is
especially prevalent among patients with burn wounds, cystic fibrosis, acute leukemia, organ transplants and intravenous-drug addiction.

**Fig. 15. Pseudomonas aeruginosa** (Tsang *et al.*, 2003)

*P. aeruginosa* (Fig. 15) is a common nosocomial contaminant and epidemics have been traced to many items in the hospital environment. Patients who are hospitalized for extended periods are frequently colonized by this organism and are at increased risk of developing infection. The most serious infections include malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia and septicaemia (Young and Armstrong, 1972).

2.5.1.6 Antifungal activity against *Candida albicans*

**Fig. 16. Candida albicans** (Staniszewska *et al.*, 2013)

*Candida albicans* (*C. albicans*) (Fig. 16) is a fungus that is normally present on the skin and in mucous membranes such as the vagina, mouth or rectum (Mayer *et al.*, 2013). The fungus can also travel through the blood stream and affect the throat, intestines and heart valves. An infection in the bloodstream can detrimental affect the kidneys, heart, lungs, eyes or organs causing high fever, chills, anaemia, rash or shock. To infect host tissue, the usual unicellular yeast-like form of *C. albicans* reacts to environmental cues and switches into an invasive, multicellular filamentous form, called dimorphism (Ryan and Ray, 2004). In addition, an overgrowth infection is considered a superinfection, usually
applied when an infection becomes opportunistic and very resistant to antifungals. Antibiotics were used to suppress or cure its effectiveness.

2.5.1.7 Antifungal activity against *Candida utilis*

![Image of Candida utilis](image1)

**Fig. 17. Candida utilis** (Waghmare et al., 2015)

*Candida utilis* (Fig. 17) is common yeast causing urinary tract infections (UTIs). The systematic disease caused by the organism is usually associated with the patients who are receiving fluconazole as an antifungal therapy, but this species was a common etiologic agent prior to the fluconazole era. The detection of these organisms in urinary tract specimens, especially clean catch or catheterized (in and out) urine samples, is vital. In the absence of uropathogen or uropathogen predominance signs, in the diagnosis of UTI, the patient may not be administered any antimicrobial therapy (Hazen et al., 1999).

2.5.2. Antioxidant activities

![Image of antioxidant activities](image2)

**Fig. 18.** The effect of antioxidants (Lobo et al., 2010)
Free radicals are highly reactive and have the potential to damage cells (Lobo et al., 2010). Free radicals are formed naturally in the body and play an important role in many normal cellular homeostatic processes. At high concentrations, however, free radicals can be hazardous to the body and can damage all major components of cells, including DNA, proteins, and cell membranes. The damage to cells caused by free radicals, especially the damage to DNA, may play a role in the development of cancer and other health conditions. Moreover, some environmental toxins, cigarette smoke, metals, and high-oxygen atmospheres, may contain large amounts of free radicals or stimulate the body’s cells to produce more free radicals (Fig. 18).

Antioxidants interact with and neutralize free radicals, thus preventing them from causing damage (Lu et al., 2013). Antioxidants are also known as “free radical scavengers”. The body makes some of the antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione (GSH). However, the body relies on external (exogenous) sources primarily from the diet to obtain the rest of the antioxidants it requires (Rahal et al., 2014). In laboratory and animal studies, the presence of increased levels of exogenous antioxidants has been shown to prevent free radical damage that has been associated with carcinogenesis. Therefore, investigations into dietary antioxidant supplementation to help lower the risk of developing or dying from cancer have been conducted. Thus, it is relevant that prior to introducing antioxidant therapy into mainstream medicine, significant advances in basic cell biology, pharmacology and clinical bioanalysis will be required.

Notably, α-diphenyl-β-picrylhydrazyl hydrate (DPPH) free radical scavenging assay offers an approach for evaluating the antioxidant potential of compounds. It is a rapid, simple, inexpensive and widely used method to measure the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. It can also be used to quantify antioxidants in complex biological systems for solid or liquid samples. The DPPH method is unique in carrying out the reaction of the sample with DPPH in methanol/water, which facilitates the extraction of antioxidant compounds from the sample. Antioxidant analysis by other methods may be limited to those compounds soluble in the selected solvents. The advantage of this method is that DPPH is allowed to react with the whole sample with sufficient time to allow DPPH to react.
slowly even with weak antioxidants (Kedare and Singh, 2011). This method may be utilized in aqueous and nonpolar organic solvents and can be used to examine both hydrophilic and lipophilic antioxidants (Prior et al., 2005).

### 2.5.3. Toxicity assessment

Toxicity testing is paramount in the screening of newly developed drugs before it can be used in humans (Parasuraman, 2011). The essence of toxicity testing is not just to determine how safe a substance/drug is, but to characterize the possible toxic effects it can produce. The guiding principles of toxicity testing are to determine the effect of the test substances/drug on laboratory animals and assess the potential hazard on a human that is exposed to a lower dose. Toxicity testing is employed in a wide range of different animal species with long-term administration of drug, regular monitoring of physiological, biochemical abnormalities and detailed post-mortem examination at the end of the trial to detect gross or histological abnormalities. The use of animals in toxicity testing is most likely to continue because of the benefits they offer in examining a substance/drug in a whole functioning organism. The toxicity test enables a dose-response curve to be determined which ensures the safety of new chemicals for use as pesticides, drugs, or food additives before they are registered for general use in industry or clinical use (Woolley and Woolley, 2008).

![Artemia salina](image.jpg)

**Fig. 19. Artemia salina** (Brine shrimp) (Hentschel and Tata, 1976)

The cytotoxicity assays are often tedious, expensive and lack simple and rapid screening procedures. Nowadays, brine shrimp lethality assays are extensively used in research and applied toxicology (Costa-Lotufo et al., 2005). There is a tendency to use *Artemia salina* in toxicological tests that screen a large number of drugs and plants (Manjili et al., 2012;
Ramazani et al., 2010; Ramazani et al., 2010; Sangian et al., 2013). *Artemia salina,* brine shrimp larvae (Fig. 19), is one of the most valuable test organisms available for toxicity testing, and research suggests that there are several applications in toxicology and ecotoxicology therefore it will continue to be used widely (Nunes et al., 2006).

### 2.5.4. Molecular Docking

The development of new drugs is one of the most challenging tasks in science today. Combined efforts of the pharmaceutical industry, academic researchers and biotech companies have not only improved the process of drug design but also contributed to the advances. Computational modeling and stimulations have become integral procedures in introducing new drugs to the market and it is safe to assume that the role of theoretical computations in this field will increase in the future (Alonso et al., 2006). Protein activity in organisms involves interactions with biomolecules, which makes them perfect targets for rationally designed drugs.

![Molecular Docking Diagram]

**Fig. 20.** Structure-based drug design (Molecular docking)

After the Human Genome Project (Burley et al., 1999) was completed, it soon became evident that a protein’s crystallographic structure alone, in general, is insufficient to
understand its function (Albert et al., 2002). In fact, a complete description of the structure and dynamics of the protein along with its interacting partners is required. Such a demand from pharmaceutical industry has recently forced transition in the development of theoretical methods for protein examination; from protein structure prediction to the modeling of whole protein complexes.

Molecular docking is a technique used for predicting the structure of a molecular complex, given the structures of its individual components and these methods play an important role in drug design. Molecular docking is a method which predicts the orientation of one macromolecule of protein to the ligand when bound to each other thus forming a stable complex at the atomic level (Meng et al., 2011). The drug discovery program is oriented towards the search for lead structures and thus the virtual screening/molecular docking program constitutes a great tool which further undergoes limited optimization to identify the promising lead molecule (Fig. 20). DNA is specifically a molecular target for many of the clinically available drugs. It was reported that small drug molecules which contain planar polyaromatic systems bind to double-stranded DNA and exhibit more than one binding mode i.e., the intercalation and covalent binding. It has been oriental as a target for many antitumor agents as well as antibiotic drugs. Hence further interaction of the drug with DNA and the rational designing of selective targets in pharmacology are important to study (Kedare and Singh, 2011; Rescifina et al., 2014).

![Fig. 21. Examples of more potent fluoroquinolone drugs](image-url)
Fluoroquinolones (Fig. 21) are an important class of broad-spectrum antibacterial agents, whose spectra of activity has been parallel to modifications in the structure of the first quinolone, nalidixic acid. Nalidixic acid (245) was initially administered to treat Gram-negative urinary tract infections in humans and animals (Fraunfelder, 1996). Fluoroquinolone drugs are active against a wide range of Gram-negative and Gram-positive pathogens and show improved oral absorption and systematic distribution. Thus, the clinical applications of these compounds have been extended to the treatment of lower respiratory tract infections, skin and soft tissue infections, sexually transmitted diseases and urinary tract infections (Chen et al., 2012).

Quinolone and quinoline antibiotics inhibit DNA synthesis by targeting two essential type II topoisomerases, DNA gyrase and topoisomerase IV. The mechanism of quinolone inhibition occurs via the formation of a ternary cleavage complex with the topoisomerase enzyme and DNA (Hiasa and Shea, 2000). It is accepted that for quinolones and quinolines to inhibit DNA gyrase activity, they must form a stable interaction with the DNA gyrase-DNA complex. To overcome the lack of crystallographic data for the ternary complex, computational tools, such as molecular docking are useful for predicting the structures of protein-ligand complexes and providing information on the modes of interaction between ligands and receptors.
References


[100] Briede, J., Stivrina, M., Vigante, B., Stoldere, D., Duburs, G. 2008. Acute effect of antidiabetic 1,4-dihydropyridine compound cerebrocrast on cardiac function


melatonin modified tacrines as cholinesterases inhibitors, direct antioxidants, and nuclear factor (erythroid-derived 2)-like 2 activators. Journal of Medicinal Chemistry, (59) 9967-9973.


demonstration of resin capture. Journal of the American Chemical Society, (118) 2574-2583.


(a) Mason, T. J. 2003. Sonochemistry and sonoprocessing: the link, the trends and (probably) the future. Ultrasonics Sonochemistry, (10) 175-179. (b) Mason,


nitride-supported Pt catalysts for the deep oxidation of benzene. Journal of
Catalysis, (210) 39-45.

Pt/boron nitride catalyst. Industrial & Engineering Chemistry Research, (42)
3225-3229.


of BN catalyst supports from molecular precursors. Influence of the precursor on
the properties of the BN ceramic. Studies in Surface Science and Catalysis, (143)
227-237.

[222] Yao, H. C., Sieg, M., Plummer, H. K. 1979. Surface interactions in the Pt/γ-

driven vehicles. Catalysis Reviews, (43) 31-84.


917-918.

Ionic liquid promoted one-pot approach for the synthesis of pyrido [1, 2-c][1, 3,
5] thia diazin-4-ones and thiazolo [3, 2-c][1, 3, 5] thia diazin-4-ones in water.
Arabian Journal of Chemistry. (11) 256-264.

three component synthesis of pyrazolo [1, 2-a][1, 2, 4] triazole-1, 3-dione and 2-
amino-3-cyano-5, 10-dioxo-4-phenyl-5, 10-dihydro-4H-benzo [g] chromene
derivatives. Scientia Iranica, (20) 571-579.


Food reservoir for Escherichia coli causing urinary tract infections. Emerging Infectious Diseases, (16) 88.


Chapter-III

Synthesis of quinolinyl and quinolonyl-lipoyl peptides under microwave irradiation and antimicrobial, antioxidant, toxicity and molecular docking studies
Chapter Three

Synthesis of quinolinyl and quinolonyl-lipoyl peptides under microwave irradiation and antimicrobial, antioxidant, toxicity and molecular docking studies

3.1. Abstract

An efficient one-pot Ugi four-component condensation reaction was implemented for the synthesis of 13 new quinolinyl-lipoyl peptides (QLPs) and one quinolonyl-lipoyl peptide (QOLP) by microwave irradiation. The new N-(2-(cyclohexylamino)-1-(2-methoxyquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-phenylpentanamides were synthesised from substrates lipoic acid, 2-methoxyquinoline-3-carbaldehyde derivatives, aniline derivatives and cyclohexyl isocyanide in methanol. The new N-(2-(cyclohexylamino)-2-oxo-1-(2-oxo-1,2-dihydroquinolin-3-yl)ethyl)-5-((R)-1,2-dithiolan-3-yl)-N-(2-methoxyphenyl)pentanamide was synthesized from lipoic acid, 2-oxo-1,2-dihydroquinoline-3-carbaldehyde, o-anisidine and cyclohexyl isocyanide in methanol. The QLPs and QOLP were characterized by FTIR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis. A total of eight peptides were subjected to antimicrobial, antioxidant and toxicity evaluation. Among them, four peptides showed good antimicrobial activity against Bacillus cereus, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Candida albicans and Candida utilis whilst three peptides showed antioxidant potential by the radical scavenging assay. In addition, the brine shrimp assay showed four peptides with mortality rate less than 50% up to 48 h. Finally, molecular docking studies of the four peptides 41, 43, 46 and 47 revealed that the binding affinity of 41 and 43 with DNA gyrase was better than the standard ciprofloxacin: a docking score of 183.24 kcal/mol and 165.01 kcal/mol were recorded for 41 and 43, respectively, compared to 151.56 kcal/mol for ciprofloxacin. The advantages of this one-pot reaction are its green approach, the use of an inexpensive solvent, short reaction times in the absence of a catalyst and excellent yield of peptides. New biologically active peptides can be synthesized using this facile method.
3.2. Introduction

Recently, the study of peptides has received increased attention due to their anticancer, antihypertensive, antimicrobial, and antioxidant properties (De Mejia et al., 2012). A new range of highly selective and non-toxic peptides have been developed and their synthesis has gained attention (Möller et al., 2008). Although there are several protocols for the synthesis of new organic compounds, the use of multi-component reactions (MCRs) have grown rapidly because they provide a highly efficient and quick approach for constructing poly-functional molecules from simple materials (Domling et al., 2012).

One such example is the Ugi four-component reaction (Ugi-4CR) which is a convenient access to peptides synthesised from substrates such as carboxylic acid, an amine, an aldehyde and an isocyanide. Presently, more feasible substrates are being used to replace traditional substrates viz. diamine (Giovenzana et al., 2006), secondary amine (Tron et al., 2013) and β-ketoamide (Liéby-Muller et al., 2005) which can replace the primary amine whilst phenol (El Kaïm et al., 2005) or enol (Castellano et al., 2012) can replace the carboxylic acid. Importantly, the selection of appropriate substrates and controlled reaction conditions can promote a cascade operation (Elders et al., 2009; Ngouansavanh et al., 2007) for synthesizing complicated structures in one-pot (Sinha et al., 2013). Isocyanides are being used in the Ugi-4CR for producing peptides with important biological (Pando et al., 2011) and chemical activities (Znabet et al., 2010; Rivera et al., 2009). The combination of various carboxylic acid, aldehyde, amine and isocyanide in the Ugi-4CR is currently being used for the synthesis of new peptides (Khoury et al., 2012; Pérez-Labrada et al., 2012).

The Ugi-4CR reaction is usually conducted under reflux, in methanol, and the reaction time can take 24 h although catalysts (Zhang et al., 2007; Shanbhag et al., 2008; Bonnaterre et al., 2008; Hügel et al., 2009) can be used. The reaction time is usually long whilst product yields are moderate. However recently, researchers have improved on the reaction time and product yields.

Kaur et al., 2011 synthesized α-acyl-amino amides (5) via Ugi-4CR at room temperature (rt) in the presence of 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim]BF₄). The methodology afforded 55-97 % of yield within 8-16 h.
Also, Kanizsai et al., 2007 reported the synthesis of a Ugi four-centre three-component reaction in water as medium to construct β-lactam (9) libraries. Substrates such as β-amino acids (6), aromatic aldehydes (7) and cyclohexyl or tert-butyl isocyanide (8) were used. The observed yield was 45-91 % in 24 h.

Recently, Shiri et al., 2016 reported the synthesis of peptide derivatives (14) containing the 2-chloroquinoline scaffolds by the Ugi-4CR. The substrates used were 2-chloroquinoline-3-carboxaldehydes (10), amines (11), carboxylic acids (12) and isocyanides (13). The reaction was carried out in methanol at room temperature for 24 h and the yield was 62-92 %.
Marcaccini et al., 2001 reported the synthesis of $N$-cyclohexyl 2-[$N$-(2-chloroacetyl)-$N$-(4-chlorobenzyl)]amino-2-(4-chlorophenyl) acetamides (19) from a mixture of aromatic aldehydes (15), amines (16), acids (17) and isocyanides (18). The reaction was conducted in methanol for 30 h at room temperature and the product yield was in the range of 68-86%.

El Kaïm et al., 2005 reported the synthesis of a series of peptides: 2-((4-chlorobenzyl)(2-nitrophenyl)amino)-$N$-cyclohexylbutanamides (24) from a mixture of nitrophenols (20), propanal (21), $p$-chlorobenzylamine (22) and cyclohexyl isocyanide (23). The reaction proceeded at 40°C, in methanol, within 24 h to provide 33-96% yield.

Váradi et al., 2015 reported the synthesis of bis-amide (28) analogues of carfentanil by using aniline (1), propanoic acid (25), $N$-alkylpiperidones (26) and various aliphatic isocyanides (27) at 55°C. The reaction was conducted in methanol, for 18 h to give 75-86% yield.
Azizi et al., 2013 synthesized $N$-(2-(cyclohexylamino)-2-oxo-1-phenylethyl)-$N$-phenylbenzamide (32) by the Ugi-4CR. The substrates were isocyanides (23), acids (29), aldehydes (30) and amines (31). Choline chloride and urea were used in the reaction. The reaction was completed within 2-5 h and the yield was 60-92%.

As previously discussed in Chapter Two, quinolines are an important class of heterocyclic compounds found in many synthetic and natural products. They possess a wide range of pharmacological activities such as antimalarial, anti-inflammatory, anti-asthmatic, antihypertensive, antibacterial, and as platelet-derived growth factor receptors which are tyrosine kinase inhibiting agents (El Sayed et al., 2011). Some examples are Neocryptolepine (34), Gatifloxacin (33) and Lenvantinib (35) (Fig. 1) which are effective against Gram-positive and Gram-negative organisms (Lu et al., 1999). They inhibit the bacterial enzymes DNA gyrase (Matsui et al., 2008) and are multi-kinase inhibitors for various types of cancers (Gatto et al., 1999).

The pharmacological activity is due to the compounds intercalating between the base pairs of DNA and interfere with the normal functioning of the enzyme topoisomerase II which is involved in the breaking and releasing of DNA strands (Sigel and Sigel, 2000). The interaction of peptides with DNA has also attracted the attention of biochemists for the development of new DNA reagents for biotechnology and medicine (Grimm et al.,

\[
\begin{align*}
\text{NC} & \quad \text{COOH} & \quad \text{CHO} & \quad \text{NH}_2 \\
23 & 29 & 30 & 31
\end{align*}
\]

\[
\begin{align*}
R_1 = & \ C_6H_5, 4-OCH_3C_6H_5, 4-ClC_6H_5 \\
R_2 = & \ C_6H_5, 4-NO_2C_6H_5, 3-OCH_3C_6H_5
\end{align*}
\]
Due to the importance of quinolines, we decided to include a quinoline scaffold into the newly synthesized peptides.

In addition to the quinoline scaffold, lipoic acid can be combined with another pharmacophore to improve its biological properties such as the inhibition of acetylcholinesterase (AChE) activity (Biewenga et al., 1997). Studies have shown the neuroprotective, anti-Alzheimer and antioxidant activities of lipoic acid (Packer, 1998; Packer et al., 1995; Tirosh et al., 1999; Rosini et al., 2005; Moller et al., 2008). Thus, we envisaged that a peptide containing the quinoline ring system and a lipoic acid moiety would display good biological activity. Hence, the synthesis of peptides using Ugi-4CR was conducted.

Traditionally, the Ugi reaction is performed at room temperature or under reflux in methanol with reaction times up to 12–24 h or more using several catalysts (Zhang et al., 2007; Shanbhag et al., 2008; Bonnaterre et al., 2008; Hugel, 2009). However, most of these methods employ long reaction times and moderate yields of products are produced.

Microwave-assisted organic synthesis is a powerful technique that is used to accelerate organic reactions. The notable features of the microwave approach are eco-friendliness, enhanced reaction rates and greater selectivity (Kuang et al., 2001). Microwave has been recognized as an important technique for green and sustainable processes and this method advantageous over the traditional thermal method [Strauss et al., 1995; Varma, 1999; Bose et al., 1997; Nuchter et al., 2000; Romanova et al., 2000; Perreux and Loupy, 2001;
Deng and Lin, 1997; Elander et al., 2000; Caddick, 1995; Lidstrom et al., 2001). Due to these advantages, microwave irradiation was selected for this study.

In view of the remarkable importance from a pharmacological, industrial, and synthetic points of view, we report the one-pot synthesis of potentially biological active peptides via the Ugi-4CR under microwave irradiation within the framework of green chemistry protocol under catalyst-free condition. Furthermore, the new peptides were screened for their biological properties such as antimicrobial and antioxidant and their toxicity and binding with DNA gyrase by molecular docking were determined.

3.3. Results and discussion

The starting material, 2-chloroquinoline-3-carbaldehyde (CFQ) was prepared by the Vilsmeier-Haack reaction (Ambika and Singh, 2005). The desired starting material 2-methoxyquinoline-3-carbaldehyde (MQC) (37) was obtained from the reaction of K₂CO₃ with CFQ in methanol solution. The quinolone starting material, 2-oxo-1,2-dihydroquinoline-3-carbaldehyde (OQC) (53) was prepared from the reaction of glacial CH₃COOH with CFQ.

Scheme 1. Synthesis of quinolinyl-lipoyl peptides (40-52) via Ugi-4CR under microwave irradiation
Scheme 2. Synthesis of quinolonyl-lipoyl peptide (55) via Ugi-4CR under microwave irradiation

In a preliminary reaction, a mixture of 37 (1 mmol), aniline (1 mmol), lipoic acid (38) (1 mmol) and cyclohexyl isocyanide (39) (1 mmol) were refluxed in methanol (Ma et al., 2006). TLC was used to monitor the progress of the reaction. After 30 h, the product was formed and purified by column chromatography with a mixture of ethyl acetate: petroleum ether (1:3). The yield of the product was 30%. The reaction was too long and the yield was quite low. Thereafter, 37 (1 mmol), aniline (1 mmol), 38 (1.5 mmol) and 39 (1.5 mmol) in methanol, was used. After 24 h reflux, the product was formed and purified by column chromatography. The yield of the product was 40%. The same mole ratio of substrates was used and carried out under microwave irradiation at 120 W (110°C) for 15-18 minutes. We were pleased to find that the one-pot reaction proceeded smoothly to deliver the desired product in 90% yield (Scheme 1 & 2). The product was fully characterized by FTIR, $^1$H-NMR, $^{13}$C-NMR, HSQC, COSY, NOESY, HMBC and elemental analysis and was identified as N-(2-(cyclohexylamino)-1-(2-methoxyquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-phenylpentanamide (40).

The IR spectrum of 40 showed stretching frequencies (cm$^{-1}$) at 1600 for C=N, 1325 for C-N, 2970 for CH, 1493 for C=C, 2595 for SH, 1625 for C=O, 722 for C-S, 1230 for OCH$_3$ and 3416 for NH (Fig. 3.1, Appendix III). The $^1$H-NMR spectrum of 40 showed two singlets at $\delta$ 7.88 and $\delta$ 6.35 for C4-H and C9-H. The NH showed a doublet at $\delta$ 5.90 and the coupling constant was found to be 8.4 Hz. Cyclohexyl isocyanide proton CH (C1") showed a multiplet at $\delta$ 3.88 ($J = 4$ Hz) and lipoic acid CH (C19) showed a multiplet at $\delta$ 3.52-3.49 ($J = 1.72$ Hz) (Fig. 3.2). The $^{13}$C-NMR spectrum (Fig. 3.3)
showed the presence of two carbonyl groups at δ 168.72 (C10) and δ 173.67 (C14). The structure was further confirmed by 2D NMR spectral studies. The selected \(^1\)H and \(^{13}\)C-NMR chemical shifts are shown in Fig. 2.

The \(^{13}\)C-\(^1\)H-COSY correlation of carbon signals at δ 140.46, 139.84, 128.91, 127.85, 126.53, 124.27, 119.01, 58.65, 28.72, 25.49 and 24.84 were assigned to C1’, C4, C3’, C2’, C8, C6, C3, C9, C17, C16 and C3”, respectively. The carbon signal at δ 139.84 was due to quinolinyl C4-carbon and the spectrum is presented in (Fig. 3.4). The \(^1\)H, \(^1\)H-COSY spectrum of the compound revealed the correlation between doublet of NH at δ 5.90 and CH of cyclohexyl isocyanide at δ 3.88 (Fig. 3.5). The \(^1\)H, \(^1\)H-NOESY spectrum of the compound revealed the doublet of NH at δ 5.90 was coupled with the multiplet of cyclohexyl isocyanide CH (C1”) at δ 3.89-3.87 (J = 4 Hz), multiplet of CH\(_2\) (C2”) at δ 1.37-1.34 (J = 5.6 Hz) and the multiplet of CH\(_2\) (C6”) at δ 1.18-1.15 (J = 3.12 Hz) which was confirmed by the nearby protons of C2” and C6” (Fig. 3.6).

Fig. 2. Selected \(^1\)H and \(^{13}\)C-NMR chemical shifts of 40
The selected \(^1\)H and \(^{13}\)C-NMR) HMBC correlation chemical shifts are shown in Fig. 3. The HMBC spectrum of 40 (Fig. 3.7) showed the long-range correlations as follows: C9-H of 40 coupled with quinolinyl carbon (C4) at \(\delta\) 139.84, C8a at \(\delta\) 159.85, carbonyl carbons C10 at \(\delta\) 168.72 and C14 at \(\delta\) 173.67 and quaternary carbon C3 at \(\delta\) 119.01. This correlation of C9-H to the quaternary carbon (C3), carbonyl carbon (C10) of amide group and carbonyl carbon (C14) of lipoic acid indicated that the three groups were attached to C9. Thus it was evident the three different moieties were bonded to a common carbon and hence added valuable information to 40. The C4-H coupled with \(\alpha\)-C of amide group (C9) at \(\delta\) 58.65 and quinolinyl carbons, (C8a) at \(\delta\) 159.85, (C1’) at \(\delta\) 140.46, and (C5) at \(\delta\) 126.53.

Fig. 3. Selected HMBC correlation chemical shifts of 40
Table 1. Selected HMBC correlations of 40

<table>
<thead>
<tr>
<th>Entry</th>
<th>Proton Correlated Carbons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C9-H (s, 1H) at $\delta$ 6.35</td>
</tr>
<tr>
<td>2</td>
<td>C4-H (s, 1H) at $\delta$ 7.88</td>
</tr>
<tr>
<td>3</td>
<td>C8-H (d, 1H, $J = 8.32$Hz) at $\delta$ 7.78</td>
</tr>
<tr>
<td>4</td>
<td>C6-H (td, 1H, $J = 1.08$ Hz) at $\delta$ 7.59</td>
</tr>
<tr>
<td>5</td>
<td>C7-H (td, 1H, $J = 1.32$ Hz) at $\delta$ 7.59</td>
</tr>
<tr>
<td>6</td>
<td>C4'-H (td, 1H, $J = 7.16$Hz) at $\delta$ 7.30</td>
</tr>
<tr>
<td>7</td>
<td>C12-H (s, 3H) at $\delta$ 4.09</td>
</tr>
<tr>
<td>8</td>
<td>C2'' (m, $J = 4$ Hz) at $\delta$ 3.89-3.87</td>
</tr>
<tr>
<td>9</td>
<td>C15-H (m, $J = 7.32$) at $\delta$ 2.11-2.08</td>
</tr>
</tbody>
</table>

Similarly, C8-H coupled with quinolinyl carbons (C6) at $\delta$ 124.27 and (C7) at $\delta$ 130.05. The C6-H correlated with quinolinyl carbons (C4) at $\delta$ 139.84 and (C5a) at $\delta$ 145.84. The C7-H coupled with quinolinyl carbons (C6) at $\delta$ 124.27, (C8) at $\delta$ 124.56, (C4) at $\delta$ 139.84 and (C5a) at $\delta$ 145.84. The C4'-H correlated with aromatic carbon of amine (C3’) at $\delta$ 128.91 and carbon of amine (C2’) at $\delta$ 127.85. The C12-H correlated with quinolinyl carbon (C8a) at $\delta$ 159.85. C2''-H correlated with carbon of cyclohexyl isocyanide (C3’’) at $\delta$ 24.84 and carbonyl carbon of amide group (C10) at $\delta$ 168.72. C15-H correlated with carbons of lipoyl moiety (C16) at $\delta$ 25.49, (C17) at $\delta$ 28.72 and (C14) at $\delta$ 173.67. Selected $^1$H and $^{13}$C-NMR and HMBC chemical shifts of 40 are mentioned in Table 1.

Based on the above spectral details and mass analysis (TOF-MS, calculated value (m/z) 577.80 [M]+, found 600.23 [M+Na]+, Fig. 3.8), the structure was confirmed as N-(2-(cyclohexylamino)-1-(2-methoxyquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-phenylpentanamide (40).

The reaction was then optimised by evaluating the effect of solvents and the results are summarized in Table 2. The synthesis of 40 was chosen as the model reaction. Various solvents like acetonitrile, dichloromethane, ethanol, water and methanol were used. It
was observed that the reaction in methanol showed the best yield of 90%. The yield decreased to 40% when the solvents were changed (Table 2, entry 4).

**Table 2. Effect of solvent on the synthesis of quinolinyl-lipoyl peptides**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time (min)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetonitrile</td>
<td>18</td>
<td>71</td>
</tr>
<tr>
<td>2</td>
<td>Dichloromethane</td>
<td>20</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol</td>
<td>18</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>Water</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>Methanol</td>
<td>15</td>
<td>90</td>
</tr>
</tbody>
</table>

a Reaction conditions: 37 (1 mmol), aniline (1 mmol), 38 (1.5 mmol) and 39 (1.5 mmol) were added to methanol as solvent (15 mL) under microwave irradiation (120 W) at 110°C.

b Isolated yields.

Conventional heating and the microwave assisted method under various conditions were compared; the results are listed in Table 3. Without MW irradiation, 40 was formed at room temperature after 24 h. Also, the reaction at 90°C and without MW for 24 h, the yield of the obtained 40 was found to be 40-52% (Table 3, entries 1 & 2). Moreover, the effect of MW irradiation of different powers was investigated. As shown in Table 3, the reaction power was increased to 250 W, the yield of 40 remains unchanged. It was observed that the MW irradiation power of 120 W afforded the best yield of product with 90% isolated yield within 15 min (Table 3, entry 4). Therefore, 120 W was selected as the optimal microwave power for the synthesis.

**Table 3. Effect of microwave irradiation on the synthesis of quinolinyl-lipoyl peptides**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Power (W)</th>
<th>Time</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Without MW (r.t)</td>
<td>24 h</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>Without MW (90 °C)</td>
<td>24 h</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>15 min</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>15 min</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>15 min</td>
<td>78</td>
</tr>
<tr>
<td>6</td>
<td>200</td>
<td>20 min</td>
<td>80</td>
</tr>
<tr>
<td>7</td>
<td>250</td>
<td>20 min</td>
<td>80</td>
</tr>
</tbody>
</table>

a Reaction conditions: 37 (1 mmol), aniline (1 mmol), 38 (1.5 mmol) and 39 (1.5 mmol) were added to methanol as solvent (15 mL) under microwave irradiation at 110°C.

b Isolated yields.
It was found that microwave could raise the rate of reaction and this protocol was used to synthesize the remaining 13 derivatives by selecting the appropriate starting substrate.

Table 4. Synthesis of peptides through Ugi-4CR under microwave irradiation

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehyde</th>
<th>Amine</th>
<th>Product</th>
<th>Time (min)</th>
<th>Yield (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C_{11}H_{9}NO_{2}</td>
<td>C_{6}H_{7}N</td>
<td>C_{32}H_{30}N_{3}O_{5}S_{2} (40)</td>
<td>15</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>C_{11}H_{9}NO_{2}</td>
<td>C_{7}H_{9}N</td>
<td>C_{33}H_{41}N_{3}O_{5}S_{2} (41)</td>
<td>15</td>
<td>82</td>
</tr>
<tr>
<td>3</td>
<td>C_{11}H_{9}NO_{2}</td>
<td>C_{7}H_{9}N</td>
<td>C_{33}H_{41}N_{3}O_{5}S_{2} (42)</td>
<td>18</td>
<td>84</td>
</tr>
<tr>
<td>4</td>
<td>C_{11}H_{9}NO_{2}</td>
<td>C_{2}H_{2}NO</td>
<td>C_{33}H_{41}N_{3}O_{5}S_{2} (43)</td>
<td>15</td>
<td>84</td>
</tr>
<tr>
<td>5</td>
<td>C_{12}H_{11}NO_{2}</td>
<td>C_{6}H_{7}N</td>
<td>C_{33}H_{41}N_{3}O_{5}S_{2} (44)</td>
<td>15</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>C_{12}H_{11}NO_{2}</td>
<td>C_{7}H_{9}N</td>
<td>C_{34}H_{43}N_{3}O_{5}S_{2} (45)</td>
<td>15</td>
<td>89</td>
</tr>
<tr>
<td>7</td>
<td>C_{12}H_{11}NO_{2}</td>
<td>C_{7}H_{9}NO</td>
<td>C_{34}H_{43}N_{3}O_{5}S_{2} (46)</td>
<td>15</td>
<td>86</td>
</tr>
<tr>
<td>8</td>
<td>C_{11}H_{9}N_{2}O_{4}</td>
<td>C_{6}H_{7}N</td>
<td>C_{32}H_{36}N_{3}O_{5}S_{2} (47)</td>
<td>15</td>
<td>88</td>
</tr>
<tr>
<td>9</td>
<td>C_{11}H_{9}N_{2}O_{4}</td>
<td>C_{6}H_{7}N</td>
<td>C_{33}H_{41}N_{3}O_{5}S_{2} (48)</td>
<td>15</td>
<td>84</td>
</tr>
<tr>
<td>10</td>
<td>C_{11}H_{9}N_{2}O_{4}</td>
<td>C_{6}H_{7}N</td>
<td>C_{32}H_{36}N_{3}O_{5}S_{2} (49)</td>
<td>15</td>
<td>80</td>
</tr>
<tr>
<td>11</td>
<td>C_{11}H_{9}N_{2}O_{4}</td>
<td>C_{7}H_{9}N</td>
<td>C_{33}H_{41}N_{3}O_{5}S_{2} (50)</td>
<td>18</td>
<td>80</td>
</tr>
<tr>
<td>12</td>
<td>C_{11}H_{9}N_{2}O_{4}</td>
<td>C_{7}H_{9}N</td>
<td>C_{33}H_{41}N_{3}O_{5}S_{2} (51)</td>
<td>18</td>
<td>81</td>
</tr>
<tr>
<td>13</td>
<td>C_{11}H_{9}N_{2}O_{4}</td>
<td>C_{2}H_{2}NO</td>
<td>C_{33}H_{41}N_{3}O_{5}S_{2} (52)</td>
<td>15</td>
<td>90</td>
</tr>
<tr>
<td>14</td>
<td>C_{10}H_{7}NO_{2}</td>
<td>C_{2}H_{2}NO</td>
<td>C_{32}H_{39}N_{3}O_{5}S_{2} (55)</td>
<td>18</td>
<td>80</td>
</tr>
</tbody>
</table>

b Isolated yield.

The reaction proceeded well with different aldehydes such as 2-methoxy-6-methylquinoline-3-carbaldehyde and 2-methoxy-6-nitroquinoline-3-carbaldehyde with aromatic amines which resulted in a chemical library of fused quinolinyl-lipoxy peptides (40-52) and quinolonyl-lipoxy peptide (55) (Table 4).

Finally, we observed that the % yield (Table 4) of the peptides synthesized with anilines which possessed electron-withdrawing groups was lower than those with electron-donating groups. It was expected when the formation of an imine by nucleophilic addition, the electron withdrawing groups deactivated the aromatic ring by decreasing the electron density on the ring through an inductive effect/ resonance effect and made the formation of imine slower than the substrates containing electron donating groups. The characterization of the peptide 40 was discussed earlier: the derivatives were then easily characterized since the main scaffold was the same with minor alteration due to simple functional groups in the aromatic structure. The FTIR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis for the 14 new peptides are presented in Appendix-III.
A plausible mechanism is shown in **Scheme 3**. The initial step was the formation of an imine from the amine (2) and aldehyde with loss of one equivalent of water. Proton exchange with lipoic acid activated the iminium ion for nucleophilic addition of cyclohexyl isocyanide with its terminal carbon to nitrilium ion (3).

![Scheme 3](image)

**Scheme 3.** Plausible mechanism of the synthesis of quinolinyl-lipoic peptide

A second nucleophilic addition occurred at this intermediate (4) with lipoic acid anion. Subsequent reaction of imine with isocyanide and lipoic acid resulted in the intermediate, imidate (5). Some equilibria involving nitrilium trapped by oxygenated anion resulted in the corresponding imidate (5). The last step was thought to be an irreversible arrangement.
(i.e. Mumm rearrangement) displacing the whole equilibria by developing a CO double bond (Chéron et al., 2012).

The antimicrobial activity of the peptides (40, 41, 43, 44, 45, 46, 47 and 48) were assessed against four bacterial strains and two yeast strains using the well diffusion assay method (Balouiri et al., 2016). To assess the antimicrobial assay, bacterial cultures were plated on Luria Bertani (LB) Agar and yeast cultures on potato dextrose (PD) agar and kept at 37°C (bacterial cultures for 16 h) and 30°C (yeast cultures for 48 h). Further, bacterial and yeast cultures were grown in LB broth and PD broth at the same condition, respectively.

**Table 5. Antimicrobial activity of the novel peptides**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zone of inhibition by different peptides</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>0</td>
<td>20 ± 0.3</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Yeast</th>
<th></th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

(1 = 45; 2 = 47; 3 = 46; 4 = 48; 5 = 40; 6 = 44; 7 = 41; 8 = 43).

**Fig. 4. Antimicrobial screening of different peptides shown by well-diffusion method**

(1 = 45; 2 = 47; 3 = 46; 4 = 48; 5 = 40; 6 = 44; 7 = 41; 8 = 43)
The peptides showed antibacterial activity against *Bacillus cereus* (*B. cereus*) (Table 5) which was determined by the zone of inhibition (Fig. 4). Amongst all the peptides 41, 43, 46 and 47 showed clear zone inhibition against *B. cereus* (Fig. 4). In addition, the minimum inhibitory concentration (MIC) of all peptides were determined: A clear zone against *B. cereus* by using broth dilution methods were observed (Table 6).

The quinolinyl-lipoyl peptides (QLPs) containing methyl group in the ortho position of benzyl moiety (41), methoxy group in the ortho position of benzyl ring (43), methoxy group in the ortho position of benzyl ring and methyl group in the quinoline moiety (46) and nitro group (47) showed potential activities towards *B. cereus*. All the other peptides tested, showed no activity against the remaining bacterial and yeast strains.

The antioxidant ability of the peptides were determined by the decolourization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH). The DPPH radical scavenging assay was used for preliminary screening of the compounds for their antioxidant activity (Shaikh et al., 2014).

![Fig. 5. Qualitative detection of antioxidant properties of different peptides with -ve (methanol) and +ve (Rutin hydrate) control in colour (3= 41; 4= 46; 7= 47).](image)

The results of the antioxidant study revealed that the peptides 41, 46 and 47 were found to be effective DPPH scavenging agents (Fig. 5).

Furthermore, different concentrations of peptides, which showed positive antioxidant activities, were used with a control (Rutin hydrate) to determine the radical-scavenging inhibitory concentration i.e. IC$_{50}$ (Table 6). QLPs containing methyl group in the ortho position of benzyl moiety (41), methoxy group in the ortho position of benzyl ring and
methyl group in the quinoline moiety (46) and nitro group (47) showed significant DPPH scavenging activity.

**Table 6.** Minimum inhibitory concentration and radical-scavenging inhibitory concentration of different peptides

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compounds</th>
<th>MIC (µg/mL) <em>(B. cereus)</em></th>
<th>Radical-scavenging IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CIPX (control)</td>
<td>0.26 ± 0.11</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>RH (control)</td>
<td>–</td>
<td>2.16 ± 1.03</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0.67 ± 0.23</td>
<td>6.34 ± 0.89</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0.79 ± 0.16</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>–</td>
<td>7.87 ± 1.02</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>0.43 ± 0.21</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>0.72 ± 0.19</td>
<td>5.23 0.98</td>
</tr>
</tbody>
</table>

(1= 41; 2= 43; 3= 46; 4= 45; 5= 47).

The brine shrimp lethality assay was used as a preliminary assessment for toxicity (Carballo *et al.*, 2002). The mortality rate (24 and 48 h) of brine shrimp *Artemia salina* treated with different peptides is presented in **Fig. 6**.

**Fig. 6.** Mortality rate (24 and 48 h) of brine shrimp *Artemia salina* treated with different peptides

1= 40; 2= 41; 3= 43; 4= 46; 5= 44; 6= 45; 7= 47; 8= 48.
The results showed that the mortality rate was below 50% for QLPs containing methyl group in the ortho position of benzyl moiety (41), methoxy group in the ortho position of benzyl ring (43), methoxy group in the ortho position of benzyl ring and methyl group in the quinoline moiety (46) and nitro group (47) when treated with 300 µg of each peptide. This indicated that QLPs are safe to use for biological applications (Meyer et al., 1982).

The peptides including 41, 43, 46 and 47 showed significant antibacterial activity. To gain more insights into their antibacterial activity, computational studies based on the molecular docking approach were conducted.

![Molecular docking](image.png)

**Fig. 7.** The binding interactions between QLP (41) and DNA-gyrase

Molecular docking was conducted in the Libdock module of Discovery studio. The 3D-models of compounds 41, 43, 46 and 47 were constructed in Chembio3D-ultra software and were optimized using the MMFF94 minimization method. The crystal structure of DNA gyrase (PDB ID: 5BS8) (Blower et al., 2016) was used as a macromolecular
therapeutic target and the missing atoms were fixed using tLEaP and Chimera software (Kholmurodov et al., 2000; Jorgensen et al., 1983; Pettersen et al., 2004).

The hotspot for ligand docking was constructed around the surrounding of the moxifloxacin (co-crystallized ligand). Ciprofloxacin was used as a reference ligand to assess the antibacterial potency of 41, 43, 46 and 47. The results of molecular docking are presented in Table 7 based on Libdock score (Rao et al., 2007). The ligand binding landscape and their interaction pattern were analysed from the top ranked binding pose.

![Fig. 8. The binding interactions between QLP (43) and DNA-gyrase](image)

Peptides 41 and 43 were docked with a better docking score of 183.24 and 165.01 kcal/mol in comparison to ciprofloxacin (151.56 kcal/mol). The Libdock score of 41 and 43 indicated a higher antibacterial potency over ciprofloxacin (Table 7). The results are in accordance with the preliminary antimicrobial assay. The hydrogen bonding and
hydrophobic forces were found to be the prominent interaction of QLPs with DNA-gyrase (Fig. 7 and Fig. 8).

**Table 7.** Libdock scores of active quinoline peptides from molecular docking

<table>
<thead>
<tr>
<th>S. No</th>
<th>Compounds</th>
<th>Absolute energy</th>
<th>Libdock Score</th>
<th>Antimicrobial activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>41</td>
<td>87.5808</td>
<td>183.237</td>
<td>12 ± 0.4</td>
</tr>
<tr>
<td>2.</td>
<td>43</td>
<td>88.3352</td>
<td>165.014</td>
<td>11 ± 0.3</td>
</tr>
<tr>
<td>3.</td>
<td>46</td>
<td>-----</td>
<td>-----</td>
<td>20 ± 0.3</td>
</tr>
<tr>
<td>4.</td>
<td>47</td>
<td>-----</td>
<td>-----</td>
<td>12 ± 0.2</td>
</tr>
<tr>
<td>5.</td>
<td>Ciprofloxacin</td>
<td>38.7128</td>
<td>151.561</td>
<td>31 ± 0.2</td>
</tr>
</tbody>
</table>

Molecular docking: Libdock scores for the active molecules of quinoline peptides were obtained from molecular docking protocol of Libdock module of Discovery Studio.

### 3.4. Conclusion

In conclusion, medicinally active 14 quinoline and quinolone based peptides were synthesised by Ugi four-component reaction under the action of microwave irradiation. All compounds were fully characterized by spectroscopic techniques. A total of eight peptides were evaluated for their antimicrobial and antioxidant activities; compounds 41, 43, 46, 47 showed positive results for antimicrobial activity and 41, 46, 47 showed positive results for antioxidant activity. Molecular docking studies of 41 and 43 showed a higher binding affinity towards DNA gyrase than ciprofloxacin based on Libdock score. The advantages for the synthesis of new peptides are the use of inexpensive solvent, eco-friendly and short reaction time. It is envisaged that the peptides will find application in the field of biopharmaceuticals and drug discovery.

### 3.5. Experimental

**General procedure for the microwave synthesis of peptides**

A mixture of amine (1 mmol), 37 (1 mmol), 38 (1 mmol) and 39 (1.5 mmol) was dissolved in MeOH (15 mL) and the reaction mixture was transferred to a microwave tube (35 mL). The reaction tube was placed into a CEM microwave discover synthesizer and irradiated at 120 W with the temperature of 110°C for 15 min. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was allowed to attain room temperature. The solvent was removed under *in vacuo* and the residue was
purified by column chromatography (eluent ethyl acetate: petroleum ether, 1:3) to give the corresponding peptide. The recrystallization of the product was performed with 5:1 EtOAc: MeOH to yield the pure desired product.

**Antimicrobial Assay**

Approximately, 108 cfu/mL was used to standardize the bacterial and yeast cell concentration. A suspension (100 μL of 108 cfu/mL) of bacteria and yeast was plated on Mueller Hinton Agar plates and PD Agar plates, respectively. A well of 6 mm diameter was made using a sterile cork borer. The peptides (30 μL) were added to each well at concentration of 3 mg/ml and kept at 37°C (bacterial cultures) for 16 h and 30°C (yeast cultures) for 48 h. The assays were carried out in triplicate. Ciprofloxacin (3 mg/ml for bacteria) and Geneticin (50 mg/mL) were used as a positive control while DMSO (100%) as a negative control.

**Antioxidant assays**

Exactly 200 μL of the compound was added to 3 mL of 0.1 mM DPPH solution, and a colour change was observed at regular intervals. Rutin hydrates were used as a positive control and methanol (95%) as negative control.

**Toxicity assessment**

The brine shrimp larvae (*Artemia salina*) were hatched in sea water for 24-48 h prior to being used in the test. An aliquot of 5 mL sea water and ten brine shrimp were added to each vial and treated with different peptides having concentration of 300 μg. Brine shrimp death were observed at regular intervals.
References


3.1. $N$-((2-(cyclohexylamino)-1-(2-methoxyquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-phenylpentanamide (40)

Brown solid, m.p = 123-125°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 1600 C=N, 1325 C-N, 2970 CH, 1493 C=C, 1625 C=O, 722 C-S, 685 S-S, 3416 NH.

$^1$H-NMR: (400 MHz, CDCl$_3$) $\delta$ (ppm) 7.88 (1H, s, Ar-H), 7.78 (1H, d, $J=8.32$ Hz, Ar-H), 7.58 (2H, td, $J=1.32$ Hz, Ar-H), 7.52 (1H, d, $J=7.96$ Hz, Ar-H), 7.32 (3H, d, $J=0.76$ Hz, Ar-H), 7.10 (2H, d, $J=6.8$ Hz, Ar-H), 6.35 (1H, s, CH), 5.90 (1H, d, $J=8.4$ Hz, N-H), 4.09 (3H, s, OCH$_3$), 3.89-3.87 (1H, m, $J=4$ Hz, CH), 3.52-3.51 (1H, m, $J=1.72$ Hz, CH), 3.12-3.05 (2H, m, $J=6.88$ Hz, CH$_2$), 2.40-2.36 (2H, m, $J=7.32$ Hz, CH$_2$), 2.10 (3H, t, $J=7.32$ Hz, 2H(CH$_2$) and 1H(CH$_2$)), 2.06 (2H, d, $J=2.6$ Hz, CH$_2$), 1.89-1.88 (2H, m, $J=4.32$ Hz, CH$_2$) 1.67-1.65 (2H, m, $J=7.6$ Hz, CH$_2$), 1.62 (2H, m, $J=7.12$ Hz, CH$_2$), 1.60-1.58 (2H, m, $J=3.36$ Hz, CH$_2$), 1.37-1.34 (3H, m, $J=5.6$ Hz, 2H(CH$_2$) and 1H(CH$_2$)), 1.18-1.15 (2H, m, $J=3.12$ Hz, CH$_2$). $^{13}$C-NMR: (100 MHz, CDCl$_3$) $\delta$ (ppm) 177.24, 173.67, 168.72, 159.85, 145.84, 140.46, 139.84, 130.05, 128.91, 128.23, 127.85, 126.53, 124.56, 124.27, 119.01, 58.65, 56.38, 53.94, 48.78, 40.17, 38.43, 34.62, 33.60, 32.87, 32.84, 28.72, 25.49, 25.07, 25.01, 24.84, 24.76, 24.49. TOF-MS $m/z$: Calculated: 577.80 [M]$^+$, Found: 600.23 [M+Na]$^+$, 577.25 [M]$^+$. After subtracting the molecular weight of Na, the obtained value was 577.25). Elemental Analysis: Anal. Calc. for C$_{32}$H$_{39}$N$_3$O$_3$S$_2$: C, 66.52%; H, 6.80%; N, 7.27%; Found: C, 66.54%; H, 6.81%; N, 7.25%; %.

3.2. $N$-((2-(cyclohexylamino)-1-(2-methoxyquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-(o-tolyl)pentanamide (41)

Dark yellow solid, m.p = 128-130°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 1625 C=N, 1360 C-N, 2949 CH, 1434 C=C, 1683 C=O, 764 C-S, 539 S-S, 3456 NH. $^1$H-NMR: (400 MHz, CD$_2$OD-d$_4$) $\delta$ (ppm) 7.96 (1H, s, Ar-H), 7.90 (1H, d, $J=7.8$ Hz, Ar-H), 7.69 (2H, t, $J=10.4$ Hz, Ar-H), 7.53-7.50 (1H, m, $J=12$ Hz, Ar-H), 7.27 (1H, t, $J=7.32$ Hz, Ar-H), 7.11 (1H, t, $J=7.68$ Hz, Ar-H), 6.98 (2H, t, $J=9.28$ Hz, Ar-H), 6.57 (1H, s, NH), 6.14 (1H, s, CH), 4.02 (3H, s, OCH$_3$), 3.32 (1H, m, $J=1.52$ Hz, CH), 3.12-3.10 (2H, m, $J=1.64$ Hz, CH$_2$), 3.10-3.07 (2H, m, $J=1.28$ Hz, CH$_2$), 2.44-2.37 (2H, m, $J=4.28$ Hz, CH$_2$), 2.32 (1H, t, $J=7.2$ Hz,
CH), 1.98-1.96 (2H, \( J=1.82 \text{ Hz, CH} \)), 1.95 (2H, s, CH), 1.87-1.83 (2H, \( J=12.27 \text{ Hz, CH} \)), 1.63-1.62 (2H, \( J=4.16 \text{ Hz, CH} \)), 1.61-1.60 (2H, \( J=7.44 \text{ Hz, CH} \)), 1.60-1.58 (2H, \( J=2.28 \text{ Hz, CH} \)), 1.32-1.30 (2H, \( J=9.28 \text{ Hz, CH} \)), 1.29-1.28 (2H, \( J=3.96 \text{ Hz, CH} \)), 1.61-1.60 (2H, \( J=7.44 \text{ Hz, CH} \)), 1.60-1.58 (2H, \( J=2.28 \text{ Hz, CH} \)), 1.32-1.30 (2H, \( J=9.28 \text{ Hz, CH} \)), 1.29-1.28 (2H, \( J=3.96 \text{ Hz, CH} \)), 1.11-1.09 (3H, \( J=9 \text{ Hz, CH} \)).

\( ^{13}\text{C-NMR: (100 MHz, CDCl}_3 \delta (ppm) 159.32, 159.29, 146.30, 145.10, 132.54, 132.08, 129.67, 127.63, 127.60, 123.39, 118.38, 114.18, 114.07, 114.05, 113.94, 77.40, 76.76, 63.90, 63.83, 63.30, 63.23, 56.13, 55.62, 55.22, 30.91, 29.69, 16.47, 16.41, 16.28, 16.23. TOF-MS m/z: Calculated: 591.26 [M]+, Found: 614.35 [M+Na]+, 591.37 [M]+. Elemental Analysis: Anal. Calc. for C\(_{33}\)H\(_{41}\)N\(_3\)O\(_3\)S\(_2\): C, 66.97; H, 6.98; N, 7.10; %. Found: C, 66.98; H, 6.96; N, 7.12; %.

3.3. \( N\)-(2-(cyclohexylamino)-1-(2-methoxyquinolin-3-yl)-2-oxoethyl)-5-\((R)\)-1,2-dithiolan-3-yl)-N-(m-tolyl)pentanamide (42)

Brown solid, m.p = 129-131°C; IR \( \nu \text{max (cm}^{-1}) \): 1616 C=N, 1325 C-N, 2943 CH, 1436 C=C, 1647 C=O, 638 C-S, 538 S-S, 3266 NH.

\( ^{1}\text{H-NMR: (400 MHz, CD}_2\text{OD-d}4 \delta (ppm) 7.96 (1H, s, Ar-H), 7.70 (2H, d, Ar-H), 7.56 (2H, td, \( J=5.52 \text{ Hz, Ar-H} \)), 7.46 (1H, t, \( J=7.48 \text{ Hz, Ar-H} \)), 7.37 (1H, t, \( J=7.6 \text{ Hz, Ar-H} \)), 6.88 (1H, d, \( J=7.6 \text{ Hz, Ar-H} \)), 6.70 (1H, d, \( J=6.6 \text{ Hz, Ar-H} \)), 6.37 (1H, s, NH), 4.09 (1H, s, CH), 3.68-3.64 (1H, m, \( J=5.2 \text{, CH} \)), 3.55 (3H, s, OCH\(_3\)), 2.97-2.95 (1H, m, \( J=1.96 \text{ Hz, CH} \)), 2.83-2.80 (3H, m, \( J=4.68 \text{ Hz, CH} \)), 2.42 (2H, d, \( J=9.76 \text{ Hz, CH}_2 \)), 2.34 (2H, d, \( J=1.4 \text{ Hz, CH}_2 \)), 2.32-2.30 (2H, t, \( J=3.52 \text{ Hz, CH}_2 \)), 1.95 (2H, d, \( J=8.04 \text{ Hz, CH}_2 \)), 1.92 (2H, d, \( J=2.64 \text{ Hz, CH}_2 \)), 1.77 (2H, s, CH\(_2\)), 1.75 (2H, t, \( J=4 \text{ Hz, CH}_2 \)), 1.67-1.65 (2H, m, \( J=8 \text{ Hz, CH}_2 \)), 1.64-1.63 (2H, m, \( J=4 \text{ Hz, CH}_2 \)), 1.35 (2H, s, CH\(_2\)), 1.31 (2H, d, \( J=7.36 \text{ Hz, CH}_2 \)). \( ^{13}\text{C-NMR: (100 MHz, DMSO-d}_6 \delta (ppm) 169.07, 154.50, 145.52, 140.64, 131.56, 128.06, 127.53, 126.62, 126.60, 113.89, 112.84, 112.61, 107.25, 49.60, 40.46, 40.08, 39.87, 39.67, 39.46, 39.04, 38.83,

3.4. N-(2-(cyclohexylamino)-1-(2-methoxyquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-(2-methoxyphenyl)pentanamide (43)

Pale brown solid, m.p = 132-134°C; IR νmax (cm⁻¹): 1599 C=N, 1139 C-N, 2970 CH, 1492 C=C, 1625 C=O, 746 C-S, 618 S-S, 3416 NH.

¹H-NMR: (400 MHz, CD₃OD-d₄) δ (ppm) 8.00 (1H, s, ArH), 7.91 (1H, s, NH), 7.36 (1H, t, J=7.68 Hz, ArH), 7.27 (1H, t, J=7.08 Hz, ArH), 7.17-7.10 (1H, m, J=9.08 Hz, ArH), 6.99 (1H, d, J=8.32 Hz, ArH), 6.89 (2H, t, J=7.64 Hz, ArH), 6.60 (1H, d, J=8.4 Hz, ArH), 6.46 (1H, s, ArH), 6.17 (1H, s, CH), 4.01 (3H, s, OCH₃), 3.86 (2H, d, J=5.16 Hz, CH₂), 3.43 (3H, s, OCH₃), 3.32 (1H, m, J=1.52 Hz, CH), 2.12-2.10 (1H, m, J=7.12 Hz, CH), 2.01 (2H, s, CH₂), 1.83 (2H, m, J=2.0 Hz, CH₂), 1.76 (2H, d, J=3.8 Hz, CH₂), 1.62 (2H, m, J=1.12 Hz, CH₂), 1.58 (2H, m, J=2.4 Hz, CH₂), 1.40 (2H, d, J=1.12 Hz, CH₂), 1.38 (2H, m, J=2.4 Hz, CH₂), 1.35 (2H, m, J=5.32 Hz, CH₂), 1.32 (2H, m, J=1.88 Hz, CH₂), 1.24 (2H, s, CH₂). ¹³C-NMR: (100 MHz, CD₃OD-d₄) δ (ppm) 171.03, 161.79, 161.34, 147.34, 132.44, 129.24, 128.87, 128.73, 127.68, 125.65, 125.27, 121.98, 121.45, 121.10, 120.65, 112.92, 112.29, 79.57, 76.74, 63.97, 62.89, 61.58, 59.82, 59.82, 37.88, 37.23, 37.20, 36.52, 36.46, 36.14, 35.96, 29.99, 28.10, 26.28, 25.90. TOF-MS m/z: Calculated: 607.25 [M]⁺, Found: 630.31 [M+Na]⁺, 607.33 [M]⁺. Elemental Analysis: Anal. Calc. for C₃₃H₄₁N₃O₄S₂: C, 65.21; H, 6.80; N, 6.91; %. Found: C, 65.20; H, 6.82; N, 6.90; %.

3.5. N-(2-(cyclohexylamino)-1-(2-methoxy-6-methylquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-phenylpentanamide (44)

Brown solid, m.p = 127-129°C; IR νmax (cm⁻¹): 1738 C=N, 1189 C-N, 2970 CH, 1492 C=C, 1600 C=O, 747 C-S, 655 S-S, 3418 NH. ¹H-NMR: (400 MHz, CDCl₃) δ (ppm) 8.63 (1H, s, ArH), 8.36 (1H, d, J=1.38 Hz, NH), 8.08 (1H, t, J=4.52 Hz, ArH), 7.53 (2H, d, J=7.88 Hz, ArH), 7.16 (2H, t, J=7.4 Hz, ArH), 7.11 (1H, d, J=11.12 Hz, ArH), 7.08 (2H, m, J=1.88 Hz, ArH), 5.80 (1H, s, CH), 4.03 (3H, s, OCH₃), 3.77-3.73 (2H, m, J=3.16 Hz, CH₂), 3.58-3.51 (1H, m, J=6.4 Hz, CH), 3.46-3.42 (2H, m, J=6.48 Hz, CH₂), 3.14-
3.11 (2H, m, J=1.28 Hz, CH₂), 3.09-3.05 (2H, m, J=4.0 Hz, CH₂), 2.35-2.33 (2H, m, J=7.52 Hz, CH₂), 2.33-2.31 (2H, m, J=7.4 Hz, CH₂), 1.88-1.87 (2H, m, J=4.44 Hz, CH₂), 1.86-1.85 (2H, m, J=7.52 Hz, CH₂), 1.67-1.65 (2H, m, J=2.0 Hz, CH₂), 1.53-1.51 (2H, m, J=1.52 Hz, CH₂), 1.31 (2H, d, J=3.84 Hz, CH₂), 1.30 (2H, d, J=4.2 Hz, CH₂), 1.14 (3H, d, J=10.32 Hz, CH₃). ¹³C-NMR: (100 MHz, CD₃OD-d₄) δ (ppm) 177.42, 176.18, 175.06, 161.62, 141.07, 138.96, 132.39, 131.55, 130.78, 130.67, 130.57, 129.96, 125.88, 125.56, 121.05, 119.61, 63.95, 57.47, 48.82, 48.60, 41.31, 41.27, 36.42, 36.32, 36.25, 35.62, 34.76, 33.57, 29.87, 29.78, 26.72, 26.50, 26.02, 25.86, 25.65. TOF-MS m/z: Calculated: 591.83 [M]+ Found: 614.24 [M+Na]+, 591.26 [M]+. Elemental Analysis: Anal. Calc. for C₃₃H₄₁N₃O₃S₂: C, 66.97; H, 6.98; N, 7.10; %. Found: C, 66.99; H, 6.96; N, 7.12; %.

3.6. N-(2-(cyclohexylamino)-1-(2-methoxy-6-methylquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-(m-tolyl)pentanamide (45)

Brown solid, m.p = 129-131°C; IR νmax (cm⁻¹): 1615 C=N, 1074 C-O, 2970 CH, 1491 C=C, 1645 C=O, 741 C-S, 698 S-S, 3393 NH. ¹H-NMR: (400 MHz, CDCl₃) δ (ppm) 8.64 (1H, d, J=11.28 Hz, ArH), 8.35 (1H, s, ArH), 8.10 (1H, s, ArH), 7.63 (1H, d, J=8.48 Hz, ArH), 7.42 (1H, s, ArH), 7.07 (2H, d, J=12.04 Hz, ArH), 6.99 (2H, d, J=7.52 Hz, ArH), 6.24 (1H, s, CH), 4.03 (3H, s, OCH₃), 3.58-3.55 (1H, m, J=7.64 Hz, CH), 2.46-2.43 (1H, m, J=6.52 Hz, CH), 2.41 (3H, s, CH₃), 2.41 (2H, m, J= 5.12 Hz, CH₂), 2.39 (2H, m, J=6.68 Hz, CH₂), 2.34 (2H, m, J=7.68 Hz, CH₂), 2.33 (2H, m, J=3.16 Hz, CH₂), 2.09 (3H, t, J=7.36 Hz, CH₃), 1.93-1.91 (2H, m, J=8.56 Hz, CH₂), 1.90-1.88 (2H, m, J=3.44 Hz, CH₂), 1.69 (2H, m, J=1.56 Hz, CH₂), 1.68 (2H, m, J=1.42 Hz, CH₂), 1.35 (2H, m, J=3.2 Hz, CH₂), 1.32 (2H, m, J=2.32 Hz, CH₂), 1.25 (2H, s, CH₂). ¹³C-NMR: (100 MHz, CDCl₃) δ (ppm) 160.01, 145.90, 145.75, 139.04, 136.84, 136.78, 129.59, 129.13, 127.76, 126.74, 125.26, 125.23, 124.22, 121.36, 121.34, 119.50, 114.54, 110.55, 77.37, 76.73,
63.55, 63.49, 63.25, 63.18, 53.95, 49.45, 47.93, 21.57, 16.50, 16.44, 16.18, 16.12. TOF-MS m/z: Calculated 605.86 [M]^+, Found: 605.86 [M+Na]^+, 605.28 [M]^+. Elemental Analysis: Anal. Calc. for C_{34}H_{43}N_{3}O_{3}S_{2}: C, 67.40; H, 7.15; N, 6.94; %. Found: C, 67.42; H, 7.16; N, 6.93; %.

3.7. N-(2-(cyclohexylamino)-1-(2-methoxy-6-methylquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-(2-methoxyphenyl)pentanamide (46)

Dark brown solid, m.p = 130-132°C; IR ν_{max} (cm⁻¹): 1599 C=N, 1335 C-N, 2970 CH, C=C, 1625 C=O, 722 C-S, 573 S-S, 3416 NH.

^1^H-NMR: (400 MHz, CD_{3}OD-d_{4}) δ (ppm) 7.84 (1H, s, ArH), 7.71 (1H, s, NH), 7.61 (1H, m, J=6.6 Hz, ArH), 7.20 (1H, t, J=6.96 Hz, ArH), 6.89 (1H, m, J=6.32 Hz, ArH), 6.20 (1H, s, CH), 3.70 (3H, s, OCH_{3}), 3.61 (3H, s, OCH_{3}), 3.48-3.44 (1H, m, J=5.96 Hz, CH), 3.21 (2H, m, J=1.72 Hz, CH_{2}), 3.20 (2H, m, J=1.56 Hz, CH_{2}), 3.06-3.03 (2H, m, J=5.36 Hz, CH_{2}), 3.00-2.95 (2H, m, J=6.92 Hz, CH_{2}), 2.37-2.32 (2H, m, J=5.76 Hz, CH_{2}), 2.21 (2H, d, J=7.36 Hz, CH_{2}), 2.20 (2H, m, J=7.24 Hz, CH_{2}), 1.82-1.79 (1H, m, J=6.88 Hz, CH), 1.54-1.52 (2H, m, J=7.52 Hz, CH_{2}), 1.52-1.50 (2H, m, J=5.64 Hz, CH_{2}), 1.38 (2H, m, J=5.08 Hz, CH_{2}), 1.36 (2H, m, J=7.04 Hz, CH_{2}), 1.18 (3H, s, CH_{3}). ^13^C-NMR: (100 MHz, CD_{3}OD-d_{4}) δ 177.42, 176.18, 175.06, 161.62, 141.07, 138.96, 132.39, 131.55, 130.74, 130.67, 129.96, 125.88, 125.56, 121.05, 119.61, 63.95, 57.47, 50.45, 49.66, 49.52, 49.03, 48.82, 41.31, 41.27, 36.32, 36.25, 35.62, 34.76, 33.57, 29.87, 26.72, 26.02, 25.65. TOF-MS m/z: Calculated: 621.27 [M]^+. Found: 644.29 [M+Na]^+, 621.31 [M]^+. Elemental Analysis: Anal. Calc. for C_{34}H_{43}N_{3}O_{3}S_{2}: C, 65.67; H, 6.97; N, 6.76; %. Found: C, 65.66; H, 6.99; N, 6.78; %.

3.8. N-(2-(cyclohexylamino)-1-(2-methoxy-6-nitroquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-phenylpentanamide (47)

Yellow solid, m.p = 147-149°C; IR ν_{max} (cm⁻¹): 1615 C=N, 1092 C-N, 2852 CH, 1464 C=C, 1721 C=O, 741 C-S, 698 S-S, 3457 NH. ^1^H-NMR: (400 MHz, CD_{3}OD-d_{4}) δ (ppm) 8.25 (1H, s, ArH), 7.99 (2H, t, J=2.0 Hz, ArH), 7.98 (2H, t, J=2.12 Hz, ArH), 7.57 (1H, d, J=7.8 Hz, NH), 6.64 (2H, t, J=1.96 Hz, ArH), 6.62 (2H, t, J=2.0 Hz, ArH), 6.20 (1H, s, CH), 4.19 (3H, s, OCH_{3}), 3.33-3.31 (1H, m, J=6.56 Hz, CH), 3.18-3.17 (2H, m, J=5.4
1H, CH2), 2.50-2.43 (1H, J=5.4 Hz, CH), 2.37 (2H, d, J=7.08 Hz, CH2), 2.35 (2H, m, J=7.16 Hz, CH2), 2.30 (2H, t, J=7.28 Hz, CH2), 1.92-1.87 (2H, m, J=6.8 Hz, CH2), 1.70-1.68 (2H, m, J=8.24 Hz, CH2), 1.68-1.67 (2H, m, J=1.48 Hz, CH2), 1.67-1.66 (2H, m, J=3.68 Hz, CH2), 1.66-1.64 (2H, m, J=7.28 Hz, CH2), 1.51 (2H, m, J=1.48 Hz, CH2), 1.49 (2H, m, J=4.16 Hz, CH2). 13C-NMR: (100 MHz, CD3OD-d4) δ (ppm) 177.65, 177.56, 162.80, 161.66, 156.81, 138.93, 138.33, 130.69, 130.09, 129.98, 125.93, 125.60, 121.07, 119.62, 116.89, 113.68, 63.98, 57.58, 41.34, 39.40, 37.79, 36.56, 36.02, 35.34, 34.84, 33.70, 32.94, 29.89, 27.84, 26.60, 26.15. TOF-MS m/z: Calculated: 622.23 [M]+, Found: 645.28 [M+Na]+, 622.30 [M]+. Elemental Analysis: Anal. Calc. for C32H38N4O5S2: C, 61.71; H, 6.15; N, 9.00; %. Found: C, 61.73; H, 6.17; N, 9.02; %.

3.9. N-(2-amino-4-nitrophenyl)-N-(2-(cyclohexylamino)-1-(2-methoxy-6-nitro quinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)pentanamide (48)

Reddish brown solid, m.p = 126-128°C; IR νmax (cm⁻¹): 1637 C=N, 1327 C-N, 2970 CH, 1497 C=C, 1738 C=O, 787 C-S, 587 S-S, 3457 NH. 1H-NMR: (400 MHz, CD3OD-d4) δ (ppm) 8.45 (1H, s, ArH), 8.23 (1H, d, J=2.28 Hz, ArH), 8.20 (1H, d, J=2.16 Hz, ArH), 7.60-7.57 (2H, m, J=1.2 Hz, ArH), 7.33-7.32 (1H, m, J=4.28 Hz, ArH), 6.91 (1H, d, J=8.2 Hz, ArH), 6.63-6.60 (2H, m, J=2.6 Hz, ArH), 5.12 (1H, s, NH), 4.49 (2H, s, CH2), 4.33 (1H, s, CH), 4.16 (3H, s, OCH3), 3.58-3.55 (1H, m, J=2.32 Hz, CH), 2.49-2.43 (1H, m, J=5.56 Hz, CH), 2.32 (2H, s, CH2), 2.29 (2H, d, J=7.32 Hz, CH2), 2.17 (2H, s, CH2), 1.93-1.89 (2H, m, J=13.72 Hz, CH2), 1.88-1.84 (2H, m, J=6.8 Hz, CH2), 1.69-1.68 (2H, m, J=2.8 Hz, CH2), 1.65-1.64 (2H, m, J=3.76 Hz, CH2), 1.63-1.62 (2H, m, J=2.68 Hz, CH2), 1.50-1.48 (2H, m, J=3.4 Hz, CH2), 1.46-1.45 (2H, m, J=3.08 Hz, CH2), 1.28 (2H, s, CH2). 13C-NMR: (100 MHz, CD3OD-d4) δ (ppm) 147.37, 134.67, 131.13, 130.24, 128.56, 126.33, 125.65, 125.32, 125.20, 124.18, 119.41, 117.89, 11.751, 117.47, 113.53, 113.15, 111.54, 109.42, 107.01, 54.34, 54.20, 54.09, 49.74, 44.12, 43.71, 35.10, 33.69, 30.76, 27.80,

3.10. N-(2-(cyclohexylamino)-1-(2-methoxy-6-nitroquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-(2-fluorophenyl)pentanamide (49)

Brown solid, m.p = 127-129°C; IR ν_{max} (cm^{-1}): 1629 C=N, 1351 C-N, 2924 CH, 1499 C=C, 1687 C=O, 788 C-S, 620 S-S, 3447 NH. ^1^H-NMR: (400 MHz, CD_{3}OD-d_{4}) δ (ppm) 8.05 (1H, d, J=6.6 Hz, ArH), 7.87 (1H, s, ArH), 7.83 (1H, td, J=14.04 Hz, ArH), 7.72 (1H, t, J=5.52 Hz, ArH), 7.44 (1H, t, J=4.44 Hz, ArH), 7.22 (1H, t, J=18.32 Hz, ArH), 6.98-6.95 (1H, m, J=9.76 Hz, ArH), 6.41 (1H, s, NH), 6.40 (1H, s, ArH), 6.12 (1H, s, CH), 4.09 (3H, s, OCH_{3}), 3.69-3.75 (1H, m, J=6.2 Hz, CH), 3.33-3.31 (2H, m, J=1.64 Hz, CH_{2}), 3.16-3.11 (2H, m, J=2.0 Hz, CH_{2}), 3.07-3.04 (2H, m, J=6.8 Hz, CH_{2}), 2.45-2.37 (2H, m, J=5.6 Hz, CH_{2}), 2.29 (2H, d, J=7.32 Hz, CH_{2}), 2.26 (1H, d, J=4.32 Hz, CH), 1.88-1.86 (2H, m, J=2.28 Hz, CH_{2}), 1.85-1.83 (2H, m, J=6.48 Hz, CH_{2}), 1.76-1.73 (2H, m, J=4.24 Hz, CH_{2}), 1.64-1.60 (2H, m, J=7.92 Hz, CH_{2}), 1.36-1.33 (2H, m, J=4.12 Hz, CH_{2}), 1.29 (2H, t, J=7.88 Hz, CH_{2}). ^1^C-NMR: (100 MHz, CDCl_{3}) δ (ppm) 175.54, 147.10, 146.77, 139.77, 138.44, 137.06, 136.56, 135.34, 133.61, 129.25, 127.63, 127.22, 126.78, 123.97, 111.93, 110.90, 106.56, 54.08, 50.42, 46.19, 45.05, 41.61, 37.07, 33.87, 32.11, 31.90, 31.42, 30.90, 30.30, 30.18, 29.45, 29.39. Elemental Analysis: Anal. Calc. for C_{32}H_{37}FN_{4}O_{5}S_{2}: C, 59.98; H, 5.82; N, 8.74; %. Found: C, 59.96; H, 5.84; N, 8.76; %.

3.11. N-(2-(cyclohexylamino)-1-(2-methoxy-6-nitroquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-(m-tolyl)pentanamide (50)

Brown solid, m.p = 148-150°C; IR ν_{max} (cm^{-1}): 1599 C=N, 1067 C-N, 2970 CH, 1585 C=C, 1679 C=O, 749 C-S, 688 S-S, 3352 NH. ^1^H-NMR: (400 MHz, CD_{3}OD-d_{4}) δ (ppm) 8.12 (1H, d, J=1.68 Hz, ArH), 7.82 (1H, s, ArH), 7.72 (1H, td, J=1.48 Hz, ArH), 7.44 (2H, dd, J=7.64 Hz, ArH), 7.35 (1H, t, J=7.6 Hz, ArH), 7.13 (2H, t, J=7.8 Hz, ArH), 6.68 (1H, d, J=7.48 Hz, NH), 4.60 (1H, s, CH), 3.90 (3H, s, OCH_{3}), 3.32 (1H, m, J=3.08 Hz, CH), 3.15-3.12 (2H, m, J=1.56 Hz, CH_{2}), 3.10-3.07 (2H, m, J=6.96 Hz, CH_{2}), 2.42 (2H,
N-(2-(cyclohexylamino)-1-(2-methoxy-6-nitroquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-(o-tolyl)pentanamide (51)

Brown solid, m.p = 128-130°C; IR \(\nu_{\text{max}}\) (cm\(^{-1}\)): 1612 C=N, 1297 C=N, 1496 C=C, 1630 C=O, 786 C-S, 639 S-S, 3457 NH. \(^1\)H-NMR: (400 MHz, CD\(_3\)OD-d\(_4\)) \(\delta\) (ppm) 8.17 (1H, td, \(J=6.52\) Hz, ArH), 8.05 (1H, d, \(J=1.56\) Hz, NH), 7.96 (1H, s, ArH), 7.73-7.69 (2H, m, \(J=3.28\) Hz, ArH), 7.64-7.61 (1H, m, \(J=3.36\) Hz, ArH), 7.31 (2H, td, \(J=7.08\) Hz, ArH), 4.57 (1H, s, CH), 4.23-4.22 (1H, m, \(J=2.08\) Hz, CH), 4.10 (3H, s, OCH\(_3\)), 3.59-3.56 (2H, m, \(J=5.96\) Hz, CH\(_2\)), 3.32 (2H, m, \(J=3.12\) Hz, CH\(_2\)), 3.31 (2H, m, \(J=3.16\) Hz, CH\(_2\)), 3.18-3.15 (2H, m, \(J=5.36\) Hz, CH\(_2\)), 3.13-3.05 (2H, m, \(J=6.76\) Hz, CH\(_2\)), 2.49-2.44 (1H, m, \(J=5.76\) Hz, CH), 2.30 (3H, t, \(J=7.3\) Hz, CH\(_3\)), 1.92-1.90 (2H, m, \(J=6.76\) Hz, CH\(_2\)), 1.89-1.87 (2H, m, \(J=6.64\) Hz, CH\(_2\)), 1.66-1.64 (2H, m, \(J=5.0\) Hz, CH\(_2\)), 1.63-1.62 (2H, m, \(J=5.4\) Hz, CH\(_2\)), 1.51-1.49 (2H, m, \(J=8.8\) Hz, CH\(_2\)), 1.47-1.46 (2H, m, \(J=1.72\) Hz, CH\(_2\)). \(^{13}\)C-NMR: (100 MHz, CDCl\(_3\)) \(\delta\) (ppm) 163.66, 146.17, 146.03, 139.05, 131.76, 129.46, 129.40, 129.38, 129.32, 129.08, 119.56, 115.67, 115.64, 115.45, 115.43, 114.75, 110.77, 77.33, 76.70, 63.40, 63.33, 63.29, 63.22, 56.10, 54.59, 21.54, 16.45, 16.40,
16.26, 16.20. Elemental Analysis: Anal. Calc. for C$_{33}$H$_{40}$N$_{4}$O$_{5}$S$_{2}$: C, 62.24; H, 6.33; N, 8.80; %. Found: C, 62.26; H, 6.34; N, 8.82; %.

3.13. N-(2-(cyclohexylamino)-1-(2-methoxy-6-nitroquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-(2-methoxyphenyl)pentanamide (52)

Brown solid, m.p = 140-142°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 1676 C=N, 1366 C-N, 2970 CH, 1495 C=C, 1691 C=O, 734 C-S, 3457 NH. $^1$H-NMR: (400 MHz, CD$_{3}$OD-d$_{4}$) $\delta$ (ppm) 8.39 (1H, s, NH), 8.24 (1H, t, $J$=2.84 Hz, Ar-H), 8.22 (1H, t, $J$=2.76 Hz, Ar-H), 7.99-7.80 (1H, t, $J$=3.28 Hz, Ar-H), 7.97 (1H, t, $J$=2.0 Hz, Ar-H), 7.83 (1H, t, $J$=3.84 Hz, Ar-H), 7.81 (1H, t, $J$=2.0 Hz, Ar-H), 6.64 (1H, t, $J$=3.24 Hz, Ar-H), 6.62 (1H, t, $J$=2.28 Hz, Ar-H), 6.12 (1H, s, CH), 4.24 (3H, s, OCH$_3$), 4.11 (3H, s, OCH$_3$), 3.90 (2H, d, $J$=3.02 Hz, CH$_2$), 3.59-3.56 (1H, m, $J$=6.0 Hz, CH), 3.33-3.21 (2H, m, $J$=3.16 Hz, CH$_2$), 3.20-3.18 (2H, m, $J$=5.4 Hz, CH$_2$), 3.16-3.09 (2H, m, $J$=13.0 Hz, CH$_2$), 2.49-2.44 (1H, m, $J$=9.0 Hz, CH), 2.38-2.35 (2H, t, $J$=14.52 Hz, CH$_2$), 2.31 (2H, t, $J$=7.32 Hz, CH$_2$), 1.94-1.87 (2H, m, $J$=6.88 Hz, CH$_2$), 1.70-1.68 (2H, m, $J$=7.16 Hz, CH$_2$), 1.68-1.66 (2H, m, $J$=5.64 Hz, CH$_2$), 1.66-1.64 (2H, m, $J$=4.56 Hz, CH$_2$), 1.62 (2H, m, $J$=2.56 Hz, CH$_2$), 1.29 (2H, d, $J$=1.36 Hz, CH$_2$). $^{13}$C-NMR: (100 MHz, CD$_{3}$OD-d$_{4}$) $\delta$ (ppm) 177.66, 164.27, 161.93, 161.90, 156.82, 150.46, 145.07, 144.91, 138.32, 127.86, 127.34, 126.53, 125.96, 125.91, 122.15, 121.59, 120.98, 120.56, 113.64, 111.76, 57.56, 56.33, 41.31, 37.78, 37.33, 35.79, 34.68, 34.56, 33.69, 30.77, 29.88, 26.14, 25.88. Elemental Analysis: Anal. Calc. for C$_{33}$H$_{40}$N$_{4}$O$_{5}$S$_{2}$: C, 60.72; H, 6.18; N, 8.58; %. Found: C, 60.74; H, 6.19; N, 8.56; %.

3.14. N-(2-(cyclohexylamino)-2-oxo-1-(2-oxo-1,2-dihydroquinolin-3-yl)ethyl)-5-((R)-1,2-dithiolan-3-yl)-N-(2-methoxyphenyl)pentanamide (55)

Yellow solid, m.p = 136-138°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 1604 C=N, 1365 C-N, 2970 CH, 1474 C=C, 1625 C=O, 746 C-S, 663 S-S, 3457 NH. $^1$H-NMR: (400 MHz, CD$_{3}$OD-d$_{4}$) $\delta$ (ppm) 8.10 (1H, s, Ar-H), 8.02 (1H, s, Ar-H), 7.96 (1H, s, NH), 7.69 (2H, dd, $J$= 8Hz, Ar-H), 7.57 (1H, t, $J$=8.4 Hz, Ar-H), 7.38 (1H, d, $J$= 8.24 Hz, Ar-H), 7.28 (1H, td, $J$=7.16 Hz, Ar-H), 6.81 (1H, d, $J$=1.6 Hz, Ar-H), 6.75 (1H, t, $J$= 2.28 Hz, Ar-H), 6.73 (1H, d, $J$=1.52
Hz, Ar-H), 3.75 (3H, s, OCH₃), 3.42 (1H, s, CH), 3.33-3.31 (1H, m, J=1.6 Hz, CH), 2.32-2.28 (1H, m, J=7.4 Hz, CH), 1.76 (2H, t, J=3.64 Hz, CH₂), 1.74-1.72 (2H, m, J=2.28 Hz, CH₂), 1.39-1.38 (2H, m, J=3.2 Hz, CH₂), 1.37-1.36 (2H, m, J=7.56 Hz, CH₂), 1.35 (2H, t, J=2.24 Hz, CH₂), 1.32-1.31 (2H, m, J=2.76 Hz, CH₂), 1.30-1.28 (2H, m, J=12.04 Hz, CH₂), 1.27-1.26 (2H, m, J=10.72 Hz, CH₂), 1.25 (2H, d, J=1.32 Hz, CH₂), 0.92-0.90 (2H, m, J=4.72 Hz, CH₂), 0.89-0.88 (2H, m, J=4Hz, CH₂). ¹³C-NMR: (100 MHz, CDCl₃) δ (ppm) 144.12, 143.98, 138.22, 138.18, 136.07, 133.22, 131.41, 131.37, 129.60, 129.57, 127.49, 127.44, 126.83, 116.56, 114.68, 77.35, 7724, 77.03, 76.71, 63.75, 63.68, 63.50, 63.43, 55.98, 54.47, 21.14, 16.40, 16.34, 16.28. Elemental Analysis: Anal. Calc. for C₃₂H₃₉N₃O₄S₂: C, 64.73; H, 6.62; N, 7.08; %. Found: C, 64.75; H, 6.64; N, 7.06; %. 
3.1. \(N\)-(2-(cyclohexylamino)-1-(2-methoxyquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-phenylpentanamide (40)

![Fig. 3.1. IR spectrum of 40](image1)

![Fig. 3.2. \(^1\)H-NMR spectrum for 40](image2)
Fig. 3.3. $^{13}$C-NMR spectrum for 40

Fig. 3.4. HSQC spectrum for 40
Fig. 3.5. COSY spectrum for 40

Fig. 3.6. NOESY spectrum for 40
Fig. 3.7. HMBC spectrum for 40

Fig. 3.8. Mass spectrum for 40
3.2. \( N-(2-(\text{cyclohexylamino})-1-(2\text{-methoxyquinolin-3-yl})-2\text{-oxoethyl})-5-((R)-1,2\text{-dithiolan-3-yl})-N-(o\text{-tolyl})\text{pentanamide (41)} \)

**Fig. 3.9.** IR spectrum of 41

**Fig. 3.10.** \(^1\text{H}-\text{NMR spectrum for 41}\)
Fig. 3.11. $^{13}$C-NMR spectrum for 41

Fig. 3.12. Mass spectrum of 41
3.3. \( \text{N-(2-(cyclohexylamino)-1-(2-methoxyquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-(m-tolyl)pentanamide (42)} \)

**Fig. 3.13.** IR spectrum of 42

**Fig. 3.14.** \(^1\text{H}-\text{NMR} \) spectrum for 42
Fig. 3.15. $^{13}$C-NMR spectrum for 42

3.4. $N$-(2-(cyclohexylamino)-1-(2-methoxyquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-$N$-(2-methoxyphenyl)pentanamide (43)

Fig. 3.16. IR spectrum of 43
Fig. 3.17. $^1$H-NMR spectrum for 43

Fig. 3.18. $^{13}$C-NMR spectrum for 43
3.5. \( \text{N-}(2-(\text{cyclohexylamino})-1-(2-\text{methoxy-6-methylquinolin-3-yl})-2-\text{oxoethyl})-5-((\text{R})-1,2-\text{dithiolan-3-yl})-\text{N-phenylpentanamide (44)} \)

Fig. 3.19. Mass spectrum of 43

Fig. 3.20. IR spectrum of 44
Fig. 3.21. $^1$H-NMR spectrum for 44

Fig. 3.22. $^{13}$C-NMR spectrum for 44
Fig. 3.23. Mass spectrum for 44

3.6. N-(2-(cyclohexylamino)-1-(2-methoxy-6-methylquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-(m-tolyl)pentanamide (45)

Fig. 3.24. IR spectrum of 45
Fig. 3.25. $^1$H-NMR spectrum for 45

Fig. 3.26. $^{13}$C-NMR spectrum for 45
Fig. 3.27. Mass spectrum for 45

3.7. \( N-(2-\text{(cyclohexylamino)}-1-(2\text{-methoxy-6-methylquinolin-3-yl})-2\text{-oxoethyl})-5\text{-((R)}-1,2\text{-dithiolan-3-yl})-N\text{-}2\text{-methoxyphenyl})\text{pentanamide (46)} \)

Fig. 3.28. IR spectrum of 46
Fig. 3.29. $^1$H-NMR spectrum for 46

Fig. 3.30. $^{13}$C-NMR spectrum for 46
Fig. 3.31. Mass spectrum of 46

3.8. \( \text{N-(2-(cyclohexylamino)-1-(2-methoxy-6-nitroquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-} \text{N-phenylpentanamide (47)} \)

Fig. 3.32. IR spectrum of 47
Fig. 3.33. $^1$H-NMR spectrum for 47

Fig. 3.34. $^{13}$C-NMR spectrum for 47
3.9. \(N\)-(2-amino-4-nitrophenyl)-\(N\)-(2-(cyclohexylamino)-1-(2-methoxy-6-nitroquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)pentanamide (48)

Fig. 3.35. Mass spectrum of 47

Fig. 3.36. IR spectrum of 48
Fig. 3.37. $^{1}$H-NMR spectrum for 48

Fig. 3.38. $^{13}$C-NMR spectrum for 48
3.10. \( N-(2-(\text{cyclohexylamino})-1-(2-\text{methoxy-6-nitroquinolin-3-yl})-2-\text{oxoethyl})-5-((\text{R})-1,2-\text{dithiolan-3-yl})-\text{N-(2-fluorophenyl)pentanamide} \) (49)
Fig. 3.41. $^1$H-NMR spectrum for 49

Fig. 3.42. $^{13}$C-NMR spectrum for 49
3.11. *N*-(2-(cyclohexylamino)-1-(2-methoxy-6-nitroquinolin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-*N*-(m-tolyl)pentanamide (50)

Fig. 3.43. IR spectrum of 50

Fig. 3.44. $^1$H-NMR spectrum for 50
3.12. \(N\)-(2-(cyclohexylamino)-1-(2-methoxy-6-nitroquinoxilin-3-yl)-2-oxoethyl)-5-((R)-1,2-dithiolan-3-yl)-N-(o-tolyl)pentanamide (51)
Fig. 3.47. $^1$H-NMR spectrum for 51

Fig. 3.48. $^{13}$C-NMR spectrum for 51
3.13. \( N-(2-(\text{cyclohexylamino})-1-(2-\text{methoxy-6-nitroquinolin-3-yl})-2-\text{oxoethyl})-5-((R)-1,2-\text{dithiolan-3-yl})-N-(2-\text{methoxyphenyl})\text{pentanamide} \) (52)

Fig. 3.49. IR spectrum of 52

Fig. 3.50. \(^1\text{H-NMR spectrum for 52}\)
Fig. 3.51. $^{13}$C-NMR spectrum for 52

3.14. $N$-(2-(cyclohexylamino)-2-oxo-1-(2-oxo-1,2-dihydroquinolin-3-yl)ethyl)-5-((R)-1,2-dithiolan-3-yl)-$N$-(2-methoxyphenyl)pentanamide (55)

Fig. 3.52. IR spectrum of 55
Fig. 3.53. $^1$H-NMR spectrum for 55

Fig. 3.54. $^{13}$C-NMR spectrum for 55
Chapter-IV

Microwave synthesis of quinolinyl, quinolonyl and indolyl pyrans by humic acid supported ionic liquid catalyst and antimicrobial, antioxidant, toxicity and molecular docking studies
Chapter Four

Microwave synthesis of quinolinyl, quinolonyl and indolyl pyrans by humic acid supported ionic liquid catalyst and antimicrobial, antioxidant, toxicity and molecular docking studies

4.1. Abstract

A series of 12 new quinolinyl-4H-pyran (QPs) were successfully synthesized via a three-component reaction of 2-methoxyquinoline-3-carbaldehyde derivatives, malononitrile and 1,3-diketone, using ethanol as the solvent in the presence of a new humic acid supported 1-butyl-3-methylimidazolium thiocyanate ionic liquid catalyst (HASIL) under microwave irradiation. Two quinolonyl-4H-pyran (QOPs) were synthesized from substrates 2-oxo-1,2-dihydroquinoline-3-carbaldehyde derivatives, malononitrile and 1,3-diketone in the presence of HASIL and one indolyl-4H-pyran (IP) was synthesized from substrates 4-methylbenzaldehyde, malononitrile and 3-(3H-indol-3-yl)-3-oxopropanenitrile in the presence of HASIL in ethanol. HASIL was analysed with various characterization techniques including XRD, SEM with EDX, TEM, TGA, DSC and FTIR. A total of 15 new pyrans were synthesized and characterized by FTIR, 1H-NMR, 13C-NMR and elemental analysis. QPs, QOPs and IP were assessed for antimicrobial and antioxidant activities. Seven QPs and one QOP showed potential antibacterial activities against Bacillus cereus, Staphylococcus aureus, Escherichia coli and Enterococcus faecalis and whilst nine QPs showed antioxidant activity effectively. QPs, QOPs and IP were then evaluated for toxicity using the brine shrimp assay and five QPs showed mortality rate less than 50% until 48 h. Molecular docking studies were performed: one QP (42) showed higher binding affinity of 96.96 kcal/mol based on LibDock score with Mtb DNA gyrase. The advantages of this synthetic protocol are mild reaction conditions, excellent yields, operational simplicity, the use of an inexpensive and environmentally benign catalyst.
4.2. Introduction

Six-membered heterocyclic compounds containing oxygen is an important class of organic molecules which play a fundamental role in bio-organic chemistry and continue to attract research interest (Abe et al., 2005; Sibi et al., 2006). The fused pyrans display biological activities which include antimicrobial (Hussein et al., 2012), antifungal (Chattapadhyay and Dureja, 2006), antitumor (Wang et al., 2011), anti-coagulant, diuretic, spasmyloytic and anti-anaphylactic properties (DeSimone et al., 2004; Safari et al., 2012; Bonsignore et al., 1993; Heravi et al., 2008; Andreani et al., 1960). Furthermore, 4H-pyans form the building blocks for important natural products Hatakeyama et al., 1988; Singh et al., 1996). A number of 2-amino-4H-pyans are used as photoactive materials (Armesto et al., 1989), pigments (Maleki et al., 1989) and potentially biodegradable agrochemicals (Kumar et al., 2009).

The importance of quinoline and its annulated derivatives are recognized by synthetic and biological chemists (Elderfield, 1960; Wright et al., 2001; Sahu et al., 2002; Bringmann et al., 2004; Kouznetsov et al., 2005). Compounds possessing this ring system have wide applications as drugs and pharmaceuticals (Peters et al., 1984). The pyrano-quinolines are an important class of compounds that contain the basic framework for a number of alkaloids of biological significance (Corral and Orazi, 1967; Sekar and Prasad, 1998; Puricelli et al., 2002; Marco and Carreiras, 2003) such as geibalansine, ribalinine and flindersine (Fig. 1). Therefore, considerable efforts have been directed towards their preparation (Ghorab et al., 2001; Morel and Larghi, 2004) resulting in a number of new compounds possessing diverse biological activities.

![Geibalansine](1) ![Ribalinine](2) ![Flindersine](3)

**Fig. 1.** Examples of pyrano-quinolines (Corral and Orazi, 1967; Sekar and Prasad, 1998; Puricelli et al., 2002; Marco and Carreiras, 2003)
The sulfur-containing molecules also have broad application in medicinal chemistry (Metzner and Thuillier, 1994; Nudelman, 1984; Chatgilialoglu and Asmus, 1991): β-mercapto diketones display pharmacological properties such as diuretic and HIV protease inhibitory activities (Bicking et al., 1976; Ding et al., 2009; Inomata et al., 2005; yamauchi et al., 1982).

Recently, growing awareness to environmental issues has led to greener and more sustainable technologies in the chemical industry. Ionic liquids (ILs), which consist of ionic compounds with low melting points and very low vapour pressures, (Wasserscheid and Welton, 2008; Weingärtner, 2008; Wassercheid and Keim, 2000) are in demand. Many different ILs have been synthesized and successfully used as solvents as well as modified catalysts. Homogeneous catalysts containing ILs usually provide the advantages of high catalytic activity and good selectivity, however, drawbacks such as difficulty of product isolation and recovery of catalyst, use of large amounts of expensive ILs and possible toxicological concerns are evident. ILs in heterogeneous form usually overcome these drawbacks especially the supported solid catalysts with ILs. These reflect good stability of the active species, ease of handling, separation and recycling. In this context, ILs are now widely used for immobilization of homogeneous catalysts (Mehnert, 2005; Riisagera et al., 2006; Gu and Li, 2009; Van Doorslaer et al., 2010).

An easy access to pyran derivatives is highly desirable as they possess unique pharmacological properties (Green et al., 1995; Jin et al., 2004). The most straightforward synthesis of this heterocyclic system involves a three-component coupling of an aromatic aldehyde, malononitrile and 1, 3-diketones. Recently, several multi-component reactions have been described. Evdokimov et al., 2007 reported a one-pot synthesis of heterocyclic privileged medicinal scaffolds by fusing malononitrile with aldehydes and thiols, in ethanol. The reaction was catalyzed by triethylamine.

Ding et al., 2013 reported the synthesis of 2-amino-3-phenylsulfonyl-4H-pyran (7) by the condensation of aromatic aldehydes (4), dimedone (5) and phenylsulfonyl acetonitrile (6) in the presence of 2-hydroxyethyl ammonium acetate (HEAA) catalyst. The reaction was achieved in water: ethanol (1:1) medium under reflux conditions within 2-12 h and afforded 71-93 % yield.
Khurana et al., 2012 synthesized 4H-pyrans (11) and 4H-pyrano [2,3-c]pyrazoles (13) from aldehydes (8), malononitrile (9) and ethylacetoacetate (10) and pyrazolone (12), respectively, in the presence of 1-butyl-3-methyl imidazolium hydroxide ([bmim]OH) catalyst. The protocol was achieved within 30-60 minutes under reflux and a maximum yield of 88-92 % was observed.

Peng et al., 2005 reported the synthesis of 4H-pyrans (16) through one-pot condensation of aromatic aldehydes (15), malononitrile (9) and 1, 3-diketones (14), using tetramethyl guanidine in 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF4]) ionic liquid as a catalyst. The reaction was achieved within 0.5-1.5 h and the yield was 78-89 %.
Nimbalkar et al., 2017 synthesized 6-amino-4-substituted-3-methyl-2,4-dihydro pyrano[2,3-c]pyrazole-5-carbonitriles (20) via a one-pot condensation reaction from aryl aldehydes (17), malononitrile (9), hydrazine hydrate (19) and ethyl acetoacetate (18). A Brønsted acid ionic liquid [BAIL] triethylammonium hydrogen sulphate [Et$_3$NH][HSO$_4$] catalyst was used to afford a 85-94% yield within 15 minutes.

Shaabani et al., 2005 synthesized 3-cyano-4H,5H-pyran derivatives (24, 26) in the presence of 1,1,3,3-$N,N,N',N'$-tetramethylguanidinium trifluoroacetate (TMGT) ionic liquid catalyst. The condensation reaction was carried out from a mixture of aldehyde (21) and alkyl nitrile (22) by heating for 1 h. The yield was 70-82%.

Wang et al., 2015 synthesized 3,4-dihydropyran-[3,2-c]chromene derivatives (29) from aromatic aldehydes (28), malononitrile (9) and 4-hydroxycoumarin (27) in the presence of poly ethylene glycol grafted $N,N$-dimethylaminopyridine functionalized dicationic
ionic liquid ([DMAP-PEG1000-DIL][BF₄]) and 68-92 % yield was afforded within 60 minutes.

Due to the attractive properties of supported ILs catalysis, we designed a novel catalyst, ie. humic acid supported ionic liquid (HASIL). This was developed by using the homogeneous nature of ILs and a cheap humic acid (HA) as the heterogeneous support to provide large interfacial reaction areas. The supported ILs phase catalysis concept was based on a classical homogeneous catalyst that was dissolved in a thin film of ILs which was further dispersed over the high internal surface area of porous support. In supported ILs phase materials, the dissolved catalyst acts microscopically as a homogeneously dissolved metal complex in its uniform ILs environment while macroscopically in dry solid form that can be recycled easily. HA is known to catalyse many reactions (Klavins et al., 2001) and in our study, it was used to enhance the basic nature of the catalyst and provide heterogeneity. Furthermore, HASIL was used to synthesize new quinolinyl-4H-pyans (QPs), quinolonyl-4H-pyans (QOPs) and indolyl-4H-pyran (IP) via a three-component reaction of malononitrile, 1,3-diketone and the appropriate aromatic aldehyde. The new compounds were further investigated for antimicrobial, antioxidant and toxicity whilst molecular docking studies were undertaken with MtbdNA gyrase.

4.3. Results and discussion

The study was initiated by the synthesis of HASIL. Briefly, a solution of 1-butyl-3-methylimidazolium thiocyanate ionic liquid ([bmim]SCN) and HA was stirred for 10 h under an inert atmosphere. The solvent was evaporated in vacuo to produce a free flowing powder. It was then reacted with a solution of HA and copper acetate to produce the catalyst, after work-up of the reaction content. A general scheme for the preparation of HASIL is presented in Fig. 2.
Fig. 2. The synthesis of humic acid supported ionic liquid

The proposed interaction of copper, humic acid and [bmim]SCN (Lu et al., 2013; Wilmer et al., 2003) is shown in Fig. 3 below.

Fig. 3. The possible interaction of humic acid supported ionic liquid (Lu et al., 2013; Wilmer et al., 2003)
HASIL was then analysed by various characterization techniques including XRD, SEM with EDX, TEM, TGA, DSC and FTIR.

The X-ray diffraction pattern in the $2\theta$ range from 10 to 70° (Fig. 4) exhibited small diffuse peaks with a few intense peaks, implying its non-crystalline nature. This was consistent with other HA based catalysts (Chilom and Rice, 2005; Visser and Mendel, 1971). The weak diffraction lines suggested HA particles were coated with ILs. The diffraction observed at 38.5°, 45°, 56° and 62.5° corresponded to (002), (111), (200) and (113) of Bragg’s reflection for the cubic structure of copper (Das et al., 2014).

![Powder XRD pattern of humic acid supported ionic liquid](image)

**Fig. 4.** Powder XRD pattern of humic acid supported ionic liquid

**Fig. 5 (A-D)** provides the SEM images of HASIL where small particles at 20 µm, 10 µm, 2 µm and 1 µm were observed. The small particles suggested that high metal adsorption (Xu et al., 2006) was promoted: Cu can form bridges with carboxyl groups of HA, interact by chemisorption with oxidized carbon atoms and form coordination compounds (Pandey et al., 2000) with other functional groups such as hydroxyl, phenol, carboxyl and methoxy functional groups. Also, compacted micro-aggregates were formed. The aggregation phenomenon is important for transport of metal ions in natural environments (Chen et al., 2007). The humic acid substances in general formed thin thread and net-like
structures that grew into larger rings and sheets with increasing humic and cationic concentrations (Zhang et al., 2009).

Fig. 5. SEM images of humic acid supported ionic liquid

Fig. 6 (A-B) showed the EDX pattern and elemental mapping of HASIL. The elements carbon, oxygen, copper and gold (Au) were present. The appearance of Au peaks was due to the coating of sample with conducting material used to reflect electrons by sputter-coating.

Fig. 6A. EDX spectrum of humic acid supported ionic liquid
Fig. 6B. Elemental mapping of humic acid supported ionic liquid

The size and morphology of HASIL was obtained by TEM (Fig. 7). The images revealed particles with an average size of 166 nm and 250 nm (standard deviation: ± 14 nm calculated by ImageJ, see: particle histogram). The particles formed aggregates with no uniform size and fractal feature, however, few particles were ellipsoidal in shape (Fig. 7A and 7C). The observed size of the smallest particle was approximately 83 nm (Fig. 7: particle histogram).
Fig. 7. TEM images of humic acid supported ionic liquid and the particle size

Fig. 8. TGA curve of humic acid supported ionic liquid
The TGA curve (Fig. 8) showed that in the temperature range 25-500°C, the mass loss reached about 60 % which suggested possible decomposition of organic components and condensation of hydroxyl groups at high temperatures (Sirbu et al., 2010). The mass loss at 110°C was due to the loss of a water molecule. It was observed that the decomposition process started at nearly 230°C and ended at 320°C with a mass loss range of 50-60 %. A temperature range of 244°C - 390°C was reported for a [bmim]SCN heterogeneous catalyst thereby suggesting a strong interaction existed between the ILs and HA (Dharaskar et al., 2016).

The DSC method is convenient as it is a rapid technique for characteristic curves, whose variation in enthalpy is associated with phase changes in mineral or soil organic matter, reflecting events related to structures and chemical compositions (Kucerík et al., 2005; Gibbs et al., 2001; Valkova et al., 2007).

![DSC curve of humic acid supported ionic liquid](image)

**Fig. 9.** DSC curve of humic acid supported ionic liquid

The areas under the DSC curves were divided into two groups representing different degrees of resistance to thermal oxidation: a) 230-338°C, which was mostly attributed to labile organic matter, mainly comprising phenolic hydrates and other aliphatic compounds. Nevertheless, decarboxylation and dehydration (removal of OH groups during the formation of the interaction between humic acid and [bmim]SCN) should be
considered and b) 35-175°C, mostly attributable to organic matter, such as polyphenols and polycondensed aromatic substances including HA and black carbon (Dell’Abate et al., 2002; Fernandez et al., 2011). The temperature of maximum combustion peaks were observed at 230°C and 338°C with two exothermic peaks evident (Fig. 9).

The FTIR spectrum (Fig. 10) exhibited a strong stretching band at 3289 cm\(^{-1}\) which was assigned to the OH stretch of HA. The other absorption frequencies (cm\(^{-1}\)) were observed at 1210 for C-N, 1652 for C=O, 1487 for C=C, 1055 for -C-O and bending frequencies at 1742 for N-H, 1368 for -C-H and 754 for -CH=CH-.

![FTIR spectrum of humic acid supported ionic liquid](image)

**Fig. 10.** FTIR spectrum of humic acid supported ionic liquid

The absorption bands (cm\(^{-1}\)) were also observed at 557 for Cu-O and 437 for Cu-N. The absorption at 937 cm\(^{-1}\) was assigned to –N-O-Cu. This confirmed the formation of –N-O---Cu bond (Sajila et al., 2014). These results suggested that Cu was adsorbed on the bulk humic material. It is suggested that the procedure adopted for the preparation of HASIL, where the hydroxyl and carboxylic groups are present in their dissociated form, could create favourable conditions for strong interactions with the positive charges of ionic liquid.

After the successful characterization of HASIL, its catalytic activity was then investigated in a one-pot three-component synthesis of quinolinyl, quinolonyl and indolyl pyran derivatives. The starting materials, 2-chloroquinoline-3-carbaldehyde (CFQ), 2-chloro-6-methylquinoline-3-carbaldehyde (CMFQ) and 2-chloro-7-fluoroquinoline-3-
carbaldehyde (CFFQ) were prepared by the Vilsmeier-Haack reaction (Ambika et al., 2005). The desired starting material, 2-methoxyquinoline-3-carbaldehyde derivatives (MQCs) (30 and 51), were synthesized from the reaction of \( \text{K}_2\text{CO}_3 \) with CFQ, CMFQ and CFFQ as discussed in Chapter Three. The quinolone starting materials, 2-oxo-1,2-dihydroquinoline-3-carbaldehyde derivatives were prepared from the reaction of glacial \( \text{CH}_3\text{COOH} \) with corresponding carbaldehyde derivatives. Indole starting material 3-(\( 3\text{H} \)-indol-3-yl)-3-oxopropanenitrile (IOPN) was synthesized by the reported literature (Venkatanarayana and Dubey, 2013).

Initially, an equimolar mixture (1 mmol) of the MQC (30), malononitrile (9) and dimedone (5), in ethanol and without catalyst, was investigated. TLC was used to monitor the reaction progress: no products were obtained even up to 24 h. When the same reaction was investigated with a catalytic amount (10 mol %) of HASIL, after 3 h a new product was observed on the TLC plate. The reaction mixture was then subjected to column chromatography for purification. A solvent ratio of 1:3 of ethyl acetate: petroleum ether solvent system resulted in a product of 52 % yield. When the reaction was investigated under microwave irradiation (MW) at 100 W, within 10 minutes the new spot was observed on TLC. Hence MW irradiation became the method of choice for synthesis of new quinolinyl, quinolonyl and indolyl pyrans (Scheme 1-5).

\[
\text{Scheme 1. Synthesis of quinolinyl-4H-pyrans (31, 32, 36, 41 and 42) in the presence of HASIL under microwave irradiation}
\]
Scheme 2. Synthesis of quinolinyl-4H-pyrans (34, 38, 35 and 44) in the presence of HASIL under microwave irradiation

\[
\begin{align*}
\text{NC} & \quad \text{CN} \\
\text{R}_1 & \quad \text{N} \quad \text{O} \quad \text{CH}_3 \\
\text{CHO} & \quad \text{R}_2 \\
\text{R}_1 & \quad \text{H; R}_2 = \text{O; (34)} \\
\text{R}_1 & \quad \text{6-NO}_2; \text{R}_2 = \text{S; (35)} \\
\text{R}_1 & \quad \text{7-F; R}_2 = \text{O; (38)} \\
\text{R}_1 & \quad \text{6-CH}_3; \text{R}_2 = \text{S; (44)}
\end{align*}
\]

Scheme 3. Synthesis of quinolonyl-4H-pyrans (39 and 40) in the presence of HASIL under microwave irradiation

\[
\begin{align*}
\text{NC} & \quad \text{CN} \\
\text{R}_1 & \quad \text{N} \quad \text{O} \\
\text{CHO} & \quad \text{R}_2 \\
\text{R}_1 & \quad \text{7-F; (48)} \\
\text{R}_1 & \quad \text{6-CH}_3; (49)
\end{align*}
\]
Scheme 4. Synthesis of quinolinyl-4H-pyrans (33, 37 and 43) in the presence of HASIL under microwave irradiation

\[ \text{NC-CN} + \text{C}_2\text{H}_5\text{O}-\text{CO} \rightarrow \text{HASIL} \]

MW, 120W 80°C
Ethanol, 10-15 min

\[ \text{R}_1 = \text{H}; (33) 
\text{R}_1 = 7-\text{F}; (37) 
\text{R}_1 = 6-\text{CH}_3; (43) \]

Scheme 5. Synthesis of indolyl-4H-pyran (44) in the presence of HASIL under microwave irradiation

The structure was confirmed by FTIR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis. As a typical characterization of structures, 31 was then selected.

The FTIR spectrum (Fig. 4.1, Appendix IV) of 31 showed stretching frequencies (cm$^{-1}$) at 2253 for C≡N, 1571 for C=N, 1216 for C-N, 2970 for CH, 1637 for C=C, 1752 for C=O, 2940 for OCH$_3$ and 3028 for NH. The $^1$H-NMR spectrum (Fig. 4.2) of 31 showed six singlets for CH (C4') at $\delta$ 4.68, NH$_2$ (C12') at $\delta$ 4.59, methyl proton broad singlet (CH$_3$, C10') at $\delta$ 1.44, broad singlet of (CH$_3$, C9') at $\delta$ 1.05, broad singlet of OCH$_3$ at $\delta$ 4.08 and quinolinyl proton singlet C4, at $\delta$ 7.91. The dimedone protons (CH$_2$) showed a
quartets at $\delta$ 2.48 and $\delta$ 2.22 for C8'-H and C6'-H, respectively. The $^{13}$C-NMR spectrum (Fig. 4.3) showed the carbonyl group C5' (C=O) at $\delta$ 195.94. The methoxy group C9 (OCH$_3$) was assigned to $\delta$ 53.47. The methyl carbon C9' at $\delta$ 27.02 and C10' methyl carbon at $\delta$ 29.30, was observed. The carbon C6' (CH$_2$) showed a peak at $\delta$ 50.58, C8' showed a peak at $\delta$ 40.79. The CH (C4') carbon showed a peak at $\delta$ 32.17 and the carbonitrile CN was observed at $\delta$ 111.83. The pyran ring carbon atoms C3' at $\delta$ 60.83 and in the same pyran ring, C2' at $\delta$ 160.15 and C8b' at $\delta$ 158.65, were observed. The dinedone carbonyl carbon C7' was identified at $\delta$ 32.77. The quinolinyl carbon C3 was observed at $\delta$ 125.28 and C=N in the quinolinyl ring showed a peak at $\delta$ 162.93 and C4 showed a peak at $\delta$ 137.87. The selected $^1$H-NMR and $^{13}$C-NMR chemical shifts of 31 is shown in Fig. 11.

![Chemical structure](image)

**Fig. 11.** Selected $^1$H-NMR and $^{13}$C-NMR chemical shifts of 31

The structure was further confirmed on the basis of 2D NMR spectral studies. The $^{13}$C, $^1$H-COSY correlation of carbon signals at $\delta$ 137.87, 129.24, 127.60, 126.61, 125.35, 124.08, 53.47, 50.58, 40.79, 32.17, 29.30 and 27.02 were assigned to C4, C7, C5, C8, C6, C4b', C9, C6', C8', C4', C10' and C9', respectively. The carbon signal at $\delta$ 137.87 was due to the quinolinyl carbon (C4) and the spectrum is shown in Fig. 4.4.
The $^1$H, $^1$H-COSY spectrum (Fig. 4.5) revealed the correlation between quartet of C6’ (CH$_2$) proton at δ 2.22 and singlet of CH$_3$ (C9’) proton δ 1.05. The C9’ (CH$_3$) proton at δ 1.05 correlated with quartet of C6’ (CH$_2$) proton at δ 2.22.

The $^1$H, $^1$H-NOESY spectrum (Fig. 4.6) revealed the singlet protons (NH$_2$) at δ 4.59 was coupled with a quartet of C6’ (CH$_2$) at δ 2.22.

The HMBC spectrum (Fig. 4.7) showed the long-range correlations as follows: the proton C4’-H, (CH) group was coupled with C3’ of pyran ring carbon at δ 60.83 and C11’ carbonitrile CN carbon at δ 111.83, quinolinyl carbon C3 at δ 125.28 and dimedone carbons C5’ at δ 195.94 and C4b’ at δ 124.08.

This correlation of C4’-H to the pyran ring carbon (C3’), carbonitrile carbon (C11’), quaternary carbon (C3) of quinoline ring and carbons C5’ and C4b’ of dimedone
indicated that the three groups were attached to C4' (CH). Thus it was evident that three different moieties were bonded to a common carbon (C4') and hence added valuable information to 31. The selected ¹H-NMR, ¹³C-NMR and HMBC chemical shifts are shown in Fig. 12.

The C4-H, quinoline proton was coupled with C4' at δ 32.17, quinolinyl carbons (C5) at δ 127.60 and (C2) at δ 163.92. The C6'-H of dimedone was coupled with C7' at δ 32.77, C8' (40.79) and C5' (195.94). The C8'-H proton was coupled with C7' at δ 32.77, C6' (CH₂) at δ 50.58 and C2' at δ 160.15. The C9'-H (CH₃) was coupled with C2 at δ 162.93. The C10'-H was coupled with C7' at δ 32.77, C8' at δ 40.79 and C6' (CH₂) carbon at δ 50.58. The quinolinyl proton C4-H was coupled with C4' (CH) carbon at δ 32.17, quinolinyl carbons (C5) at δ 127.60 and C2 at δ 162.93. The selected HMBC correlations of 31 is shown in Table 1.

**Table 1. Selected HMBC correlations of 31**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Protons</th>
<th>Correlated carbons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C4'-H (s, 1H) at δ 4.68 ppm</td>
<td>C3' (60.83), C11' (111.83), C3 (125.28), C5' (195.94) and C4b' (124.08)</td>
</tr>
<tr>
<td>2</td>
<td>C4-H (s, 1H) at δ 7.91 ppm</td>
<td>C4' (32.17), C5 (127.60) and C2 (163.92)</td>
</tr>
<tr>
<td>3</td>
<td>C6'-H (q, 2H) at δ 2.22 ppm</td>
<td>C7' (32.77), C8' (40.79) and C5' (195.94)</td>
</tr>
<tr>
<td>4</td>
<td>C8'-H (q, 2H) at δ 2.48 ppm</td>
<td>C7' (32.77), C6' (50.58) and C2' (160.15)</td>
</tr>
<tr>
<td>5</td>
<td>C9'-H (brs, 3H) at δ 1.05 ppm</td>
<td>C2 (162.93)</td>
</tr>
<tr>
<td>6</td>
<td>C10'-H (brs, 3H) at δ 1.44 ppm</td>
<td>C7' (32.77), C8' (40.79) and C6' (50.58)</td>
</tr>
<tr>
<td>7</td>
<td>C4-H (s, 1H) at δ 7.91 ppm</td>
<td>C4' (32.17), C5 (127.60) and C2 (162.93)</td>
</tr>
</tbody>
</table>

Based on the above spectral details and its elemental analysis (Anal. Calc. for C₂₂H₂₁N₃O₃: C, 70.38; H, 5.64; N, 11.19; %, Found: C, 70.36; H, 5.65; N, 11.20; %), the structure was confirmed as 2-amino-4-(2-methoxyquinolin-3-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (31).

To determine the effect of solvents, acetonitrile, dichloromethane, ethanol, water and ethanol-water (1:1) were investigated (Table 2). It was found that ethanol gave a high yield of 97 %. The yield decreased to 35 % when other solvents were used. Ethanol is a very polar molecule due to its hydroxyl (OH) group, with the high electronegativity of
oxygen allowing hydrogen bonding to take place with other molecules (Narten and Habenschuss, 1984). This could enable the reaction to occur faster resulting in a maximum yield based on a set time frame.

**Table 2. Effect of solvents on the synthesis of quinolinyl-4H-pyrans**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time (min)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetonitrile</td>
<td>15</td>
<td>73</td>
</tr>
<tr>
<td>2</td>
<td>Dichloromethane</td>
<td>15</td>
<td>71</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol</td>
<td>10</td>
<td>97</td>
</tr>
<tr>
<td>4</td>
<td>Methanol</td>
<td>15</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>Water</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
<td>Ethanol-Water</td>
<td>15</td>
<td>40</td>
</tr>
</tbody>
</table>

*a Reaction conditions: aldehyde (1 mmol), malononitrile (1 mmol), 1,3-diketone (1 mmol) and HASIL (10 mol%) were added to the ethanol as solvent (15 mL) under microwave irradiation (120 W) at 80°C.

*b Isolated yields.

When the model reaction was conducted in the presence of 5 mol % of HASIL, the isolated yield of 31 was 71 %. In another attempt, a trace amount of 31 was obtained when HA was used (Table 3, entry 1). It was observed that in the presence of 10 mol % of HASIL, the yield of 31 substantially increased to 97 % within 10 minutes (Table 3, entry 3). However, on further increasing the amount of the HASIL catalyst to 15 mol % and 20 mol %, no improvements in the yield were observed (Table 3, entries 4 and 5). This showed that the best yield of 31 was obtained when 10 mol % of HASIL was used.

**Table 3. Optimization of humic acid supported ionic liquid (HASIL) catalyst**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Mol %</th>
<th>Time (min)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HA</td>
<td>25</td>
<td>20</td>
<td>Trace</td>
</tr>
<tr>
<td>2</td>
<td>HASIL</td>
<td>5</td>
<td>15</td>
<td>71</td>
</tr>
<tr>
<td>3</td>
<td>HASIL</td>
<td>10</td>
<td>10</td>
<td>97</td>
</tr>
<tr>
<td>4</td>
<td>HASIL</td>
<td>15</td>
<td>15</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>HASIL</td>
<td>20</td>
<td>15</td>
<td>90</td>
</tr>
</tbody>
</table>

*b Isolated yield

To report the effect of MW irradiation, evaluate and compare normal refluxing with MW assisted method, various conventional and unconventional conditions were screened. The results are listed in Table 4. Without microwave, 31 was formed in the presence of
HASIL under stirring at room temperature after 24 h. Also when the reaction was conducted at 90°C and without microwave irradiation for 3 h the yield of the obtained 31 was found to be 38-52 % (Table 4, entries 1 and 2). Moreover, the effect of MW irradiation of different powers was investigated. It was observed that the irradiation power of 120 W at 80°C, afforded the best yield of 31, with 97 % yield after 10 min (Table 4, entry 4).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Power (W)</th>
<th>Time (min)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Without MW (r.t)</td>
<td>24 h</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>Without MW (90 °C)</td>
<td>3 h</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>20</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>10</td>
<td>97</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>15</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>200</td>
<td>15</td>
<td>90</td>
</tr>
</tbody>
</table>

a Reaction conditions: aldehyde (1 mmol), malononitrile (1 mmol), 1,3-diketone (1 mmol) and HASIL (10 mol %) were added to the ethanol as solvent (15 mL) under microwave irradiation (120 W) at 80°C.

b Isolated yields.

Following the optimisation of the reaction conditions for the synthesis of 31 with high yield, an investigation on the recyclability of HASIL was conducted.

Fig. 13. Recyclability of humic acid supported ionic liquid
Briefly, after synthesis, the reaction mixture was filtered and the solid was washed with MeOH followed by acetone and dried in an oven at 100°C. Five successive cycles of the model reaction were conducted. The activity of HASIL did not show any significant decrease in the yields after five successive runs. It was found that the catalyst displayed good recyclability as there was loss of only 15 % in catalytic activity after five cycles of re-use (Fig. 13).

HASIL was further compared with previously reported catalysts (Table 5, entries 1-13). The catalysts including montmorillonite K10, Fe(HSO₄)₃, TCT, [DMAP-PEG₁₀₀₀-DIL][BF₄], [PhNMe₂CH₂NMe₂]Cl· and DABCO offered more than 90 % yield, however they had drawbacks of long reaction times.

Table 5. Effect of optimization of reported catalysts with HASIL catalyst

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Temp (°C)</th>
<th>Time</th>
<th>Yield (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Montmorillonite K10</td>
<td>125</td>
<td>30 min</td>
<td>96</td>
<td>Kantevi et al., 2007</td>
</tr>
<tr>
<td>2</td>
<td>Fe(HSO₄)₃</td>
<td>85</td>
<td>25 min</td>
<td>97</td>
<td>Shaterian et al., 2008</td>
</tr>
<tr>
<td>3</td>
<td>Zwitterionic salt</td>
<td>80</td>
<td>90 min</td>
<td>88</td>
<td>Kundu et al., 2010</td>
</tr>
<tr>
<td>4</td>
<td>Iodine</td>
<td>125</td>
<td>300 min</td>
<td>87</td>
<td>Das et al., 2007</td>
</tr>
<tr>
<td>5</td>
<td>TCT</td>
<td>100</td>
<td>40 min</td>
<td>95</td>
<td>Zhang et al., 2009</td>
</tr>
<tr>
<td>6</td>
<td>p-TSA</td>
<td>125</td>
<td>240 min</td>
<td>90</td>
<td>Khodaei et al., 2006</td>
</tr>
<tr>
<td>7</td>
<td>K₃CoW₁₂O₄₀·3H₂O</td>
<td>125</td>
<td>180 min</td>
<td>78</td>
<td>Nagarapu et al., 2007</td>
</tr>
<tr>
<td>8</td>
<td>[FemSILP]-L-Prolinate</td>
<td>100</td>
<td>300 min</td>
<td>87</td>
<td>Rashikar et al., 2010</td>
</tr>
<tr>
<td>9</td>
<td>[TEBSA][HSO₄]</td>
<td>120</td>
<td>10 min</td>
<td>89</td>
<td>Hajipour et al., 2009</td>
</tr>
<tr>
<td>10</td>
<td>[bmim][PF₆]</td>
<td>80</td>
<td>2 hours</td>
<td>90</td>
<td>Rao et al., 2012</td>
</tr>
<tr>
<td>11</td>
<td>[DMAP-PEG₁₀₀₀-DIL][BF₄]</td>
<td>100</td>
<td>30 min</td>
<td>97</td>
<td>Wang et al., 2015</td>
</tr>
<tr>
<td>12</td>
<td>[PhNMe₂CH₂NMe₂]Cl·</td>
<td>80</td>
<td>40 min</td>
<td>95</td>
<td>Chen et al., 2009</td>
</tr>
<tr>
<td>13</td>
<td>DABCO</td>
<td>90</td>
<td>2 hours</td>
<td>97</td>
<td>Tahmassebi et al., 2011</td>
</tr>
<tr>
<td>14</td>
<td>HASIL</td>
<td>80</td>
<td>10 min</td>
<td>97</td>
<td>Present work</td>
</tr>
</tbody>
</table>

It was found that the protocol used in our study was better suited than that reported with respect to time, temperatures and yields.
Following optimization of the reaction conditions for 31, the protocol was used to synthesize new quinolinyl pyran derivatives. In summary, 15 novel compounds were synthesized (Table 6) and characterized. The FTIR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis for all the synthesized compounds are available in Appendix IV. In summary, the one-pot multi-component synthesis by microwave irradiation in the presence of a catalytic amount of a new catalyst HASIL was successfully achieved.

Table 6. Synthesis of quinolinyl-4H-pyran by using HASIL under MW conditions

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehydes</th>
<th>1,3-Diketones</th>
<th>Product</th>
<th>Product</th>
<th>Time (min)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>31</strong></td>
<td>10</td>
<td>97</td>
</tr>
<tr>
<td>2</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>32</strong></td>
<td>10</td>
<td>96</td>
</tr>
<tr>
<td>3</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>33</strong></td>
<td>10</td>
<td>96</td>
</tr>
<tr>
<td>4</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>34</strong></td>
<td>12</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>35</strong></td>
<td>15</td>
<td>88</td>
</tr>
<tr>
<td>6</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>36</strong></td>
<td>12</td>
<td>89</td>
</tr>
<tr>
<td>7</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>37</strong></td>
<td>14</td>
<td>92</td>
</tr>
<tr>
<td>8</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>38</strong></td>
<td>15</td>
<td>87</td>
</tr>
<tr>
<td>9</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>39</strong></td>
<td>12</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>40</strong></td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>11</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>41</strong></td>
<td>12</td>
<td>89</td>
</tr>
<tr>
<td>12</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>42</strong></td>
<td>10</td>
<td>93</td>
</tr>
<tr>
<td>13</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>43</strong></td>
<td>10</td>
<td>95</td>
</tr>
<tr>
<td>14</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>44</strong></td>
<td>10</td>
<td>93</td>
</tr>
<tr>
<td>15</td>
<td>C$_{11}$H$_8$NO$_2$</td>
<td>C$<em>{6}$H$</em>{12}$O$_2$</td>
<td>C$<em>{22}$H$</em>{21}$N$_5$O$_3$</td>
<td><strong>45</strong></td>
<td>10</td>
<td>92</td>
</tr>
</tbody>
</table>

* Isolated yields

A suggested mechanism is presented in Scheme 6. The active methylene group of malononitrile (2) attacked the carbonyl carbon of aldehyde (1) and underwent a Knoevenagel condensation to form 3 with loss of water. The methine carbon of the Knoevenagel product (3) was activated by HASIL and it reacted with C-H activated compound by a Michael addition to produce intermediate 5. Thereafter, 5 underwent intramolecular cyclization to form 6 which then underwent tautomerism to produce 7.
The antibacterial activity was evaluated for QPs by determining the zone of inhibition against a range of Gram-positive (Bacillus cereus (B. cereus), Staphylococcus aureus (S. aureus) and Enterococcus faecalis (E. faecalis)) and Gram-negative (Escherichia coli (E. coli)) bacteria (Fig. 14). It was found that 31, 34, 37, 38 and 43 had preferential activity towards all Gram-positive species tested (Fig. 14B).

Moreover, compounds 40 and 44 showed activity towards S. aureus only. Interestingly, compound 42 showed its potential antibacterial activity against all species tested. Furthermore, compound 42 was effective against one of Gram-negative (E. coli) species (Table 7).
Fig. 14. Growth inhibition of bacterial strains caused by: (A) positive (ciprofloxacin) and negative (DMSO) control, (B) different derivatives of the (quinolinyl, quinolonyl and indolyl)-4H-pyran derivatives

Table 7. Antibacterial screening test of quinolinyl-4H-pyran derivatives against Gram-positive and Gram-negative bacterial strains

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zone of inhibition by (quinolinyl, quinolonyl and indolyl)-4H-pyran derivatives</th>
<th>Ciprofloxacin (Positive control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1  2  3  4  5  6  7  8  9  10  11  12  13  14  15</td>
<td></td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>0  0  0  0  19 ± 0.2  0  14 ± 0.4  14 ± 0.4  0  16 ± 0.3  0  0  13 ± 0.4  12 ± 0.3</td>
<td>25 ± 0.2</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0  0  0  0  16 ± 0.2  0  17 ± 0.3  16 ± 0.3  15 ± 0.2  21 ± 0.2  0  12 ± 0.4  0  19 ± 0.2  19 ± 0.2</td>
<td>24 ± 0.3</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0  0  0  0  12 ± 0.3  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0</td>
<td>25 ± 0.4</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>0  0  0  0  14 ± 0.4  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0</td>
<td>20 ± 0.6</td>
</tr>
</tbody>
</table>

Data are means of three replicates (n = 3) ± standard error.

QPs containing methoxy groups (31, 43 and 44), sulfur heterocycles (34 and 38) fluorinated quinolines (37 and 38) and QOP containing quinolonyl ring and methyl group (40) showed remarkable antibacterial activity against Gram-positive strains. Interestingly
the QP containing methoxy and methyl groups (42) showed potential against both Gram positive and Gram-negative bacteria.

The DPPH radical scavenging assay was used for initial screening of the compounds for their antioxidant activity (Sowndhararajan et al., 2013). An antioxidant assay was conducted for all QP derivatives and it was found that compounds 32, 34, 35, 36, 37, 38, 42, 43 and 44 were effective DPPH scavenging agents. QPs containing a benzyl group (32), fused sulfur heterocycles (34, 35, 38 and 44), fluorinated quinolines (36, 37 and 38) and methyl quinolines (42 and 43) showed significant DPPH scavenging activity.

**Fig. 15.** Toxicity assessment of (quinolinyl, quinolonyl and indolyl)-4H-pyrans (31, 34, 37, 38, 40, 42, 43 and 44) at different time intervals (24 h and 48 h) in *Artemia salina* (Brine shrimp)

(5= 42; 7= 31; 8= 34; 9= 44; 10= 38; 12= 40; 14= 43; 15= 37)

Brine shrimp is one of the most valuable test organisms available for preliminary assessment of toxicity (Rajabi et al., 2015). Therefore, the toxicity of all compounds at different intervals (24 h and 48 h), which were active against Gram-positive, Gram-negative or both were evaluated. Amongst all compounds tested, 34, 37, 42, 43 and 44 showed mortality rates below 50% (Fig. 15). This suggests that these compounds were less toxic and can be used for further biological and pharmaceutical applications since
less than 50% of brine shrimp mortality rate is considered as safe (Meyer et al., 1982). Sulfur-containing QPs (34 and 44), fluorinated quinolines (37) and methyl quinolines (43) showed mortality rates less than 50%.

The biological importance of quinolinyl pyrans were assessed based on molecular docking. The crystal structure of Mtb gyrase (PDB ID: 4PLB) was used to determine the antibacterial activity of quinolinyl-4H-pyran derivatives (Singh et al., 2014). The three dimensional structure of Mtb gyrase was prepared in the Chimera software package to carry out molecular docking (Pettersen et al., 2004). The ligands (QPs) were built in Chem3D Biodraw and were energetically minized by MM2 method integrated with the same software. Further, ligands were prepared for ionization, conformation and stereoisomers in Discover Studio software packages (Biovia, 2015). Thereafter, molecular docking was conducted using the LibDock module of the Discovery Studio software packages around the bound ligand.

Fig. 16. Ligand binding mode of 42 inside Mtb DNA gyrase

The docking pose of the ligands were ordered based on LibDock score (Table 8). The ligands were docked with the scores varying from 74.59 to 96.96 kcal/mol: 31, 36, 37, 41 and 42 showed higher binding affinity with the LibDock scores of 96.69, 90.51, 96.30,
92.11 and 96.96, toward Mtb gyrase. Fig. 16 shows the binding interaction of the ligand (42) inside Mtb DNA gyrase.

**Table 8.** Molecular docking scores of (quinolinyl, quinolonyl and indolyl)-4H-pyran derivatives

<table>
<thead>
<tr>
<th>Entry</th>
<th>Molecule</th>
<th>Absolute Energy</th>
<th>LibDock Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>43.92</td>
<td>96.69</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>67.44</td>
<td>88.08</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>59.67</td>
<td>74.59</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>47.82</td>
<td>91.58</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>58.46</td>
<td>85.14</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>43.95</td>
<td>90.51</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>66.08</td>
<td>96.30</td>
</tr>
<tr>
<td>8</td>
<td>38</td>
<td>38.40</td>
<td>88.41</td>
</tr>
<tr>
<td>9</td>
<td>39</td>
<td>61.69</td>
<td>78.97</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>56.36</td>
<td>79.23</td>
</tr>
<tr>
<td>11</td>
<td>41</td>
<td>41.66</td>
<td>92.11</td>
</tr>
<tr>
<td>12</td>
<td>42</td>
<td>49.82</td>
<td>96.96</td>
</tr>
<tr>
<td>13</td>
<td>43</td>
<td>67.50</td>
<td>82.32</td>
</tr>
<tr>
<td>14</td>
<td>44</td>
<td>51.36</td>
<td>86.55</td>
</tr>
<tr>
<td>15</td>
<td>45</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>16</td>
<td>Reference Ligand</td>
<td>83.08</td>
<td>176.61</td>
</tr>
</tbody>
</table>

### 4.4. Conclusion

In conclusion, a simple, energy-efficient, convenient, practical method was developed for easy access to a wide range of pharmaceutically functionalized quinolinyl-4H-pyrans (QPs): QPs were successfully synthesized via a three-component reaction of 2-methoxyquinoline-3-carbaldehydes, malononitrile and 1,3-diketone in the presence of a new humic acid supported 1-butyl-3-methylimidazolium thiocyanate ionic liquid catalyst under microwave irradiation. Seven QPs (31, 34, 37, 38, 42, 43 and 44) and one QOP (40) showed good potential against *B. cereus, S. aureus, E. coli* and *E. faecalis* whilst nine (32, 34, 35, 36, 37, 38, 42, 43 and 44) QPs showed antioxidant activity. The brine shrimp test showed five QPs (34, 37, 42, 43 and 44) with mortality rate less than 50% (48 h). Molecular docking showed higher binding affinity of 96.96 kcal/mol for 42 based on Libdock score with Mtb DNA gyrase. Mild reaction conditions, excellent yields, operational simplicity, clean reaction profiles, energy-efficiency, the use of an
inexpensive and environmentally benign catalyst are the key advantages of the present method of synthesis. Moreover, the reusability of the catalyst is an added advantage.

4.5. Experimental

General synthesis of HASIL

A mixture of [bmim]SCN (5 mmol), in methanol (5 mL), and humic acid (5g) was placed in a round-bottomed flask (100 mL) and stirred at room temperature for 10 h under an inert atmosphere and then dried under reduced pressure until a free flowing powder was obtained. In another 100 mL round-bottomed flask, [bmim]SCN (10 mmol), copper acetate (0.5 mmol) were dissolved in methanol (10 mL) and stirred for 5 minutes. Thereafter, the humic acid pre-treated with ionic liquid was suspended in this solution and the mixture was refluxed for 10 h under an inert atmosphere. The solvent was subsequently evaporated under reduced pressure to produce a free flowing powder which was dried in an oven at 200°C for 5 h.

Typical procedure for the microwave synthesis of 4H-pyran

A catalytic amount (10 mol %) of HASIL was added to a mixture of 30 (1 mmol) and 9 (1 mmol) in ethanol (15 mL) followed by the addition of 1,3-diketone (1 mmol). The reaction tube was placed into a CEM microwave Discover Synthesizer and irradiated at 120 W at a temperature of 80°C for 10 minutes. The progress of the reaction was monitored by TLC. Following completion, the catalyst was separated by simple filtration. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography from the eluent (ethyl acetate: petroleum ether, 50 %). The crystallization of 4H-pyran was performed in ethanol to yield the pure product.

Bacterial strains

The antibacterial activity of each synthesized compound was assessed using four bacterial strains. Two strains of each Gram-positive (*Bacillus cereus, Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative (*Escherichia coli*) bacteria were selected. The bacterial strains were provided from the culture collection at Department of Biotechnology and Food Technology, Durban University of Technology, South Africa.
**Inoculum preparation**

Each bacterial strain was sub-cultured overnight at 37°C on a Mueller-Hilton agar plate. Further, bacterial cultures were grown in a Mueller-Hilton broth at 37°C, 200 rpm in order to attain the viable count of approximately $10^8$ cfu/mL.

**Antibacterial activity**

The agar well diffusion method was used to evaluate the antibacterial activity. Hundred microliter of $\sim 10^8$ cfu/mL bacterial suspension was plated on Mueller-Hinton Agar plates. A well of 6 mm diameter was made using a sterile cork borer and 30 μL of each compound (3 mg/ml) was added in each well and kept at 37 °C for 16 h. The assays were carried out in triplicate. Ciprofloxacin (3 mg/ml) was used as positive control and DMSO (100%) as a negative control.

**Antioxidant activity**

The antioxidant ability of the quinolinyl-4H-pyran derivatives were determined by the decolourization of a methanol solution of 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH). Hundred microliter of each compound was added separately to 1 mL of 0.1 mM DPPH solution, and a colour change was observed at regular intervals. Rutin hydrate was used as a positive control and methanol (95%) as a negative control.

**Toxicity assessment**

The brine shrimp larvae (*Artemia salina*) were hatched in sea water for 24-48 h prior to being used. An aliquot of 5 mL sea water containing ten brine shrimp was added to each vial and supplemented with different derivatives of quinolinyl-4H-pyran. Derivative concentrations of 300 μg were used in individual vials. Brine shrimp death was observed at regular intervals (24h and 48h) in order to determine the toxic nature of each compound. The data was expressed as % mortality.
References


novel bacterial topoisomerase inhibitors as broad spectrum antibacterial agents.
ACS Medicinal Chemistry Letters, (5) 609-614.

[78] Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M.,
Meng, E. C., Ferrin, T. E. 2004. UCSF Chimera-a visualization system for
1605-1612.

Systemes.
4.1. 2-Amino-4-(2-methoxyquinolin-3-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (31)

Pale yellow crystals, m.p = 182-184°C; IR ν<sub>max</sub> (cm<sup>-1</sup>): 2253 C≡N, 1571 C≡N, 1216 C-N, 2970 CH, 1637 C=C, 1752 C=O, 2940 OCH<sub>3</sub>, 3028 NH. <sup>1</sup>H-NMR: (400 MHz, CDCl<sub>3</sub>) δ ppm 7.91 (1H, s, Ar-H), 7.79 (1H, d, J= 8.32 Hz, Ar-H), 7.65 (1H, d, J= 1.4 Hz, Ar-H), 7.58 (1H, td, J= 1.24 Hz, Ar-H), 7.38 (1H, td, J= 8.28 Hz, Ar-H), 4.68 (1H, s, CH), 4.59 (2H, brs, NH<sub>2</sub>), 4.08 (3H, brs, OCH<sub>3</sub>), 2.48 (2H, q, J= 1.04 Hz, CH<sub>2</sub>), 2.22 (2H, q, J= 18 Hz, CH<sub>2</sub>), 1.14 (3H, brs, CH<sub>3</sub>), 1.05 (3H, brs, CH<sub>3</sub>). <sup>13</sup>C-NMR: (100 MHz, CDCl<sub>3</sub>) δ ppm 195.94, 162.93, 160.15, 158.65, 137.87, 129.24, 127.60, 126.61, 126.61, 125.35, 125.28, 124.08, 111.83, 60.83, 53.47, 50.58, 40.79, 32.77, 32.17, 29.30, 27.02. Elemental Analysis: Anal. Calc. for C<sub>22</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>: C, 70.38; H, 5.64; N, 11.19; %. Found: C, 70.36; H, 5.65; N, 11.20; %.

4.2. 2-Amino-7,7-dimethyl-5-oxo-4-(2-(o-tolyloxy)quinolin-3-yl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (32)

Yellow solid, m.p = 209-211°C; IR ν<sub>max</sub> (cm<sup>-1</sup>): IR ν<sub>max</sub> (cm<sup>-1</sup>): 2246 C≡N, 1577 C≡N, 1207 C-N, 2924 CH, 1631 C=C, 1751 C=O, 3327 NH. <sup>1</sup>H-NMR: (400 MHz, DMSO-d<sub>6</sub>) δ ppm 8.69 (1H, s, Ar-H), 8.05 (2H, d, J=7.84 Hz, Ar-H), 7.92 (2H, d, J=8.32 Hz, Ar-H), 7.84 (2H, td, J=1.36 Hz, Ar-H), 7.63 (2H, td, J= 1.04 Hz, Ar-H), 5.21 (1H, s, CH), 4.98 (2H, s, NH<sub>2</sub>), 3.01 (3H, brs, CH<sub>3</sub>), 2.70 (2H, q, J=17.88 Hz, CH<sub>2</sub>), 2.41 (2H, q, J=16.16 Hz, CH<sub>2</sub>), 1.14 (3H, brs, CH<sub>3</sub>), 1.12 (3H, brs, CH<sub>3</sub>). <sup>13</sup>C-NMR: (100 MHz, DMSO-d<sub>6</sub>) δ ppm 195.65, 169.07, 154.50, 145.52, 14064, 131.56, 128.06, 127.53, 126.62, 126.60, 113.89, 112.84, 112.61, 107.25, 49.60, 40.46, 40.08, 39.87, 39.67, 39.46, 39.25, 38.83, 33.26, 31.77, 30.50, 28.99, 27.17, 26.06. Elemental Analysis: Anal. Calc. for C<sub>28</sub>H<sub>25</sub>N<sub>3</sub>O<sub>3</sub>: C, 74.48; H, 5.58; N, 9.31; %. Found: C, 74.47; H, 5.59; N, 9.33; %.
4.3. Ethyl 6-amino-5-cyano-4-(2-methoxyquinolin-3-yl)-2-methyl-4H-pyran-3-carboxylate (33)

Yellow solid, m.p = 209-211°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 2254 C≡N, 1552 C=N, 1226 C-N, 2970 CH, 1672 C=C, 1722 C=O, 2876 OCH$_3$, 3361 NH. $^1$H-NMR: (400 MHz, DMSO-d$_6$) $\delta$ ppm 8.92 (1H, s, Ar-H), 8.21 (1H, d, $J$= 6.40 Hz, Ar-H), 7.78 (1H, dd, $J$=2.52 Hz, Ar-H), 7.52 (1H, dt, $J$=1.12 Hz, Ar-H), 7.30 (1H, t, $J$= 5.56 Hz, Ar-H), 6.98 (2H, brs, NH$_2$), 4.59 (1H, s, CH), 3.76 (3H, s, OCH$_3$), 2.59 (2H, q, $J$= 13.52 Hz, CH$_2$), 1.95 (3H, s, OCH$_3$), 2.59 (2H, q, $J$= 13.52 Hz, CH$_2$), 1.95 (3H, s, CH$_3$), 1.23 (3H, s, CH$_3$). $^{13}$C-NMR: (100 MHz, DMSO-d$_6$) $\delta$ ppm 165.40, 158.43, 156.55, 144.83, 128.39, 127.14, 126.77, 119.68, 107.20, 60.10, 57.21, 40.07, 39.86, 39.65, 39.02, 38.82, 38.78, 30.62, 18.07, 13.66. Elemental Analysis: Anal. Calc. for C$_{20}$H$_{19}$N$_3$O$_4$: C, 65.74; H, 5.24; N, 11.50; %. Found: C, 65.76; H, 5.26; N, 11.51; %.

4.4. 5-Amino-7-(2-methoxyquinolin-3-yl)-2-oxo-3,7-dihydro-2H-pyrano[2,3-d]thiazole-6-carbonitrile (34)

Yellow solid, m.p = 218-220°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 2191 C≡N, 1604 C≡N, 1215 C-N, 2970 CH, 1654 C=C, 1751 C=O, 2863 OCH$_3$, 714 C-S, 2596 S-H, 3393 NH. $^1$H-NMR: (400 MHz, DMSO-d$_6$) $\delta$ ppm 10.25 (2H, s, NH$_2$), 8.41 (1H, s, Ar-H), 7.79 (1H, d, $J$=7.44 Hz, Ar-H), 7.56 (1H, t, $J$=7.12 Hz, Ar-H), 7.34 (1H, d, $J$=8.28 Hz, Ar-H), 7.17 (1H, t, $J$=7.24 Hz, Ar-H), 5.13 (1H, s, NH), 4.42 (1H, s, CH), 2.51 (3H, s, OCH$_3$). $^{13}$C-NMR: (100 MHz, CDCl$_3$) $\delta$ ppm 159.89, 159.85, 153.12, 150.74, 145.87, 136.85, 136.79, 134.43, 134.29, 134.18, 129.73, 127.76, 126.78, 125.18, 125.16, 124.55, 124.51, 124.33, 120.83, 120.81, 118.20, 118.13, 114.80, 114.62, 113.13, 113.10, 63.63, 63.57, 63.30, 63.23, 54.01, 49.37, 47.84, 16.48, 16.42, 16.20, 16.14. Elemental Analysis: Anal. Calc. for C$_{17}$H$_{12}$N$_3$O$_3$S: C, 57.95; H, 3.43; N, 15.90; %. Found: C, 57.97; H, 3.44; N, 15.92; %.
4.5. 5-Amino-7-(2-methoxy-6-nitroquinolin-3-yl)-2-thioxo-3,7-dihydro-2H-pyrano[2,3-d]thiazole-6-carbonitrile (35)

Brown solid, m.p = 249-251°C; IR ν_max (cm⁻¹): 2254 C≡N, 1588 C=N, 1047 C-N, 2880 CH, 1608 C=C, 1709 C=O, 2944 OCH₃, 2602 S-H, 1268 C=S, 3232 NH. ¹H-NMR: (400 MHz, DMSO-d₆) δ ppm 8.15 (1H, s, NH), 7.78 (1H, s, Ar-H), 7.65 (2H, d, J= 10.24 Hz, Ar-H), 7.55 (1H, d, J= 8.44 Hz, Ar-H), 6.80 (2H, brs, NH₂), 4.25 (1H, s, CH), 4.03 (3H, s, OCH₃). ¹³C-NMR: (100 MHz, CDCl₃) δ ppm 150.21, 150.15, 147.07, 146.98, 145.30, 145.16, 139.30, 137.83, 137.78, 130.78, 129.29, 129.12, 129.11, 128.06, 127.90, 127.32, 127.28, 119.92, 114.47, 110.60, 63.99, 63.92, 63.57, 63.49, 52.58, 51.06, 16.49, 16.44, 16.14, 16.14, 16.08. Elemental Analysis: Anal. Calc. for C₁₇H₁₁N₅O₄S₂: C, 49.39; H, 2.68; N, 16.94 %. Found: C, 49.41; H, 2.70; N, 16.96 %.

4.6. 2-Amino-4-(7-fluoro-2-methoxyquinolin-3-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (36)

White solid, m.p = 195-197°C; IR ν_max (cm⁻¹): 2181 C≡N, 1602 C=N, 1135 C-N, 2970 CH, 1638 C=C, 1678 C=O, 2653 OCH₃, 1007 C-F, 3368 NH. ¹H-NMR: (400 MHz, DMSO-d₆) δ ppm 8.03 (1H, s, Ar-H), 7.96 (1H, t, J=6.44 Hz, Ar-H), 7.46 (1H, d, J=2.52 Hz, Ar-H), 7.33 (1H, t, J=6.20 Hz, Ar-H), 7.01 (2H, brs, NH₂), 4.52 (1H, s, CH), 3.96 (3H, s, OCH₃), 2.49 (2H, q, J=17.80 Hz, CH₂), 2.24 (2H, q, J=16.04 Hz, CH₂), 1.04 (3H, s, CH₃), 0.96 (3H, s, CH₃). ¹³C-NMR: (100 MHz, CDCl₃) δ ppm 155.69, 148.57, 146.43, 146.31, 137.33, 129.20, 129.08, 123.08, 123.04, 122.99, 122.96, 119.70, 118.59, 114.02, 63.67, 63.59, 63.34, 63.27, 58.30, 56.80, 16.43, 16.37, 16.25, 16.19. ¹⁹F-NMR: (400 MHz, CDCl₃) -107.33. Elemental Analysis: Anal. Calc. for C₂₂H₂₀FN₃O₃: C, 67.17; H, 5.12; N, 10.68 %. Found: C, 67.19; H, 5.14; N, 10.70 %.
4.7. Ethyl 6-amino-5-cyano-4-(7-fluoro-2-methoxyquinolin-3-yl)-2-methyl-4H-pyran-3-carboxylate (37)

Yellow solid, m.p = 191-193°C; IR ν\textsubscript{max} (cm\textsuperscript{-1}): 2229 C≡N, 1580 C=\texttext{N}, 1219 C-N, 2919 CH, 1614 C=C, 1708 C=O, 1021 C-F, 3400 NH.
\textsuperscript{1}H-NMR: (400 MHz, DMSO-d\textsubscript{6}) δ ppm 8.87 (1H, s, Ar-H), 8.59 (1H, s, Ar-H), 8.02 (1H, d, J= 8.04 Hz, Ar-H), 7.83 (2H, s, NH\textsubscript{2}), 7.54 (1H, t, J=2.88 Hz, Ar-H), 4.43 (1H, s, CH), 4.07 (3H, s, OCH\textsubscript{3}), 3.91-3.89 (2H, m, J= 16.88 Hz, CH\textsubscript{2}), 2.50 (3H, s, CH\textsubscript{3}), 2.39 (3H, s, CH\textsubscript{3}). \textsuperscript{13}C-NMR: (100 MHz, CDCl\textsubscript{3}) δ ppm 176.04, 176.00, 156.24, 155.42, 155.36, 151.51, 151.40, 139.27, 134.26, 126.20, 125.98, 125.80, 123.28, 119.55, 118.32, 112.30, 64.05, 63.98, 63.91, 63.84, 45.62, 44.05, 29.68, 16.45, 16.40, 16.31, 16.26, 14.11. Elemental Analysis: Anal. Calc. for C\textsubscript{20}H\textsubscript{18}FN\textsubscript{3}O\textsubscript{4}: C, 62.66; H, 4.73; N, 10.96; %. Found: C, 62.68; H, 4.75; N, 10.95; %.

4.8. 5-Amino-7-(7-fluoro-2-methoxyquinolin-3-yl)-2-oxo-3,7-dihydro-2H-pyrano[2,3-d]thiazole-6-carbonitrile (38)

Brown solid, m.p = 205-207°C; IR ν\textsubscript{max} (cm\textsuperscript{-1}): 2252 C≡N, 1557 C≡N, 1292 C-N, 2917 CH, 1621 C=C, 1690 C=O, 2849 OCH\textsubscript{3}, 693 C-S, 2606 S-H, 1393 C-F, 3362 NH. \textsuperscript{1}H-NMR: (400 MHz, CDCl\textsubscript{3}) δ ppm 8.42 (1H, s, NH), 8.35 (1H, d, J= 3.12 Hz, Ar-H), 8.17 (2H, s, NH\textsubscript{2}), 7.80 (1H, d, J = 5.68 Hz, Ar-H), 7.68 (1H, t, J= 7.08 Hz, Ar-H), 7.42 (1H, t, J= 7.24 Hz, Ar-H), 4.91 (1H, s, CH), 2.25 (3H, s, OCH\textsubscript{3}). \textsuperscript{13}C-NMR: (100 MHz, CDCl\textsubscript{3}) δ ppm 206.98, 163.55, 147.90, 147.81, 145.98, 142.30, 137.93, 129.18, 122.84, 121.31, 113.86, 32.97, 32.97, 32.76, 30.92, 22.20, 13.79. Elemental Analysis: Anal. Calc. for C\textsubscript{17}H\textsubscript{11}FN\textsubscript{4}O\textsubscript{3}S: C, 55.13; H, 2.99; N, 15.13; %. Found: C, 55.15; H, 2.98; N, 15.15; %.
4.9. 2-Amino-4-(7-fluoro-2-oxo-1,2-dihydroquinolin-3-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (39)

Pale yellow, m.p = 129-131°C; IR ν<sub>max</sub> (cm<sup>-1</sup>): 2183 C≡N, 1572 C=N, 1152 C-N, 2979 CH, 1679 C=C, 1722 C=O, 1098 C-F, 3446 NH. ¹H-NMR: (400 MHz, DMSO-d<sub>6</sub>) δ ppm 8.69 (1H, s, NH), 8.40 (1H, s, Ar-H), 8.10-8.04 (1H, m, J= 6.24 Hz, Ar-H), 7.73 (2H, s, NH<sub>2</sub>), 7.50 (1H, t, J=5.84 Hz, Ar-H), 7.42 (1H, s, Ar-H), 4.09 (1H, s, CH), 2.70-2.57 (2H, m, J=17.92 Hz, CH<sub>2</sub>), 2.40-2.28 (2H, m, J=16.20 Hz, CH<sub>2</sub>), 1.25 (3H, s, CH<sub>3</sub>), 1.05 (3H, s, CH<sub>3</sub>). ¹³F-NMR: (400 MHz, DMSO-d<sub>6</sub>) δ ppm -108.37. ¹³C-NMR: (100 MHz, CDCl<sub>3</sub>) δ ppm 195.67, 191.20, 161.94, 140.51, 138.18, 130.13, 126.39, 126.18, 123.93, 122.47, 121.00, 120.52, 119.06, 117.65, 109.32, 108.72, 108.59, 96.60, 37.70, 29.08, 24.84, 19.80, 13.82, 13.72. Elemental Analysis: Anal. Calc. for C<sub>21</sub>H<sub>18</sub>F<sub>3</sub>O<sub>3</sub>N: C, 66.48; H, 4.78; N, 11.08; %. Found: C, 66.50; H, 4.79; N, 11.10; %.

4.10. 7-Amino-5-(6-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-carbonitrile (40)

Brown solid, m.p = 237-239°C; IR ν<sub>max</sub> (cm<sup>-1</sup>): 2222 C≡N, 1572 C≡N, 1217 C-N, 2925 CH, 1651 C=C, 1750 C=O, 3026 NH. ¹H-NMR: (400 MHz, DMSO-d<sub>6</sub>) δ ppm 12.20 (1H, s, NH), 10.78 (1H, s, NH), 9.42 (1H, s, NH), 8.12 (1H, s, Ar-H), 7.55 (1H, d, J = 1.44 Hz, Ar-H), 7.46 (2H, t, J=8.44 Hz, Ar-H), 7.29 (2H, s, NH<sub>2</sub>), 4.44 (1H, s, CH), 2.36 (3H, brs, CH<sub>3</sub>). ¹³C-NMR: (100 MHz, CDCl<sub>3</sub>) δ ppm 159.88, 159.84, 145.96, 145.94, 144.53, 144.39, 136.74, 136.68, 129.71, 129.11, 127.70, 126.87, 125.16, 125.13, 124.32, 123.21, 120.66, 120.64, 114.76, 63.60, 63.53, 63.40, 63.32, 53.91, 49.68, 48.15, 16.48, 16.43, 16.16, 16.11. Elemental Analysis: Anal. Calc. for C<sub>18</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>: C, 59.50; H, 3.61; N, 19.28; %. Found: C, 59.52; H, 3.63; N, 19.30; %.
4.11. 2-Amino-4-(2-chloro-6-methylquinolin-3-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (41)

Yellow solid, m.p = 187-189°C; IR νmax (cm⁻¹): 2227 C≡N, 1562 C≡N, 1216 C-N, 2923 CH, 1657 C=C, 1751 C=O, 733 C-Cl, 3039 NH. ¹H-NMR: (400 MHz, DMSO-d₆) δ ppm 11.38 (2H, s, NH₂), 7.71 (1H, d, J= 3.2 Hz, Ar-H), 7.47 (1H, s, Ar-H), 7.35-7.32 (1H, t, J=2.52 Hz, Ar-H), 7.30-7.27 (1H, d, J=5.56 Hz, Ar-H), 4.51 (1H, s, CH), 2.52 (3H, brs, CH₃), 2.39 (2H, q, J= 16.25 Hz, CH₂), 2.25 (2H, q, J=12.36 Hz, CH₂), 1.32 (3H, s, CH₃), 0.92 (3H, s, CH₃). ¹³C-NMR: (100 MHz, CDCl₃) δ ppm 176.28, 176.24, 156.26, 155.35, 155.29, 145.21, 145.08, 133.87, 129.37, 125.94, 124.44, 123.41, 120.02, 119.17, 118.28, 114.02, 63.86, 63.79, 63.53, 63.45, 46.20, 44.65, 30.92, 16.44, 16.29, 16.24. Elemental Analysis: Anal. Calc. for C₂₂H₂₀ClN₃O₂: C, 67.09; H, 5.12; N, 10.67; %; Found: C, 67.10; H, 5.14; N, 10.69; %.

4.12. 2-Amino-4-(2-methoxy-6-methylquinolin-3-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (42)

Yellow solid, m.p = 174-176°C; IR νmax (cm⁻¹): 2223 C≡N, 1579 C≡N, 1236 C-N, 2840 CH, 1614 C=C, 1697 C=O, 2970 OCH₃, 3446 NH. ¹H-NMR: (400 MHz, CDCl₃) δ ppm 7.72 (1H, s, Ar-H), 7.60 (1H, d, J= 8.48 Hz, Ar-H), 7.39 (1H, s, Ar-H), 7.32 (1H, dd, J= 1.60 Hz, Ar-H), 4.68 (2H, brs, NH₂), 4.54 (1H, s, CH), 3.98 (3H, s, OCH₃), 2.37 (3H, s, CH₃), 2.34 (2H, q, J= 12.20 Hz, CH₂), 2.17 (2H, q, J= 16.44 Hz, CH₂), 1.02 (3H, s, CH₃), 0.93 (3H, s, CH₃). ¹³C-NMR: (100 MHz, CDCl₃) δ ppm 196.03, 162.96, 159.77, 158.78, 144.09, 137.26, 133.59, 131.22, 126.70, 126.32, 125.37, 125.31, 119.01, 111.86, 60.45, 53.34, 50.57, 40.75, 32.67, 32.12, 29.26, 27.00, 21.26. Elemental Analysis: Anal. Calc. for C₂₃H₂₁N₃O₃: C, 70.93; H, 5.95; N, 10.79; %. Found: C, 70.95; H, 5.96; N, 10.81; %.
4.13. Ethyl 6-amino-5-cyano-4-(2-methoxy-6-methylquinolin-3-yl)-2-methyl-4H-pyran-3-carboxylate (43)

Brown solid, m.p = 188-190°C; IR νmax (cm⁻¹): 2338 C≡N, 1512 C≡N, 1240 C-N, 2917 CH, 1627 C=C, 1697 C=O, 2879 OCH₃, 3233 NH. ¹H-NMR: (400 MHz, DMSO-d₆) δ ppm 8.30 (1H, s, Ar-H), 7.46 (1H, d, J= 3.12 Hz, Ar-H), 7.11 (2H, dt, J= 10.20 Hz, Ar-H), 7.05 (2H, brs, NH₂), 4.17 (1H, s, CH), 2.49 (3H, s, OCH₃), 2.06 (2H, q, J= 16.04 Hz, CH₂), 1.48 (3H, s, CH₃), 1.02 (3H, s, CH₃), 0.94 (3H, s, CH₃). ¹³C-NMR: (100 MHz, CDCl₃) δ ppm 165.08, 163.08, 160.37, 150.68, 145.77, 142.95, 135.32, 130.50, 130.48, 129.90, 129.19, 128.12, 127.44, 126.80, 125.45, 124.02, 120.55, 103.47, 62.02, 61.01, 60.87, 53.58, 33.68, 13.87, 13.47. Elemental Analysis: Anal. Calc. for C₂₁H₂₁N₃O₄: C, 66.48; H, 5.58; N, 11.08; %. Found: C, 66.50; H, 5.59; N, 11.10; %.

4.14. 5-Amino-7-(2-methoxy-6-methylquinolin-3-yl)-2-thioxo-3,7-dihydro-2H-pyra[n2,3-d]thiazole-6-carbonitrile (44)

Brown solid, m.p = 198-200°C; IR νmax (cm⁻¹): 2226 C≡N, 1557 C≡N, 1229 C-N, 2927 CH, 1688 C=C, 1751 C=O, 2826 OCH₃, 1074 C=S, 615 C-S, 2511 S-H, 3407 NH. ¹H-NMR: (400 MHz, DMSO-d₆) δ ppm 12.07 (1H, brs, NH), 8.49 (1H, s, Ar-H), 7.82 (1H, d, J=5.88 Hz, Ar-H), 7.74 (2H, dd, J=3.96 Hz, Ar-H), 7.46 (2H, brs, NH₂), 4.69 (1H, s, CH), 3.63 (3H, s, OCH₃), 2.39 (3H, s, CH₃). ¹³C-NMR: (100 MHz, CDCl₃) δ ppm 190.41, 189.36, 135.25, 134.93, 128.95, 126.68, 115.73, 77.40, 47.08, 46.45, 40.92, 32.42, 31.42, 29.65, 29.29, 27.40, 20.90. Elemental Analysis: Anal. Calc. for C₁₈H₁₄N₄O₂S₂: C, 56.53; H, 3.69; N, 14.65; %. Found: C, 56.55; H, 3.71; N, 14.67; %.
**4.15. 2-Amino-6-(2H-indol-3-yl)-4-(p-tolyl)-4H-pyran-3,5-dicarbonitrile (45)**

Dark yellow solid, m.p = 201-203°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 2216 C≡N, 1568 C=N, 1289 C-N, 2918 CH, 1614 C=C, 3259 NH. $^1$H-NMR: (400 MHz, CDCl$_3$) $\delta$ ppm 8.72 (2H, brs, NH$_2$), 8.52 (1H, d, $J$= 3.2 Hz, Ar-H), 8.43 (1H, t, $J$=5.04 Hz, Ar-H), 7.93 (2H, d, $J$=8.12 Hz, Ar-H), 7.44 (1H, t, $J$=4.88 Hz, Ar-H), 7.34-7.29 (3H, dd, $J$ = 6.56 Hz, Ar-H), 4.62 (1H, s, CH), 2.43 (2H, s, CH$_2$), 2.15 (3H, s, CH$_3$). $^{13}$C-NMR: (100 MHz, CDCl$_3$) $\delta$ ppm 149.29, 145.99, 145.86, 142.53, 142.50, 129.21, 119.06, 114.10, 110.81, 110.79, 108.90, 108.83, 63.58, 63.51, 63.40, 63.33, 51.12, 49.54, 16.46, 16.41, 16.32, 16.26. Elemental Analysis: Anal. Calc. for C$_{22}$H$_{16}$N$_4$O: C, 74.98; H, 4.58; N, 15.90; %. Found: C, 74.99; H, 4.57; N, 15.92; %.
4.1. 2-Amino-4-(2-methoxyquinolin-3-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (31)

Fig. 4.1. IR spectrum of 31

Fig. 4.2. $^1$H-NMR spectrum of 31
Fig. 4.3. $^{13}$C-NMR spectrum of 31

Fig. 4.4. HSQC spectrum of 31
Fig. 4.5. COSY spectrum of 31

Fig. 4.6. NOESY spectrum of 31
Fig. 4.7. HMBC spectrum of 31

4.2. 2-Amino-7,7-dimethyl-5-oxo-4-(2-(o-tolyloxy)quinolin-3-yl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (32)

Fig. 4.8. IR spectrum of 32
Fig. 4.9. $^1$H-NMR spectrum of 32

Fig. 4.10. $^{13}$C-NMR spectrum of 32
4.3. Ethyl 6-amino-5-cyano-4-(2-methoxyquinolin-3-yl)-2-methyl-4H-pyran-3-carboxylate (33)

Fig. 4.11. IR spectrum of 33

Fig. 4.12. $^1$H-NMR spectrum of 33
Fig. 4.13. $^{13}$C-NMR spectrum of 33

4.4. 5-Amino-7-(2-methoxyquinolin-3-yl)-2-oxo-3,7-dihydro-2H-pyrano[2,3-d]thiazole-6-carbonitrile (34)

Fig. 4.14. IR spectrum of 34
Fig. 4.15. $^1$H-NMR spectrum of 34

Fig. 4.16. $^{13}$C-NMR spectrum of 34
4.5. 5-Amino-7-(2-methoxy-6-nitroquinolin-3-yl)-2-thioxo-3,7-dihydro-2H-pyrano[2,3-d]thiazole-6-carbonitrile (35)

Fig. 4.17. IR spectrum of 35

Fig. 4.18. $^1$H-NMR spectrum of 35
4.6. 2-Amino-4-(7-fluoro-2-methoxyquinolin-3-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (36)

Fig. 4.19. $^{13}$C-NMR spectrum of 35

Fig. 4.20. IR spectrum of 36
Fig. 4.21. $^1$H-NMR spectrum of 36

Fig. 4.22. $^{13}$C-NMR spectrum of 36
Fig. 4.23. $^{19}$F-NMR spectrum of 36

4.7. Ethyl 6-amino-5-cyano-4-(7-fluoro-2-methoxyquinolin-3-yl)-2-methyl-4H-pyran-3-carboxylate (37)

Fig. 4.24. IR spectrum of 37
Fig. 4.25. $^1$H-NMR spectrum of 37

Fig. 4.26. $^{13}$C-NMR spectrum of 37
4.8. 5-Amino-7-(7-fluoro-2-methoxyquinolin-3-yl)-2-oxo-3,7-dihydro-2H-pyrano[2,3-d]thiazole-6-carbonitrile (38)

Fig. 4.27. IR spectrum of 38

Fig. 4.28. $^1$H-NMR spectrum of 38
Fig. 4.29. $^{13}$C-NMR spectrum of 38

4.9. 2-Amino-4-(7-fluoro-2-oxo-1,2-dihydroquinolin-3-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (39)

Fig. 4.30. IR spectrum of 39
Fig. 4.31. $^1$H-NMR spectrum of 39

Fig. 4.32. $^{13}$C-NMR spectrum of 39
Fig. 4.33. $^{19}$F-NMR spectrum of 39

4.10. 7-Amino-5-(6-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-carbonitrile (40)

Fig. 4.34. IR spectrum of 40
Fig. 4.35. $^1$H-NMR spectrum of 40

Fig. 4.36. $^{13}$C-NMR spectrum of 40
4.11. 2-Amino-4-(2-chloro-6-methylquinolin-3-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (41)

Fig. 4.37. IR spectrum of 41

Fig. 4.38. $^1$H-NMR spectrum of 41
Fig. 4.39. $^{13}$C-NMR spectrum of 41

4.12. 2-Amino-4-(2-methoxy-6-methylquinolin-3-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (42)

Fig. 4.40. IR spectrum of 42
Fig. 4.41. $^1$H-NMR spectrum of 42

Fig. 4.42. $^{13}$C-NMR spectrum of 42
4.13. Ethyl 6-amino-5-cyano-4-(2-methoxy-6-methylquinolin-3-yl)-2-methyl-4H-pyran-3-carboxylate (43)

Fig. 4.43. IR spectrum of 43

Fig. 4.44. $^1$H-NMR spectrum of 43
Fig. 4.45. $^{13}$C-NMR spectrum of 43

4.14. 5-Amino-7-(2-methoxy-6-methylquinolin-3-yl)-2-thioxo-3,7-dihydro-2H-pyrano[2,3-d]thiazole-6-carbonitrile (44)

Fig. 4.46. IR spectrum of 44
Fig. 4.47. $^1$H-NMR spectrum of 44

Fig. 4.48. $^{13}$C-NMR spectrum of 44
4.15. 2-Amino-6-(2H-indol-3-yl)-4-(p-tolyl)-4H-pyran-3,5-dicarbonitrile (45)

Fig. 4.49. IR spectrum of 45

Fig. 4.50. $^1$H-NMR spectrum of 45
Fig. 4.51. $^{13}$C-NMR spectrum of 45
Chapter-V

Synthesis of $\alpha$-aminobenzylthioquinolinyl phosphonates catalysed by an iron-loaded boron nitride material and their antimicrobial, antioxidant, toxicity assessment and molecular docking studies
Chapter Five

Synthesis of α-aminobenzylthioquinolinyl phosphonates catalysed by an iron-loaded boron nitride material and their antimicrobial, antioxidant, toxicity assessment and molecular docking studies

5.1. Abstract

A series of novel α-aminobenzylthioquinolinyl phosphonates (BTQPs) were synthesized via the Kabachnik-Fields reaction of 2-(benzylthio)quinoline-3-carbaldehyde derivatives, arylamines and diethylphosphite in the presence of a catalytic amount of iron-loaded boron nitride (Fe/BN) catalyst. The catalyst was prepared by simple stirring of Fe(OAc)₂ and boron nitride under a nitrogen atmosphere. Fe/BN was characterized by XRD, SEM with EDX, TEM, TGA, DSC and FTIR. The efficiency of Fe/BN, the effects of solvent and recyclability of the catalyst were optimized. All the synthesized BTQPs were characterized by FTIR, ¹H-NMR, ¹³C-NMR and elemental analysis. A total of 10 BTQPs were subjected to antimicrobial, antioxidant and toxicity assessment studies. Among them, eight BTQPs showed potential antibacterial activities against Bacillus cereus, Staphylococcus aureus, Escherichia coli and Enterococcus faecalis whilst six BTQPs showed antioxidant activity effectively. 10 BTQPs were evaluated for toxicity by using brine shrimp and it was observed that eight BTQPs showed mortality rate less than 50% until 48 h. Molecular docking studies were performed to determine the medicinal application of these molecules based on the Libdock score. Among them, compound 31 showed more potency towards Staphylococcus aureus gyrase with Libdock score 149.97 kcal/mol. Eco-friendly methodology, water as solvent, easy work up, mild reaction conditions, shorter reaction time with higher yields, recyclability of the catalyst and non-hazardous solvents are the significant features of the study.

5.2. Introduction

Among the mimics of natural bioactive molecules, α-amino phosphonates (APs) are important due to their vast applications in medicinal chemistry (Moonen et al., 2004;
As a result of continuous efforts in this area, utilization of APs is expanding into many new research areas (Popa et al., 2015; Ménard et al., 1994). The APs exhibit a wide range of activities including being used as an agent against cancer (Ye et al., 2014; Huang et al., 2013; Huang et al., 2013; Huang et al., 2017), tuberculosis (Mulla et al., 2014; Subbedar et al., 2016), HIV/AIDS (Stowasser et al., 1992; Lacbay et al., 2014) and bacteria (Ali et al., 2012; Sampath et al., 2016; Sivala et al., 2016). They have been used as mimics of peptides, enzyme inhibitors and also as therapeutic agents for many diseases (De Schutter et al., 2014; Ewa et al., 2012; Leon et al., 2006; Leung et al., 2013; Leung et al., 2013; Wang et al., 2012).

Quinolines are important constituents of pharmacologically active plant extracts and their synthesis has been reported regularly. The quinoline scaffold is also frequently found in the structure of numerous naturally occurring alkaloids and has been associated with a broad spectrum of biological activities. Pharmacological activities such as antituberculosis (Lilienkampf et al., 2009), antiproliferation (Sedic et al., 2008), anti-inflammatory (Sujatha et al., 2017), anticancer (Gakhar et al., 2008) and antioxidant (Chung and Woo, 2001) activity have been reported.

The preparation of sulphur-containing compounds is also important in organic synthesis because of their broad application in organic and medicinal chemistry (Metzner and Thuillier, 2013; Nudelman, 1984; Chatgilialoglu and Asmus, 1991). The β-mercapto diketone is a vital sub-class as the compounds display remarkable pharmacological properties such as diuretic and HIV protease inhibitory activities (Bicking et al., 1976; Ding et al., 2009; Inomata et al., 2005; Yamauchi et al., 1982). Recently, several MCRs based on sulphur have been described. Evdokimov et al., 2007 reported a one-step synthesis of privileged medicinal sulphur based compounds from a mixture of malononitrile, aldehydes and thiols which was catalyzed by triethylamine. Based on the properties of quinolines, phosphonates and sulphur, their fusion into a single heterocycle could lead to new compounds with improved biological activities.

Generally, APs are synthesized by the nucleophilic addition of phosphite to imine in the presence of a Bronsted acid (Petrov et al., 1974) or Lewis acids like ZnCl₂ (Zawadzki, 1987), BF₃•Et₂O (Laschat and Kunz, 1992) and CdI₂/benzene (Kabachnik et al., 2002). The one-pot synthesis of APs have been carried out in organic solvents using lanthanide triflate (Qian and Huang, 1998), InCl₃ (Ranu et al., 1999), ZrCl₄ (Yadav et al., 2001),
In(OTf)$_3$/MgSO$_4$ (Ghosh et al., 2004), GaI$_3$ (Sun et al., 2004), BiCl$_3$ (Zhan and Li, 2005), Cu(OTf)$_2$ (Paraskar and Sudalai, 2006), SbCl$_3$/Al$_2$O$_3$ (Kumar et al., 2008) and an ionic liquid (Yadav et al., 2002). The transformations could also be accomplished in the presence of LiClO$_4$•Et$_2$O (Saidi and Azizi, 2002; Azizi and Saidi, 2003), TFA (Akiyama et al., 2003), LiClO$_4$ (Azizi et al., 2004), metal triflate (Firouzabadi et al., 2004), Na$_2$CaP$_2$O$_7$ (Elmakssoudi et al., 2005), ZrOCl$_2$•8H$_2$O or ZrO(ClO$_4$)$_2$•6H$_2$O (Bhagat and Chakraborti, 2008) and TsCl (Kaboudin and Jafari, 2008). Recently Krishna et al., 2010 reported the synthesis of APs (5) by condensation of imines (2) and dialkyl phosphite (4) without a catalyst. The reaction was carried out for six h to afford yields of 66-82 %.

Abdel-Megeed et al., 2012 developed a three-component reaction for the synthesis of diphenyl-1-(arylamino)-1-(pyridin-3-yl)ethyl phosphonates (9) by using LiClO$_4$, at room temperature. The disadvantage was the long reaction time of 20-30 h to afford the yield of 73-90 %, in the presence of DCM as a solvent.

Badadhe et al., 2011 showed the synthesis of new functionalized APs (12) from various substituted anilines (11) and 1-phenyl-3-(pyridin-4-yl)-1H-pyrazole-4-carbaldehyde (10) under reflux. The reaction was carried out for 24 h to provide a maximum yield of 58 - 83 %.
Chinnam et al., 2013 synthesized a series of APs (16) by a one-pot three-component reaction of equimolar quantities of 4-amino-N-2-thiazolyl-benzene sulphonamide (13), dimethyl phosphite (15) and various aldehydes (14) under reflux. The reaction was carried out within 5-6 h in absence of a catalyst; the yield was 70-80%.

Recently, Ahmed et al., 2016 reported the synthesis of aminophenylaminoquinoline analogues (20) from phenylenediamine derivatives (17) in the presence of a Lewis acid and LiClO₄ as a catalyst. The one-pot reaction was carried out within 24-48 h using dry acetonitrile as a solvent with 85-93% yield.
The majority of syntheses had several drawbacks including long reaction times, low product yield, stoichiometric amount of catalyst and costly metal ion catalyst. Therefore, we synthesized new APs with the aid of a new catalyst. Boron nitride was used as a support for iron which resulted in the heterogeneous catalyst.

5.3. Results and discussion

Scheme 1. The preparation of iron loaded boron nitride catalyst
Firstly, the iron-loaded boron nitride catalyst was prepared by simply mixing iron acetate and boron nitride, in methanol, at room temperature for three days. This was performed under a nitrogen atmosphere. A schematic illustration is shown in Scheme 1. The novel heterogeneous catalyst was characterized by using techniques including XRD, SEM with EDX, TEM, TGA, DSC and FTIR.

The XRD pattern (Fig. 1) showed the diffraction at 33.2°, 42°, 43.7°, 49.3°, 54°, 62.5° and 63.9° which corresponded to the spinel structure of Fe. The average particle size, calculated by using the Debye-Scherrer formula from the main reflection peak (311) at 42° 2θ, was approximately 75 nm. The Bragg’s diffraction reflection was observed at approximately 26.5°, 35.2°, 50°, 54.9° and 75.8° which corresponded to the crystalline planes (002), (100), (102), (004) and (220). These peaks confirmed the hexagonal crystal structure of boron nitride (Trivedi et al., 2015).

**Fig. 1.** X-Ray diffraction pattern of iron loaded boron nitride catalyst

The morphologies of Fe/BN is shown in Fig. 2 (A-D). The SEM images showed small uniform pseudo cubic shape particles. The particles were fairly regular. The aggregation of the particles indicated the possible interaction of hexagonal boron nitride (h-BN) with metal. Fig. 2 (C-D) shows particles were agglomerated and consisted of irregular rod and plate-like crystals.
Fig. 2. Scanning electron microscopy images of iron loaded boron nitride catalyst

The dimension of crystals were about 80, 160 nm and 200 nm (Fig. 4, see: histogram) and the thickness of sheet was approximately 20-50 nm. The average crystallite size, calculated from Scherrer’s formula (Scherrer, 1948), was ~24 nm, thus the observed SEM images suggested that the catalyst consisted of many small crystallites.

Fig. 3 displays the SEM images of Fe/BN showing the elements iron, boron, nitrogen, carbon and gold (Au). The iron peaks were at 0.35, 6.20 and 6.50 keV. The elemental mapping was obtained while running the sample in EDS and is illustrated in Fig. 3.
Fig. 3. Energy dispersive X-ray spectrum and elemental mapping of iron loaded boron nitride catalyst

The appearance of Au was due to the coating of the sample with conducting Au material to reflect electrons known as sputter coating.

The particle size and morphology was obtained from TEM analysis and is shown in Fig. 4 (A-E). TEM indicated the presence of two distinct size modes. Particles in the larger size mode were non-agglomerated and displayed a log-normal size distribution.
The count median diameter of the log-normal size distribution was 160 nm and 200 nm. The average size of the particles was 80 nm (size variation: ± 9 nm by ImageJ, Fig. 4: histogram) with an initial process of particle sinterization. The shape of h-BN particles appeared spherical and irregular. The layered appearance of the particles suggested Fe packed within BN. The closely packed aggregated particles and multilayer appearance was possible due to the cavity and movement of atoms towards the interior sphere of h-BN as reported by Chien et al., which focused on boron nitride coated Rhodium (Chien et al., 2020).
and Van Bokhoven, 2015). Wang et al., 2009 also showed the crystallization of boron nitride spheres prepared by vapor phase pyrolysis of ammonia borane.

The degradation of Fe/BN was analysed by thermogravimetric analysis. The TGA curve (Fig. 5) indicated that the sample encountered an initial weight loss of ~52 %, when the temperature was increased from room temperature to 130°C, which corresponded to the elimination of adsorbed and bound water molecules. The weight of the sample stabilized in the range of 180°C - 547°C. The Fe/BN showed further degradation at approximately 725°C which was ascribed to partial decomposition of metal loaded boron nitride material.

![TGA curve of iron loaded boron nitride catalyst](image)

**Fig. 5.** Thermogravimetric curve of iron loaded boron nitride catalyst

The decomposition and subsequent active oxidation started at temperatures greater than 725°C. This could possibly be attributed to BN oxidation and subsequent formation of boron trioxide (B₂O₃) on its surface. As the powder decomposed, boron reacted with the atmospheric oxygen and produced B₂O₃ while nitrogen gas was released (Oda and Yoshio, 1993). The process can be explained by a chemical reaction below:

\[
2\text{BN} + \frac{3}{2} \text{O}_2 \rightarrow \text{B}_2\text{O}_3 + \text{N}_2
\]
TGA analysis was completed at 1000°C: about 44 % mass loss was observed in the range of 725°C - 1000°C (Fig. 5).

The DSC curve (Fig. 6), provided information on decomposition and oxidation. A broad endothermic peak occurred between the temperatures of 200°C - 960°C. This confirmed the crystallization of the Fe phase (Sneha and Sundaram, 2015). The appearance of the peak was directly related to the decomposition of the powder (h-BN) as detected by the thermogravimetric technique (at 725°C) i.e., it absorbed the heat and confirmed the transformation of BN to B₂O₃. Also, a small endothermic peak appeared with a specific band width in temperature range of 95°C - 200°C. The appearance of the peak could be due to crystallization of the powder and the strong interaction between iron and the support (Sneha and Sundaram, 2015).

![DSC curve of iron loaded boron nitride catalyst](image)

**Fig. 6.** Differential scanning calorimetry curve of iron loaded boron nitride catalyst

The FTIR spectrum (Fig. 7) showed O-H and B-H stretching peaks at 3287 cm⁻¹ and 2193 cm⁻¹, respectively. The peaks observed at 1646 cm⁻¹ and 828 cm⁻¹ were assigned to the stretching frequencies of N-B-N and B-N-B, respectively (Muthu et al., 2016). The absorption frequency for Fe-N was observed at 1735 cm⁻¹ (Miller and Wilkins, 1952). The O-H absorption was possibly due to moisture.
After the successful characterization of Fe/BN, the heterogeneous catalytic activity was investigated in a one-pot three-component Kabachnik-Fields reaction.

Initially, chloroformylquinoline (CFQ) was synthesized via the Vilsmeier-Haack reaction which is a well-established protocol in our laboratory (Srivastava and Singh, 2005). The starting material, 2-(benzylthio)quinoline-3-carbaldehyde (BTQC), was prepared by heating CFQ with 2-benzyl mercaptan in the presence of NaH at 90°C for 2-3 h. It was then separated from DCM and water solvent system (1:3) followed by column chromatography. The desired starting material BTQC (21) was used for the preparation of APs via the Kabachnik-Fields reaction. At first, a trace amount of APs was obtained when an equivalent mixture of 21 (1 mmol), m-toluidine (1 mmol) and diethyl phosphite (4) (1 mmol) was used in acetonitrile medium. The reaction was carried out under reflux conditions (90°C) for 3-6 h in the presence of available catalysts: Mg(ClO₄)₂, InCl₃, CuI, L-proline and FeCl₃ according to the reported procedures (Maghsoodlou et al., 2009). The reaction was monitored by TLC every 5 minutes. Thereafter, the same mole ratio was used in the presence of Fe/BN (10 mol %) in MeCN medium under reflux condition which resulted in a 60 % yield within 3 h. Microwave irradiation was then used to produce a yield of 65 %. However a maximum yield of 98 % was obtained when water was used as the solvent (Scheme 2). The synthesis of 23 which used m-toluidine as the variable substrate was the template reaction. The structure of 23 was characterized by FTIR, ¹H-NMR, ¹³C-NMR, HSQC, COSY, NOESY, HMBC and elemental analysis.
Scheme 2. Synthesis of α-aminobenzylthioquinoline phosphonates (23-36) by microwave irradiation

The IR spectrum (Fig. 5.1, Appendix V) of 23 showed stretching frequencies (cm⁻¹) at 1216 for C-N, 2970 for CH, 1651 for C=C, 1273 for P=O, 1022 for P-O-C, 2585 for S-H, 695 for C-S and 3285 for NH. The ¹H-NMR spectrum (Fig. 5.2) of 23 showed two singlets: (brs, NH (C1')) at δ 12.46 and methyl group of m-toluidine (CH₃ (C15)) at δ 2.20. The C9-H proton of (CH) group showed a doublet at δ 5.61. Quinolinyl proton showed a doublet at δ 8.11 with a coupling constant value of J=3.72 Hz. The benzylthio group proton of CH₂ (C2") showed a multiplet at δ 4.33-4.29 with coupling constant value J=7.32 Hz. The ¹³C-NMR spectrum (Fig. 5.3) of 23 showed a peak at δ 21.55 for methyl group (CH₃) of m-toluidine (C15). The methyl carbons (C14) of the phosphoryl group showed a peak at δ 16.50 and C12 was at δ 16.27. The carbons C11 and C13 from ethoxy groups of the phosphoryl group (-OCH₂) showed peaks at δ 63.78 and 63.36. The carbon of the CH₂ (C2") group of benzyl thio group showed a peak at δ 47.29. The quinolinyl carbon C4 showed a peak at δ 130.51 and C9, (CH) group carbon showed a peak at δ 48.82. The quinolinyl carbon (C=N) showed a peak at δ 163.29. The structure was further confirmed on the basis of 2D NMR spectral studies. The selected ¹H and ¹³C-NMR chemical shifts for 23 are shown in Fig. 8.
Fig. 8. $^1$H and $^{13}$C-NMR chemical shifts of 23

The $^{13}$C, $^1$H-COSY correlation of carbon signals at $\delta$ 145.91, 139.09, 138.45, 128.70, 128.69, 128.17, 122.71, 119.97, 119.94, 115.73, 114.62, 110.58, 63.78, 63.36, 48.82, 47.29, 21.55, 16.50 and 16.27 were assigned to C8a, C4', C3", C7", C5", C8", C5a, C3, C4", C6", C5', C7', C11, C13, C9, C2", C15, C14 and C12, respectively. The carbon signal at $\delta$ 130.51 was due to quinolinyl carbon (C4), is shown in Fig. 5.5.

The $^1$H, $^1$H-COSY spectrum of 23 (Fig. 5.6) showed the correlation between CH (C9) at $\delta$ 5.61 with phenyl protons of C3', C6' and C7' at $\delta$ 6.59 with coupling constant value J=9.84 Hz was observed. CH$_3$ (C14) at $\delta$ 1.36. The $^1$H, $^1$H-NOESY spectrum (Fig. 5.7) of 23 showed the singlet of NH at $\delta$ 12.46 was coupled with the doublet of C9 (CH) at $\delta$ 5.61.

The $^1$H and $^{13}$C-NMR chemical shifts of HMBC correlation is illustrated in Fig. 9. The HMBC spectrum of 23 (Fig. 5.8) showed the long-range correlations as follows: the proton C9-H$_9$ (CH) was coupled with m-toluidine ring carbons, C6’ at $\delta$ 129.18, C4’ at $\delta$ 139.09 and C2’ at $\delta$ 146.05, quinolinyl carbons C8a at $\delta$ 145.91 and C2 at $\delta$ 163.29. This correlation of C9-H$_9$ to the m-toluidine ring carbons C6’, C4’ and C2’ and carbons C8a and C2 of quinolinyl moiety indicated that the two groups were attached to C9 (CH).
Thus it was evident that the two different moieties (amine and aldehyde) were bonded to a common carbon and hence added valuable information to 23.

The C4-H proton was coupled with carbons C9 at δ 48.82, C2" at δ 47.29, C8 at δ 138.39 and C2 at δ 163.29. The C4"-H proton was coupled with carbons C6" at δ 119.50, C7" at δ 128.70 and C8" at δ 128.17 of benzyl mercaptan ring. The C3'-H coupled with carbons C15 at δ 21.55, C7' at δ 110.58 and C5' at δ 115.73 of m-toluidine ring. The C15-H proton was coupled with m-toluidine ring carbons C3’ at δ 114.62 and C5’ at δ 115.73 and C4’ at δ 139.09. The C8-H proton was coupled with quinolinyl carbon C7 at δ 129.18. The C5”-H proton was correlated with C3” at δ 138.45 of benzyl mercaptan ring. The C12-H proton was correlated with carbon C11 at δ 63.78 of phosphoryl group. The C14-H proton was coupled with carbon C13 at δ 63.36. The selected ¹H and ¹³C-NMR HMBC correlated chemical shifts of 23 are shown in Table 1.

Table. 1. HMBC correlation and chemical shifts
<table>
<thead>
<tr>
<th>Entry</th>
<th>Protons</th>
<th>Correlated carbons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C9-H (s, 1H, CH) at δ 5.61 ppm</td>
<td>C6’ (129.18), C4’ (139.09), C8a (145.91), C2’ (146.05) and C2 (163.29)</td>
</tr>
<tr>
<td>2</td>
<td>C4-H (d, 1H, J= 3.72 Hz) at δ 8.11 ppm</td>
<td>C9 (48.82), C2” (47.29), C8 (138.39) and C2 (163.29)</td>
</tr>
<tr>
<td>3</td>
<td>C4’-H (t, 1H, CH, J= 6.8 Hz) at δ 7.18 ppm</td>
<td>C6” (119.50), C7” (128.70) and C8” (128.17)</td>
</tr>
<tr>
<td>4</td>
<td>C3’-H (t, 1H, J= 9.84 Hz) at δ 6.59 ppm</td>
<td>C15 (21.55), C7’ (110.58) and C5’ (115.73)</td>
</tr>
<tr>
<td>5</td>
<td>C15-H (s, 3H, CH3) at δ 2.20 ppm</td>
<td>C3’ (114.62), C5’ (115.73) and C4’ (139.09)</td>
</tr>
<tr>
<td>6</td>
<td>C8-H (d, 1H, J= 9.6Hz) at δ 7.50 ppm</td>
<td>C7 (129.18)</td>
</tr>
<tr>
<td>7</td>
<td>C5”-H (t, 1H, J= 7.72 Hz) at δ 7.00 ppm</td>
<td>C3” (138.45)</td>
</tr>
<tr>
<td>8</td>
<td>C12-H (t, 3H, J= 1.76 Hz) at δ 1.36 ppm</td>
<td>C11 (63.78)</td>
</tr>
<tr>
<td>9</td>
<td>C14-H (t, 3H, J= 2.6 Hz) at δ 1.14 ppm</td>
<td>C13 (63.36)</td>
</tr>
</tbody>
</table>

Fig. 5.4, showed a sharp peak clearly at δ 22.82 denoting the presence of phosphorous in 23. Based on the above spectral details and elemental analysis results (Anal. Calc. for C_{28}H_{31}N_{2}O_{3}PS: C, 66.39; H, 6.17; N, 5.53; %. Found: C, 66.40; H, 6.19; N, 5.55; %), the structure was confirmed as diethyl ((2-(benzylthio)quinolin-3-yl)(m-tolylamino)methyl) phosphonate (23).

To determine the effect of the catalyst, the optimum mole ratio of Fe/BN was investigated. The model reaction was carried out with different mole percentages of Fe/BN: 5 mol %, 10 mol %, 15 mol % and 20 mol %, separately. A trace amount of 23 was obtained when 25 mol % of BN was used. Among the various mole percentages, the best yield of 23 was obtained at 10 mol % of Fe/BN, (Table 2, entry 3).

### Table 2. Catalyst optimization for the synthesis of 23

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Mol %</th>
<th>Time (min)</th>
<th>Yield (%) b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BN</td>
<td>25</td>
<td>20</td>
<td>Trace</td>
</tr>
<tr>
<td>2</td>
<td>Fe/BN</td>
<td>5</td>
<td>10</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>Fe/BN</td>
<td>10</td>
<td>5</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>Fe/BN</td>
<td>15</td>
<td>12</td>
<td>91</td>
</tr>
<tr>
<td>5</td>
<td>Fe/BN</td>
<td>20</td>
<td>12</td>
<td>91</td>
</tr>
</tbody>
</table>

b Isolated yields

Also, the model reaction, in the presence of 10 mol % of Fe/BN, was investigated in different solvents to determine its effect on the reaction yield (Table 3). Solvents
including acetonitrile, toluene, ethanol, methanol and water were used. The yield of 23 decreased to 63 % when the solvent was changed and the low yield was due to the presence of some unreacted substances (Table 3, entry 2). The best yield of 98 % was obtained when the model reaction was carried out in a water medium (Table 3, entry 5).

Water has unique physical and chemical properties such as amphiphilicity, hydrogen bonding capability and can influence the reactivity of chemicals in addition to the selectivity of reactions (Hayashi et al., 2008; Lindström, 2002; Head-Gordon and Hura, 2002). The use of hexagonal boron nitride as a catalyst in water solvent requires a basic understanding of the interaction between water and h-BN surface and is important in the understanding of the water-BN interface. Cheng et al., 2017 reported the h-BN showed a strong interaction between the O-H bonds of the water molecule at 3420 cm\(^{-1}\) in the ligand free C-C coupling reactions using h-BN supported palladium (II) as catalyst and allowed the reactions with high efficiencies and excellent yields when performed in water.

Table 3. Effect of solvents on the synthesis of BTQPs

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time (min)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetonitrile</td>
<td>15</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>Toluene</td>
<td>12</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol</td>
<td>10</td>
<td>78</td>
</tr>
<tr>
<td>4</td>
<td>Methanol</td>
<td>10</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>Water</td>
<td>5</td>
<td>98</td>
</tr>
</tbody>
</table>

*Reaction conditions: aldehyde (1 mmol), amine (1 mmol), diethyl phosphite (1 mmol) and Fe/BN (10 mol %) were added to the water as a solvent (15 mL) under microwave irradiation at 90°C.

Fe/BN was easily separated by simple filtration from the reaction mixture. After washing with a CHCl₃: MeOH (1:1) combination, followed by acetone, the solid was dried in an oven for 1-2 h and subsequently re-used.

The reusability of catalysts is an important aspect of green chemistry which makes them useful for commercial applications. To investigate the catalytic efficacy of the recycled catalyst, five successive cycles were run on the model reaction under the optimal reaction conditions. The yield of 23 decreased in small increments with a total loss of 10 %
activity after five cycles of re-use (Fig. 10). This indicated that Fe/BN displays good reusability.

![FE/BN](image)

**Fig. 10.** Reusability of Fe/BN

The next objective was to compare conventional heating with the microwave assisted method: the results are summarized in Table 4. Without MW, 23 resulted in 50 % yield in the presence of Fe/BN, under stirring at room temperature and 64 % yield under reflux conditions within 24 h (Table 4, entries 1 & 2).

**Table 4.** Effect of microwave irradiation for the synthesis of 23

<table>
<thead>
<tr>
<th>Entry</th>
<th>Power (W)</th>
<th>Time</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Without MW (r.t)</td>
<td>24 h</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Without MW (110 °C)</td>
<td>24 h</td>
<td>64</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>15 min</td>
<td>71</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>5 min</td>
<td>98</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>12 min</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>200</td>
<td>15 min</td>
<td>90</td>
</tr>
</tbody>
</table>

*a Reaction conditions: aldehyde (1 mmol), amine (1 mmol), diethyl phosphite (1 mmol) and Fe/BN (10 mol %) were added to the water as a solvent (15 mL) under microwave irradiation at 90°C.

*b Isolated yields.

In addition, different power of MW irradiation was investigated. It was observed that a maximum percentage (98%) of 23 was obtained at 120 W (with 90°C) power at room
temperature within 5 minutes. The yield of 23 remained unchanged with an increase in the power of MW (Table 4, entries 5 & 6). It was found that MW irradiation increased the rate of the reaction leading to more product yield than conventional heating in the same time frame. It might be possible that higher temperatures were obtained by localised heating due to MW irradiation and hence the product yield was increased.

The efficiency of Fe/BN was also compared to previously reported catalysts and these observations are summarised in Table 5. It was observed that many catalysts afforded more than 70% of yield however longer reaction times were noted.

Table 5. Comparison of reported catalysts with Fe/BN catalyst

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>Temp (°C)</th>
<th>Time</th>
<th>Yield (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mg(ClO$_4$)$_2$</td>
<td>EtOH</td>
<td>50</td>
<td>5h/12h</td>
<td>85</td>
<td>Wu et al., 2006</td>
</tr>
<tr>
<td>2</td>
<td>Li(Otf)$_n$</td>
<td>Free</td>
<td>80</td>
<td>20 m-3.5 h</td>
<td>72-95</td>
<td>Firouzabadi et al., 2004</td>
</tr>
<tr>
<td>3</td>
<td>GaI$_3$</td>
<td>CH$_2$Cl$_2$</td>
<td>26</td>
<td>3-6 h</td>
<td>74-92</td>
<td>Sun et al., 2004</td>
</tr>
<tr>
<td>4</td>
<td>In(Otf)$_3$</td>
<td>THF</td>
<td>66</td>
<td>21-35 h</td>
<td>47-85</td>
<td>Ghosh et al., 2004</td>
</tr>
<tr>
<td>5</td>
<td>BiNO$_3$</td>
<td>Free</td>
<td>26</td>
<td>10 h</td>
<td>93</td>
<td>Bhattacharya et al., 2007</td>
</tr>
<tr>
<td>6</td>
<td>BiCl$_3$</td>
<td>MeCN</td>
<td>80</td>
<td>6-15 h</td>
<td>80-92</td>
<td>Zhan et al., 2005</td>
</tr>
<tr>
<td>7</td>
<td>YbCl$_3$</td>
<td>MeCN</td>
<td>26</td>
<td>24 h</td>
<td>63-96</td>
<td>Xu et al., 2006</td>
</tr>
<tr>
<td>8</td>
<td>SmI$_2$</td>
<td>MeCN</td>
<td>80</td>
<td>24 h</td>
<td>18-92</td>
<td>Xu et al., 2003</td>
</tr>
<tr>
<td>9</td>
<td>(NH)$_4$Ce(NO$_3$)$_6$</td>
<td>MeCN</td>
<td>26</td>
<td>3 h</td>
<td>86</td>
<td>Ravinder et al., 2004</td>
</tr>
<tr>
<td>10</td>
<td>InCl$_3$</td>
<td>THF</td>
<td>66</td>
<td>9-12 h</td>
<td>81-93</td>
<td>Ranu et al., 1999</td>
</tr>
<tr>
<td>11</td>
<td>Ln(Otf)$_3$</td>
<td>[bmim][PF$_6$]</td>
<td>26</td>
<td>27 h</td>
<td>92</td>
<td>Lee et al., 2001</td>
</tr>
<tr>
<td>12</td>
<td>TaCl$_5$-SiO$_2$</td>
<td>CH$_2$Cl$_2$</td>
<td>RT</td>
<td>22 h</td>
<td>92</td>
<td>Chandrasekhar et al., 2001</td>
</tr>
<tr>
<td>13</td>
<td>ZnO</td>
<td>Free</td>
<td>RT</td>
<td>9 h</td>
<td>90</td>
<td>Hou et al., 2011</td>
</tr>
<tr>
<td>14</td>
<td>CaCl$_2$</td>
<td>Free</td>
<td>60</td>
<td>2-4 h</td>
<td>80-96</td>
<td>Kaboudin et al., 2008</td>
</tr>
<tr>
<td>15</td>
<td>Fe/BN</td>
<td>Water</td>
<td>RT</td>
<td>5 min</td>
<td>98</td>
<td>Present work</td>
</tr>
</tbody>
</table>

The adopted procedure offered a way to compare the effectiveness of Fe/BN with the reported catalysts and 98% yield was obtained in a relatively shorter reaction time.

Following establishing the optimum reaction conditions for the synthesis of 23 and identifying its correct structure, another 13 derivatives (24-36) were synthesised by varying the aniline substrate. Table 6 summarises the derivatives that were synthesized and the % yield obtained. The FTIR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis for all the synthesized compounds are presented in Appendix V.
Table 6. Synthesis of α-aminobenzylthioquinoline phosphonates under MW irradiation

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehyde</th>
<th>Amine</th>
<th>Time (min)</th>
<th>Product</th>
<th>Product</th>
<th>Yield (%)</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C_{17}H_{13}NOS</td>
<td>C_{6}H_{5}N</td>
<td>5</td>
<td>C_{26}H_{31}N_{2}O_{3}PS</td>
<td>23</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>C_{17}H_{13}NOS</td>
<td>C_{6}H_{5}N</td>
<td>5</td>
<td>C_{26}H_{31}N_{2}O_{3}PS</td>
<td>24</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C_{17}H_{13}NOS</td>
<td>C_{6}H_{5}NO</td>
<td>5</td>
<td>C_{26}H_{31}N_{2}O_{3}PS</td>
<td>25</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>C_{17}H_{13}NOS</td>
<td>C_{6}H_{5}N</td>
<td>5</td>
<td>C_{26}H_{31}N_{2}O_{3}PS</td>
<td>26</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>C_{17}H_{13}NOS</td>
<td>C_{6}H_{5}FN</td>
<td>6</td>
<td>C_{27}H_{32}FNO_{2}PS</td>
<td>27</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>C_{17}H_{13}NOS</td>
<td>C_{6}H_{5}ClFN</td>
<td>8</td>
<td>C_{27}H_{32}ClFNO_{2}PS</td>
<td>28</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>C_{17}H_{13}NOS</td>
<td>C_{6}H_{5}N_{2}O_{2}</td>
<td>10</td>
<td>C_{28}H_{34}N_{6}O_{3}PS</td>
<td>29</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>C_{17}H_{13}NOS</td>
<td>C_{6}H_{5}N_{2}</td>
<td>5</td>
<td>C_{28}H_{34}N_{6}O_{3}PS</td>
<td>30</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>C_{17}H_{13}NOS</td>
<td>C_{6}H_{5}NO</td>
<td>5</td>
<td>C_{28}H_{34}N_{6}O_{3}PS</td>
<td>31</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>C_{17}H_{13}NOS</td>
<td>C_{6}H_{5}N_{2}</td>
<td>10</td>
<td>C_{29}H_{36}N_{6}O_{3}PS</td>
<td>32</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>C_{17}H_{13}NOS</td>
<td>C_{6}H_{5}BrN</td>
<td>8</td>
<td>C_{29}H_{36}BrN_{2}O_{3}PS</td>
<td>33</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>C_{17}H_{13}NOS</td>
<td>C_{6}H_{5}ClN</td>
<td>8</td>
<td>C_{29}H_{36}ClN_{2}O_{3}PS</td>
<td>34</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>C_{17}H_{13}NOS</td>
<td>C_{6}H_{5}ClN</td>
<td>10</td>
<td>C_{30}H_{38}ClN_{2}O_{3}PS</td>
<td>35</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>C_{17}H_{2}FNOS</td>
<td>C_{6}H_{5}NO</td>
<td>6</td>
<td>C_{29}H_{36}FN_{2}O_{3}PS</td>
<td>36</td>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>

b  Isolated yields

The presence of electron-donating groups on the amine increased the reaction rate as well as increased the product yield. Those substrates which possessed electron-withdrawing groups had an opposite effect. This electronic effect is common for electrophilic aromatic substitution reactions. However, this particular one-pot reaction is an addition reaction therefore one would generally not expect an electronic effect to play a role in the reaction.

A plausible mechanism is presented in Scheme 3 for the synthesis of BTQPs. Similar to graphene oxide (Ruifang et al., 2016), the isoelectronic nature of BN increased the electrophilicity of the catalyst. The mechanism involved the activation of carbonyl carbon of the aldehyde group (1) by Fe/BN followed by the nucleophilic addition of amine (2) to produce the imine (3) by the removal of water. The subsequent activation of imine (3) by Fe/BN facilitated the addition of diethyl phosphite to produce an activated phosphonium intermediate (4), which then resulted in the desired product (5).
The rapid emergence of resistant bacteria is occurring worldwide, endangering the efficacy of antibiotics, which have transformed medicine and saved millions of lives (Golkar et al., 2014; Gould and Bal, 2013). Many decades after the first patients were treated with antibiotics, bacterial infections have again become a threat (Spellberg et al., 2014). In the recent years, some of the α-aminophosphonate derivatives containing heterocycle moieties have been synthesized which have shown biological activities (Luo et al., 2012; Li et al., 2015; Shitre et al., 2014; Gu and Jin, 2012; Wu et al., 2011). It seems that the existence of heterocyclic moieties in the structure of the α-aminophosphonate molecule influenced the biological activity significantly. In this

Scheme 3. A plausible mechanism for the synthesis of BTQPs promoted by Fe/BN
present study, the aminophosphonates containing a heterocyclic ring: quinoline and sulfur bearing starting material in the synthesis of novel derivatives exhibited significant biological activity.

![Antibacterial activity of BTQPs](image)

**Fig. 11.** Antibacterial activity of BTQPs

1= 23; 2= 24; 3= 25; 4= 26; 5= 27; 6= 28; 7= 29; 8= 31; 9= 32; 10=36.
Benzylthioquinolinyl phosphonate derivatives were tested for antibacterial efficacy by determining the zone of inhibition against a range of Gram-positive (Bacillus cereus (B. cereus), Staphylococcus aureus (S. aureus) and Enterococcus faecalis (E. faecalis)) and Gram-negative (Escherichia coli (E. coli)) bacteria (Fig. 11). The present investigation which focused on three Gram-positive and one Gram-negative bacteria showed that compounds 24, 25, 27, 28, 29, 31 and 36 had preferential activity towards all Gram-positive species tested (Fig. 11).

Moreover, compound 27 and 32 showed activity against B. cereus only. Interestingly, compounds 24, 31 and 36 showed their potential against all species (both Gram-positive and Gram-negative). Furthermore, compounds 24, 25, 31 and 36 were also found to be effective against one of the Gram-negative species (E. coli) (Table 7).

**Table 7. Antibacterial activity of BTQP derivatives**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zone of inhibition by α-aminobenzylthioquinolinyl phosphonate (BTQP) derivatives</th>
<th>Ciprofloxacin (Positive control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus</td>
<td>0 13 ± 0.3 15 ± 0.2 0 13 ± 0.2 15 ± 0.3 10 ± 0.3 27 ± 0.4 10 ± 0.4 12 ± 0.3</td>
<td>27 ± 0.3</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0 11 ± 0.2 15 ± 0.4 0 0 19 ± 0.4 13 ± 0.4 30 ± 0.3 0 16 ± 0.3</td>
<td>25 ± 0.4</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0 14 ± 0.4 12 ± 0.3 0 0 0 0 30 ± 0.4 0 10 ± 0.2</td>
<td>25 ± 0.3</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>0 20 ± 0.3 0 0 0 0 0 21 ± 0.4 0 17 ± 0.3</td>
<td>24 ± 0.4</td>
</tr>
</tbody>
</table>

1= 23; 2= 24; 3= 25; 4= 26; 5= 27; 6= 28; 7= 29; 8= 31; 9= 32; 10= 36.

BTQPs that contained sulfur-based quinoline as core structure with phenyl group (24), methoxy group (25 and 31), fluorine and chlorine (28) and nitro group (29) showed antibacterial activity against Gram-positive species. Furthermore, BTQP (36) containing methyl group in the quinoline core structure with the sulfur atom of benzyl mercaptan showed preferential activity against Gram-positive bacteria. The potential activity of BTQPs toward B. cereus might be due to the presence of fluorine (27) and the pyridinyl group with the benzylthioquinoline substrate (32). The presence of a phenyl group (24), methoxy group (31) and methyl-containing benzylthioquinoline substrate with methoxy group (36) of BTQP showed potential towards Gram-positive and Gram-negative species. BTQPs that contain the methoxy group in the benzylthioquinoline compound (25) showed potential activity against E. coli.
Antioxidants are considered important nutraceuticals due to many health benefits (Valko et al., 2007). Antioxidant activity was assessed using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay. The scavenging assay was used for initial screening of the compounds for their antioxidant activity. A total of 10 BTQPs were tested. Among them, BTQPs containing sulfur-based quinoline as core structure with methyl groups (23) and (26), phenyl group (24), fluorine (27), nitro group (29) and methoxy group in the quinoline core structure with sulfur atom of benzyl mercaptan (36) showed significant scavenging activities.

Brine shrimp is used for preliminary assessment of toxicity (Rajabi et al., 2015). The toxicity was tested for all BTQPs at different intervals (24h and 48h), which displayed activity towards Gram-positive, Gram-negative or both. It was found that among all compounds tested, 23, 24, 25, 26, 27, 29, 31 and 36 have mortality rate below 50% (Fig. 12), which suggested that these compounds were safe for further biological applications (Meyer et al., 1982).

![Fig. 12. Toxicity assessment of BTQPs](image)

BTQPs that contained sulfur-based quinoline as core structure with methyl group (23), phenyl group (24), methoxy group (25), methyl group (26), fluorine (27), nitro group...
(29), methoxy group (31) and methyl group in the quinoline core structure with sulphur atom of benzyl mercaptan (36) showed mortality rate below 50%.

Molecular docking is an in-silico approach to estimate the binding free energies in a simplest way. The approach also helps to identify the orientation and conformation of the ligand inside the binding site. In the present study, molecular docking was carried out for α-aminobenzylthioquinoline phosphonates (BTQPs) into the ligand binding domain of S. aureus gyrase enzyme. The three-dimensional structure of S. aureus gyrase was retrieved from protein data bank (PDB ID: 4PLB) (Singh et al., 2014). The ligand series of BTQPs were built in Chem 3D Biodraw and the conformation of all the ligands were optimized by MM2 method. Further, the ligands were prepared in Discovery Studio software (Biovia, 2015) to include the ionization state, conformational analysis and stereoisomers, etc. The three-dimensional structure of S. aureus gyrase was also prepared in Chimera software (Pettersen et al., 2004). The presence of water molecules was removed and missing hydrogen atoms were added. The LibDock module of the Discovery Studio software was employed to perform molecular docking. All the ligands were docked at the 15Å sphere which was generated around the bound ligand of S. aureus gyrase (Table 8).

Table 8. Molecular docking scores of α-aminobenzylthioquinoline phosphonates with Staphylococcus aureus gyrase

<table>
<thead>
<tr>
<th>Entry</th>
<th>BTQP</th>
<th>Absolute Energy</th>
<th>LibDock Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>72.56</td>
<td>127.32</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>69.90</td>
<td>133.27</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>84.36</td>
<td>126.70</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>81.65</td>
<td>123.38</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>73.52</td>
<td>131.36</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>73.87</td>
<td>130.92</td>
</tr>
<tr>
<td>7</td>
<td>29</td>
<td>77.80</td>
<td>121.93</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>73.69</td>
<td>129.34</td>
</tr>
<tr>
<td>9</td>
<td>31</td>
<td>81.02</td>
<td>149.97</td>
</tr>
<tr>
<td>10</td>
<td>32</td>
<td>69.68</td>
<td>134.40</td>
</tr>
<tr>
<td>11</td>
<td>Reference Ligand</td>
<td>83.08</td>
<td>176.61</td>
</tr>
</tbody>
</table>
The docked molecules showed LibDock scores from 121.93 to 149.97 kcal/mol. The molecule (31) showed more potency amongst the series of BTQPs toward S. aureus gyrase by forming hydrogen bonding interaction with DC31 (Fig. 13).

![Hydrogen bonding interaction of (31) with Staphylococcus aureus gyrase](image)

**Fig. 13.** Hydrogen bonding interaction of (31) with *Staphylococcus aureus* gyrase

### 5.4. Conclusion

In conclusion, a microwave irradiated one-pot three-component synthesis of α-aminobenzylthioquinolinyl phosphonates was developed by using a novel iron-loaded boron nitride catalyst. The catalyst was characterized by XRD, SEM with EDX, TEM, TGA, DSC and FTIR. A total of 14 compounds were synthesized and characterized with FTIR, NMR and elemental analysis. 10 BTQP derivatives were evaluated for antibacterial and antioxidant activities: Compounds 24, 25, 27, 28, 29, 31, 32 and 36 showed positive results for antibacterial activity and 23, 24, 26, 27, 29 and 36 showed positive results for antioxidant potential, respectively. Since these new BTQPs showed antibacterial, antioxidant and less toxicity, they could be useful for further pharmaceutical applications. Among the synthesized compounds, 31 exhibited potential binding affinity with *S. aureus* gyrase based on *in-silico* molecular docking studies. The study displayed the advantages of green synthesis using water as solvent, shorter reaction times, mild conditions, high yields and reusability of the catalyst.
5.5. Experimental

Preparation of iron loaded boron nitride catalyst

To a solution of anhydrous Fe(OAc)$_2$ (28.1 mg, including 32.2 % (9.02 mg) of Fe metal; 0.5 wt % metal vs BN) in methanol (50 mL), boron nitride (2.66 g, 0.1 mol) was added and the mixture was stirred at room temperature for three days under nitrogen atmosphere. The resulting suspension was filtered and the solid was washed with MeOH, dried under reduced pressure to produce 0.3 % Fe/BN catalyst as a white powder, yield: 2.680 g (99%).

General procedure for the microwave synthesis of α-aminobenzylthioquinolinyl phosphonates

Catalytic amount (10 mol %) of Fe/BN was added to a mixture of aryl amine (1 mmol) and 21 (1 mmol) in water (15 mL) followed by the addition of 4 (1 mmol). The reaction tube was placed in a CEM microwave discover synthesizer and irradiated at 120 W at a temperature of 110°C for 5 minutes. The reaction was monitored by TLC. Following completion, the catalyst was isolated by simple filtration. The residue was purified by column chromatography from eluent (ethyl acetate: petroleum ether, 25 %) followed by EtOAc: H$_2$O (1:2) separation. The crystallization of the product was performed in ethanol to yield the pure product.

Bacterial strains

The antibacterial activity of each synthesized compound was assessed using four bacterial strains. Two strains of each Gram-positive (Bacillus cereus, Staphylococcus aureus and Enterococcus faecalis) and Gram-negative (Escherichia coli) bacteria were used. The bacterial strains were provided from the culture collection of Department of Biotechnology and Food Technology, Durban University of Technology, South Africa.

Inoculum preparation

Each bacterial strain was sub-cultured overnight at 37°C on Mueller-Hilton agar plate. Further, bacterial cultures were grown in Mueller-Hilton broth at 37°C, 200 rpm in order to attain the viable count of approximately 10$^8$ cfu/mL.
Antibacterial activity

The agar well diffusion method was used to evaluate the antibacterial activity. Hundred microliter of $10^8$ cfu/mL bacterial suspension was plated on Mueller-Hinton Agar plates. A well of 6 mm diameter was made using a sterile cork borer and 30 μL of each compound (3 mg/ml) was added in each well and kept at 37°C for 16 h. The assays were carried out in triplicate. Ciprofloxacin (3 mg/ml) was used as positive control and DMSO as the negative control.

Antioxidant assays of BTQP derivatives

The antioxidant ability of the BTQPs were determined by the decolourization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH). Hundred microliter of each compound was added separately to 1 mL of 0.1 mM DPPH solution, and a colour change was observed at regular intervals. Rutin hydrate was used as the positive control and methanol (95%) as negative control.

Toxicity assessment

The brine shrimp larvae (*Artemia salina*) were hatched in sea water for 24-48 h prior to being used. An aliquot of 5 mL sea water containing ten brine shrimp was added to each vial and supplemented with different derivatives of BTQP. Derivative concentrations of 300 μg were used in individual vial. Brine shrimp death was observed at regular intervals (24 h and 48 h) in order to determine the toxic nature of each compound. The data was expressed as % mortality.
References


bis-α-aminophosphonates catalyzed by heterogeneous reusable silica supported dodecatungstophosphoric acid (DTP/SiO$_2$) at ambient temperature and their antitubercular evaluation against Mycobacterium Tuberculosis. RSC Advances, (15) 7666-7672.


amino phosphonates from carbonyl compounds. Synthetic Communications, (34) 1677-1683.


5.1. Diethyl ((2-(benzylthio)quinolin-3-yl)(m-tolylamino)methyl)phosphonate (23)

Pale yellow crystals, m.p = 216-218°C; IR ν\textsubscript{max} (cm\textsuperscript{-1}): 1216 C-N, 2970 CH, 1651 C=C, 1273 P=O, 1022 P-O-C, 2585 S-H, 695 C-S, 3285 NH. \textsuperscript{1}H-NMR: (400 MHz, CDCl\textsubscript{3}) δ (ppm) 12.46 (1H, brs, NH), 8.11 (1H, d, J = 3.72 Hz, Ar-H), 7.50 (2H, d, J = 9.6 Hz, Ar-H), 7.45 (2H, t, J = 7.6 Hz, Ar-H), 7.18 (2H, t, J = 6.8 Hz, Ar-H), 7.00 (2H, t, J = 7.72 Hz, Ar-H), 6.59 (3H, t, J = 9.84 Hz, Ar-H), 6.52 (2H, d, J = 7.20 Hz, Ar-H), 5.61 (1H, d, J = 24.88 Hz, CH), 4.33-4.29 (2H, m, J = 7.32 Hz, CH\textsubscript{2}), 4.12-4.08 (2H, m, J = 7.20 Hz, CH\textsubscript{2}), 4.04-4.01 (2H, m, J = 7.36 Hz, CH\textsubscript{2}), 2.20 (3H, brs, CH\textsubscript{3}), 1.36 (3H, t, J = 1.76 Hz, CH\textsubscript{3}), 1.14 (3H, t, J = 2.6 Hz, CH\textsubscript{3}). \textsuperscript{13}C-NMR: (100 MHz, CDCl\textsubscript{3}) δ (ppm) 163.29, 146.05, 145.91, 139.09, 138.45, 138.39, 137.90, 137.88, 130.51, 129.18, 128.70, 128.69, 128.17, 122.71, 119.97, 119.94, 119.50, 115.73, 114.62, 110.58, 63.78, 63.36, 48.82, 47.29, 21.55, 16.50, 16.27. \textsuperscript{31}P-NMR: (400 MHz, CDCl\textsubscript{3}) δ (ppm) 22.82. Elemental Analysis: Anal. Calc. for C\textsubscript{28}H\textsubscript{31}N\textsubscript{2}O\textsubscript{3}PS: C, 66.39; H, 6.17; N, 5.53; %. Found: C, 66.40; H, 6.19; N, 5.55; %.

5.2. Diethyl ((2-(benzylthio)quinolin-3-yl)(phenylamino)methyl)phosphonate (24)

Brown solid, m.p = 207-209°C; IR ν\textsubscript{max} (cm\textsuperscript{-1}): 1218 C-N, 2918 CH, 1614 C=C, 1227 P=O, 1081 P-O-C, 2438 S-H, 704 C-S, 3390 NH. \textsuperscript{1}H-NMR: (400 MHz, DMSO-d\textsubscript{6}) δ (ppm) 11.97 (1H, s, NH), 8.50 (1H, s, Ar-H), 8.09 (1H, d, J = 3.72 Hz, Ar-H), 7.57 (1H, d, J = 7.76 Hz, Ar-H), 7.47 (1H, t, J = 7.68 Hz, Ar-H), 7.31 (1H, d, J = 8.24 Hz, Ar-H), 7.15 (1H, t, J = 7.56 Hz, Ar-H), 7.04 (3H, t, J = 7.48 Hz, Ar-H), 6.70 (2H, d, J = 7.88 Hz, Ar-H), 6.55 (2H, t, J = 7.32 Hz, Ar-H), 6.31 (1H, s, Ar-H), 5.96 (1H, s, Ar-H), 5.30 (1H, d, J = 10.04 Hz, CH), 4.14-4.07 (2H, m, J = 7.08 Hz, CH\textsubscript{2}), 4.01-3.95 (2H, m, J = 7.36 Hz, CH\textsubscript{2}), 3.92-3.85 (2H, m, J = 3.04 Hz, CH\textsubscript{2}), 1.25 (3H, t, J = 3.12 Hz, CH\textsubscript{3}), 1.15 (3H, t, J = 2.24 Hz, CH\textsubscript{3}). \textsuperscript{13}C-NMR: (100 MHz, DMSO-d\textsubscript{6}) δ (ppm) 164.81, 161.50, 161.09, 147.85, 146.95, 146.81, 137.98, 137.97, 137.24, 137.18, 134.15, 130.34, 130.01,
5.3. Diethyl ((2-(benzylthio)quinolin-3-yl)((2-methoxyphenyl)amino)methyl)phosphonate (25)

Dark brown solid, m.p = 211-213°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 1217 C-N, 2970 CH, 1672 C=C, 1228 P=O, 1015 P-O-C, 2395 S-H, 708 C-S, 2841 OCH$_3$, 3395 NH. $^1$H-NMR: (400 MHz, DMSO-d$_6$) $\delta$ (ppm) 8.24 (1H, s, NH), 7.95 (1H, s, Ar-H), 7.66 (1H, d, $J$ = 7.76 Hz, Ar-H), 7.46 (3H, t, $J$ = 4.08 Hz, Ar-H), 7.31 (1H, d, $J$ = 8.24 Hz, Ar-H), 7.21 (1H, td, $J$ = 7.72 Hz, Ar-H), 7.13 (1H, t, $J$ = 7.52 Hz, Ar-H), 6.82 (2H, t, $J$ = 8.92 Hz, Ar-H), 6.69 (2H, t, $J$ = 6.80 Hz, Ar-H), 6.61 (1H, td, $J$ = 2.52 Hz, Ar-H), 6.44 (1H, dd, $J$ = 6.60 Hz, Ar-H), 5.31 (1H, d, $J$ = 16.52 Hz, CH), 4.30 (2H, s, CH$_2$), 4.14-4.01 (2H, m, $J$ = 7.20 Hz, CH$_2$), 3.99-3.90 (2H, m, $J$ = 2.68 Hz, CH$_2$), 3.88 (3H, s, OCH$_3$), 1.22 (3H, t, $J$ = 7.04 Hz, CH$_3$), 1.07 (3H, t, $J$ = 7.00 Hz, CH$_3$). $^{13}$C-NMR: (100 MHz, DMSO-d$_6$) $\delta$ (ppm) 164.18, 161.16, 161.11, 153.79, 147.02, 146.85, 137.98, 136.72, 136.65, 135.52, 135.48, 135.34, 130.40, 130.16, 128.75, 128.73, 127.89, 127.58, 127.29, 122.04, 121.95, 120.99, 120.91, 120.80, 118.82, 117.72, 117.61, 114.96, 112.90, 110.73, 110.18, 110.12, 62.84, 62.76, 62.76, 62.69, 61.42, 55.64, 55.53, 55.24, 18.50, 16.29, 16.19, 16.13, 16.03, 15.97. $^{31}$P-NMR: (400 MHz, DMSO-d$_6$) $\delta$ (ppm) 21.00. Elemental Analysis: Anal. Calc. for C$_{28}$H$_{31}$N$_2$O$_4$PS: C, 64.35; H, 5.98; N, 5.36; %. Found: C, 64.37; H, 5.97; N, 5.38; %.

5.4. Diethyl ((2-(benzylthio)quinolin-3-yl)(o-tolylamino)methyl)phosphonate (26)

Brown solid, m.p = 219-221°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 1217 C-N, 2970 CH, 1616 C=C, 1229 P=O, 1040 P-O-C, 2520 S-H, 666 C-S, 3266 NH. $^1$H-NMR: (400 MHz, CDCl$_3$) $\delta$ (ppm) 9.11 (1H, s, NH), 8.46 (1H, s, Ar-H), 7.97 (2H, t, $J$ = 7.96 Hz, Ar-H), 7.75 (1H, d, $J$ = 8.20 Hz, Ar-H), 7.67 (1H, t, $J$ = 7.48 Hz, Ar-H), 7.44 (1H, t, $J$ = 7.56 Hz, Ar-H), 7.28 (1H, d,
\[
J = 8.00 \text{ Hz, Ar-H), 7.14 (3H, t, } J = 7.12 \text{ Hz, Ar-H), 7.02 (2H, d, } J = 8.32 \text{ Hz, Ar-H), 6.64 (1H, t, } J = 7.52 \text{ Hz, Ar-H), 6.55 (1H, d, } J = 8.48 \text{ Hz, Ar-H), 5.58 (1H, d, } J = 26.68 \text{ Hz, CH}_2, 4.30-4.25 (2H, m, } J = 3.08 \text{ Hz, CH}_2, 4.08-4.02 (2H, m, } J = 7.44 \text{ Hz, CH}_2, 3.96-3.90 (2H, m, } J = 9.56 \text{ Hz, CH}_2, 2.23 (3H, s, CH}_3, 1.32 (3H, t, } J = 7.04 \text{ Hz, CH}_3, 1.16 (3H, t, } J = 7.04 \text{ Hz, CH}_3). \]

\[ ^{13}\text{C-NMR: (100 MHz, CDCl}_3 \] \( \delta \) (ppm) 159.53, 159.42, 146.12, 143.61, 143.48, 136.76, 136.70, 136.30, 133.40, 129.95, 127.74, 126.93, 126.90, 125.14, 125.11, 124.50, 124.46, 120.03, 119.99, 116.85, 114.36, 63.78, 63.71, 63.59, 63.52, 54.04, 49.44, 47.90, 16.45, 16.40, 16.30, 16.25. \[ ^{31}\text{P-NMR: (400 MHz, CDCl}_3 \] \( \delta \) (ppm) 22.12. Elemental Analysis: Anal. Calc. for C\(_{28}\)H\(_{31}\)N\(_2\)O\(_3\)P\(_5\): C, 66.39; H, 6.17; N, 5.53; %. Found: C, 66.40; H, 6.19; N, 5.55; %.

5.5. Diethyl ((2-(benzylthio)quinolin-3-yl)((3-fluorophenyl)amino)methyl) phosphonate (27)

Pale yellow solid, m.p = 229-231°C; IR \( \nu_{max} \) (cm\(^{-1}\)): 1216 C-N, 2970 CH, 1624 C=C, 1304 P=O, 1052 P-O-C, 2570 S-H, 636 C-S, 1366 C-F, 3457 NH. \[ ^1\text{H-NMR: (400 MHz, CDCl}_3 \] \( \delta \) (ppm) 8.23 (1H, s, NH), 8.14 (1H, s, Ar-H), 7.91-7.86 (1H, d, } J = 6.32 \text{ Hz, Ar-H), 7.82 (2H, t, } J = 3.36 \text{ Hz, Ar-H), 7.64 (1H, t, } J = 6.68 \text{ Hz, Ar-H), 7.40 (2H, t, } J = 2.48 \text{ Hz, Ar-H), 7.11 (2H, t, } J = 7.76 \text{ Hz, Ar-H), 6.95 (2H, t, } J = 4.72 \text{ Hz, Ar-H), 6.71 (2H, t, } J = 7.00 \text{ Hz, Ar-H), 6.67 (1H, d, } J = 7.64 \text{ Hz, Ar-H), 5.30 (1H, d, } J = 12.32 \text{ Hz, CH}_2, 4.25 (2H, s, CH}_2, 4.09-3.98 (2H, m, } J = 2.72 \text{ Hz, CH}_2, 3.63-3.51 (2H, m, } J = 7.12 \text{ Hz, CH}_2, 1.30 (3H, t, } J = 7.08 \text{ Hz, CH}_3, 1.16 (3H, t, } J = 7.04 \text{ Hz, CH}_3). \[ ^{13}\text{C-NMR: (100 MHz, CDCl}_3 \] \( \delta \) (ppm) 176.28, 176.24, 156.26, 155.35, 155.29, 145.21, 145.08, 133.87, 129.37, 125.94, 125.44, 123.41, 120.02, 119.17, 118.28, 114.02, 63.86, 63.79, 63.53, 63.45, 46.20, 44.65, 30.92, 16.44, 16.38, 16.29, 16.24. \[ ^{31}\text{P-NMR: (400 MHz, CDCl}_3 \] \( \delta \) (ppm) 22.09. Elemental Analysis: Anal. Calc. for C\(_{27}\)H\(_{28}\)FN\(_3\)O\(_3\)P\(_5\): C, 63.52; H, 5.53; N, 5.49; %. Found: C, 63.54; H, 5.55; N, 5.51; %.
5.6. Diethyl ((2-(benzylthio)quinolin-3-yl)((3-chloro-4-fluorophenyl)amino)methyl)phosphonate (28)

Yellow solid, m.p = 234-236°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 1216 C=N, 2970 CH, 1625 C=C, 1259 P=O, 1034 P-O-C, 2529 S-H, 640 C-S, 1287 C-F, 663 C-Cl, 3457 NH. $^1$H-NMR: (400 MHz, DMSO-d$_6$) $\delta$ (ppm) 8.81 (1H, s, NH), 8.75 (1H, s, Ar-H), 8.32 (1H, d, $J$ = 1.48 Hz, Ar-H), 8.27 (1H, d, $J$ = 4.40 Hz, Ar-H), 8.10 (2H, d, $J$ = 11.16 Hz, Ar-H), 7.64 (2H, t, $J$ = 7.88 Hz, Ar-H), 7.53 (2H, t, $J$ = 7.12 Hz, Ar-H), 7.32 (2H, d, $J$ = 7.40 Hz, Ar-H), 7.21 (2H, t, $J$ = 7.60 Hz, Ar-H), 5.13 (1H, s, CH), 4.27 (2H, s, CH$_2$), 4.10-3.98 (2H, m, $J$ = 7.08 Hz, CH$_2$), 3.94-3.76 (2H, m, $J$ = 8.32 Hz, CH$_2$), 1.49 (3H, t, $J$ = 7.32 Hz, CH$_3$), 1.37 (3H, t, $J$ = 7.12 Hz, CH$_3$). $^{13}$C-NMR: (100 MHz, CDCl$_3$) $\delta$ (ppm) 159.89, 159.84, 145.73, 142.96, 142.82, 136.88, 136.82, 132.54, 129.80, 128.48, 127.81, 126.67, 125.14, 124.39, 120.66, 120.63, 119.19, 112.34, 110.51, 63.74, 63.68, 63.38, 63.31, 54.19, 49.93, 48.40, 16.52, 16.46, 16.26, 16.20. $^{31}$P-NMR: (400 MHz, DMSO-d$_3$) $\delta$ (ppm) 20.82. Elemental Analysis: Anal. Calc. for C$_{27}$H$_{27}$ClFN$_2$O$_3$PS: C, 59.50; H, 4.99; N, 5.14; %. Found: C, 59.52; H, 4.98; N, 5.16; %.

5.7. Diethyl ((2-(benzylthio)quinolin-3-yl)((2-nitrophenyl)amino)methyl)phosphonate (29)

Brown solid, m.p = 226-228°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 1293 C=N, 2970 CH, 1614 C=C, 1137 P=O, 1030 P-O-C, 2451 S-H, 707 C-S, 3381 NH. $^1$H-NMR: (400 MHz, CDCl$_3$) $\delta$ (ppm) 8.39 (1H, s, NH), 8.15 (1H, s, Ar-H), 8.00 (1H, s, Ar-H), 7.82 (1H, d, $J$ = 8.36 Hz, Ar-H), 7.65 (1H, d, $J$ = 8.00 Hz, Ar-H), 7.55 (1H, t, $J$ = 7.40 Hz, Ar-H), 7.30 (1H, t, $J$ = 7.52 Hz, Ar-H), 7.02 (2H, t, $J$ = 7.32 Hz, Ar-H), 6.98 (2H, t, $J$ = 7.12 Hz, Ar-H), 6.74 (3H, t, $J$ = 7.48 Hz, Ar-H), 6.44 (1H, d, $J$ = 7.60 Hz, Ar-H), 5.37 (1H, d, $J$ = 24.76 Hz, CH), 4.19 (2H, s, CH$_2$), 3.98-3.92 (2H, m, $J$ = 7.20 Hz, CH$_2$), 3.82-3.74 (2H, m, $J$ = 4.52 Hz, CH$_2$), 1.30 (3H, t, $J$ = 7.04 Hz, CH$_3$), 1.06 (3H, t, $J$ = 7.00 Hz, CH$_3$). $^{13}$C-
NMR: (100 MHz, CDCl3) δ (ppm) 160.07, 160.02, 147.37, 145.93, 145.91, 136.75, 136.70, 135.82, 129.50, 127.76, 126.79, 125.32, 125.29, 124.13, 121.39, 121.37, 121.08, 117.82, 110.64, 63.55, 63.49, 63.17, 63.10, 55.50, 53.86, 49.29, 47.76, 16.48, 16.43, 16.21, 16.15. 31P-NMR: (400 MHz, CDCl3) δ (ppm) 21.80.

Elemental Analysis: Anal. Calc. for C27H28N3O5PS: C, 60.33; H, 5.25; N, 7.82%; %. Found: C, 60.35; H, 5.27; N, 7.83%; %.

5.8. Diethyl ((2-(benzylthio)quinolin-3-yl)((2,4-dimethylphenyl)amino)methyl)phosphonate (30)

Dark yellow crystals, m.p = 222-224°C; IR νmax (cm⁻¹): 1225 C-N, 2971 CH, 1614 C=C, 1318 P=O, 1022 P-O-C, 2384 S-H, 658 C-S, 3971 NH. 1H-NMR: (400 MHz, CDCl3) δ (ppm) 7.99 (1H, s, NH), 7.52 (1H, d, J= 8.60 Hz, Ar-H), 7.33 (2H, d, J= 2.16 Hz, Ar-H), 7.11 (2H, d, J= 8.04 Hz, Ar-H), 6.69 (2H, t, J= 7.76 Hz, Ar-H), 6.49 (1H, d, J= 7.48 Hz, Ar-H), 6.43 (1H, s, Ar-H), 6.36 (1H, dd, J= 1.88 Hz, Ar-H), 4.69 (1H, d, J= 18.32 Hz, CH), 4.18-4.03 (2H, m, J= 7.12 Hz, CH2), 3.99-3.83 (2H, m, J= 6.32 Hz, CH2), 3.71-3.62 (2H, m, J= 9.12 Hz, CH2), 2.19 (3H, s, CH3), 1.41 (3H, s, CH3), 1.27 (3H, t, J= 4.16 Hz, CH3), 1.11 (3H, t, J= 7.04 Hz, CH3). 13C-NMR: (100 MHz, CDCl3) δ (ppm) 165.12, 162.70, 159.87, 159.82, 147.86, 147.75, 147.72, 147.61, 145.99, 145.97, 136.74, 136.68, 130.41, 130.31, 129.70, 127.71, 126.89, 125.71, 125.14, 124.30, 120.75, 120.73, 109.45, 109.42, 105.13, 104.92, 100.61, 100.36, 63.57, 63.50, 63.41, 63.34, 53.92, 49.48, 47.96, 16.48, 16.42, 16.16, 16.10. 31P-NMR: (400 MHz, CDCl3) δ (ppm) 22.18. Elemental Analysis: Anal. Calc. for C29H33N2O3PS: C, 66.90; H, 6.39; N, 5.38%; %. Found: C, 66.92; H, 6.40; N, 5.39%; %.

5.9. Diethyl ((2-(benzylthio)quinolin-3-yl)((4-methoxyphenyl)amino)methyl)phosphonate (31)

Dark brown solid, m.p = 239-241°C; IR νmax (cm⁻¹): 1217 C-N, 2970 CH, 1615 C=C, 1230 P=O, 995 P-O-C, 2419 S-H, 700 C-S, 2790 OCH3, 3390 NH. 1H-NMR: (400 MHz, CDCl3) δ (ppm) 9.01 (1H, s, NH), 8.26 (1H, s, Ar-H), 8.12 (1H, d, J= 8.40 Hz, Ar-H),
7.70 (3H, t, J= 8.20 Hz, Ar-H), 7.51 (2H, d, J= 7.08 Hz, Ar-H), 7.44 (1H, t, J= 7.40 Hz, Ar-H), 7.31 (2H, t, J= 6.88 Hz, Ar-H), 6.59 (2H, d, J= 8.92 Hz, Ar-H), 6.49 (2H, d, J= 7.32 Hz, Ar-H), 4.71 (1H, d, J= 13.24 Hz, CH), 4.28-4.20 (2H, m, J= 7.20 Hz, CH2), 3.76-3.69 (2H, m, J= 7.04 Hz, CH2), 3.66 (3H, s, OCH3), 1.34 (3H, t, J= 3.24 Hz, CH3), 1.02 (3H, t, J= 7.04 Hz, CH3).

13C-NMR: (100 MHz, CDCl3) δ (ppm) 149.07, 149.01, 145.76, 145.73, 145.29, 145.14, 137.52, 137.15, 137.10, 132.22, 132.95, 130.76, 128.01, 127.79, 127.22, 127.19, 126.53, 121.46, 115.45, 112.78, 64.08, 64.00, 63.80, 63.72, 52.54, 51.01, 21.48, 16.53, 16.47, 16.16, 16.10. 31P-NMR: (400 MHz, CDCl3) δ (ppm) 21.34.

Elemental Analysis: Anal. Calc. for C28H31N2O4PS: C, 64.35; H, 5.98; N, 5.36 %. Found: C, 64.37; H, 6.00; N, 5.38 %.

5.10. Diethyl ((2-(benzylthio)quinolin-3-yl)((pyridin-2-ylmethyl)amino)methyl) phosphonate (32)

Brown solid, m.p = 197-199°C; IR νmax (cm⁻¹): 1218 C-N, 2970 CH, 1615 C=C, 1556 C-N, 2395 S-H, 1050 P=O, 701 C-S, 3395 NH. 1H-NMR: (400 MHz, CDCl3) δ (ppm) 8.58 (1H, s, Ar-H), 8.06 (1H, s, Ar-H), 7.87 (1H, d, J= 4.20 Hz, Ar-H), 7.63 (1H, t, J= 6.20 Hz, Ar-H), 7.48 (1H, d, J= 6.84 Hz, Ar-H), 7.16 (1H, t, J= 4.76 Hz, Ar-H), 7.10 (2H, t, J= 7.40 Hz, Ar-H), 6.90 (3H, t, J= 7.04 Hz, Ar-H), 6.69 (3H, td, J= 7.28 Hz, Ar-H), 5.24 (1H, s, NH), 5.01 (1H, d, J= 22.24 Hz, CH), 4.36 (2H, s, CH2), 4.14-4.08 (2H, m, J= 7.32 Hz, CH2), 4.04-3.98 (2H, m, J= 7.28 Hz, CH2), 3.90-3.83 (2H, m, J= 7.08 Hz, CH2), 1.24 (3H, t, J= 7.04 Hz, CH3), 1.13 (3H, t, J= 6.88 Hz, CH3). 13C-NMR: (100 MHz, CDCl3) δ (ppm) 155.69, 148.57, 146.43, 146.31, 137.33, 129.20, 129.08, 123.08, 123.04, 122.99, 122.96, 119.70, 118.59, 114.02, 77.39, 77.27, 76.75, 63.67, 63.59, 63.34, 63.27, 58.30, 56.80, 16.43, 16.37, 16.25, 16.19. 31P-NMR: (400 MHz, CDCl3) δ (ppm) 21.00. Elemental Analysis: Anal. Calc. for C27H30N3O4PS: C, 63.89; H, 5.96; N, 8.28 %. Found: C, 63.90; H, 5.98; N, 8.30 %.
5.11. Diethyl ((2-(benzylthio)quinolin-3-yl)((4-bromophenyl)amino)methyl)phosphonate (33)

Dark yellow solid, m.p = 183-185°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 1217 C-N, 2970 CH, 1614 C=C, 1230 P=O, 995 P-O-C, 705 C-S, 521 C-Br, 3421 NH. $^1$H-NMR: (400 MHz, CDCl$_3$) $\delta$ (ppm) 8.51 (1H, s, NH), 8.20 (1H, s, Ar-H), 8.02 (1H, d, $J=8.32$ Hz, Ar-H), 7.90 (2H, s, Ar-H), 7.69 (2H, t, $J=8.64$ Hz, Ar-H), 7.49 (2H, d, $J=7.04$ Hz, Ar-H), 7.43 (1H, t, $J=7.48$ Hz, Ar-H), 7.31 (3H, t, $J=2.84$ Hz, Ar-H), 7.08 (1H, d, $J=8.72$ Hz, Ar-H), 6.43 (1H, d, $J=8.84$ Hz, Ar-H), 4.68 (1H, d, $J=14.96$ Hz, CH), 4.27-4.20 (2H, m, $J=7.16$ Hz, CH$_2$), 4.14-4.07 (2H, m, $J=7.24$ Hz, CH$_2$), 3.92-3.82 (2H, m, $J=7.20$ Hz, CH$_2$), 1.34 (3H, t, $J=7.08$ Hz, CH$_3$), 1.00 (3H, t, $J=7.04$ Hz, CH$_3$). $^{13}$C-NMR: (100 MHz, CDCl$_3$) $\delta$ (ppm) 157.48, 144.60, 144.45, 138.37, 134.71, 131.98, 130.19, 129.30, 129.21, 128.50, 127.97, 127.51, 127.23, 125.96, 125.84, 115.31, 110.43, 63.86, 63.80, 51.55, 50.02, 35.17, 16.45, 16.39, 16.12, 16.07. $^{31}$P-NMR: (400 MHz, CDCl$_3$) $\delta$ (ppm) 19.47. Elemental Analysis: Anal. Calc. for C$_{27}$H$_{28}$BrN$_2$O$_3$PS: C, 56.75; H, 4.94; N, 4.90; %. Found: C, 56.77; H, 4.96; N, 4.91; %.

5.12. Diethyl ((2-(benzylthio)quinolin-3-yl)((4-chlorophenyl)amino)methyl)phosphonate (34)

Yellow solid, m.p = 195-197°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 1218 C-N, 2971 CH, 1613 C=C, 1260 P=O, 951 P-O-C, 707 C-S, 752 C-Cl, 3304 NH. $^1$H-NMR: (400 MHz, CDCl$_3$) $\delta$ (ppm) 8.48 (1H, s, NH), 8.10 (1H, s, Ar-H), 7.91 (1H, d, $J=8.40$ Hz, Ar-H), 7.58 (2H, t, $J=8.60$ Hz, Ar-H), 7.39 (2H, d, $J=7.04$ Hz, Ar-H), 7.31 (1H, t, $J=7.52$ Hz, Ar-H), 7.22 (1H, d, $J=6.80$ Hz, Ar-H), 7.16 (2H, d, $J=5.96$ Hz, Ar-H), 6.84 (2H, d, $J=8.80$ Hz, Ar-H), 6.37 (2H, d, $J=8.84$ Hz, Ar-H), 4.56 (1H, d, $J=6.56$ Hz, CH), 4.17-4.10 (2H, m, $J=7.20$ Hz, CH$_2$), 3.82-3.72 (2H, m, $J=4.44$ Hz, CH$_2$), 3.58-3.51 (2H, m, $J=7.08$ Hz, CH$_2$), 1.23 (3H, t, $J=7.04$ Hz, CH$_3$), 0.90 (3H, t, $J=7.08$ Hz, CH$_3$). $^{13}$C-NMR: (100 MHz, CDCl$_3$) $\delta$ (ppm) 157.51, 157.45, 147.32, 147.30, 144.20, 144.06, 138.46, 134.67, 134.61, 130.08,
129.42, 129.34, 129.29, 129.21, 129.09, 128.49, 127.97, 127.64, 127.20, 125.98, 125.95, 125.76, 123.26, 115.16, 114.84, 63.83, 63.76, 63.46, 51.65, 50.12, 35.05, 16.47, 16.41, 16.14, 16.08. $^{31}\text{P-NMR: (400 MHz, CDCl}_3$ $\delta$ (ppm) 22.39. Elemental Analysis: Anal. Calc. for C$_{27}$H$_{28}$ClN$_2$O$_3$: C, 61.53; H, 5.36; N, 5.32; %. Found: C, 61.55; H, 5.38; N, 5.33; %.

5.13. Diethyl ((2-(benzylthio)quinolin-3-yl)((3,4-dichlorophenyl)amino)methyl)phosphonate (35)

Brown solid, m.p = 200-202°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 1225 C-N, 2977 CH, 1673 C=, 1132 P=O, 1021 P-O-C, 700 C-S, 747 C-Cl, 3307 NH. $^1\text{H-NMR: (400 MHz, CDCl}_3$ $\delta$ (ppm) 8.49 (1H, s, NH), 8.23 (1H, s, Ar-H), 8.01 (1H, d, $J= 8.36$ Hz, Ar-H), 7.68 (2H, t, $J= 7.72$ Hz, Ar-H), 7.53 (1H, d, $J= 4.80$ Hz, Ar-H), 7.48 (2H, s, Ar-H), 7.32 (2H, t, $J= 6.96$ Hz, Ar-H), 7.27-7.26 (1H, td, $J= 2.64$ Hz, Ar-H), 7.00 (1H, d, $J= 8.76$ Hz, Ar-H), 6.80 (1H, d, $J= 2.72$ Hz, Ar-H), 6.39 (1H, d, $J= 4.48$ Hz, Ar-H), 4.78 (1H, d, $J= 13.20$ Hz, CH), 4.28-4.21 (2H, m, $J= 7.20$ Hz, CH$_2$), 3.91-3.85 (2H, m, $J= 4.40$ Hz, CH$_2$), 3.67-3.60 (2H, m, $J= 2.84$ Hz, CH$_2$), 1.34 (3H, t, $J= 7.04$ Hz, CH$_3$), 1.01 (3H, t, $J= 2.40$ Hz, CH$_3$). $^{13}\text{C-NMR: (100 MHz, CDCl}_3$ $\delta$ (ppm) 157.48, 157.42, 147.39, 145.29, 145.14, 138.18, 134.64, 134.59, 132.80, 130.66, 130.16, 129.26, 128.50, 128.04, 128.02, 127.93, 127.70, 127.22, 125.90, 125.87, 125.79, 121.24, 115.33, 113.03, 63.95, 63.88, 63.80, 51.44, 49.91, 35.06, 16.46, 16.41, 16.13, 16.08. $^{31}\text{P-NMR: (400 MHz, CDCl}_3$ $\delta$ (ppm) 22.57. Elemental Analysis: Anal. Calc. for C$_{27}$H$_{28}$ClN$_2$O$_3$: C, 57.76; H, 4.85; N, 4.99; %. Found: C, 57.78; H, 4.87; N, 4.98; %.

5.14. Diethyl ((2-(benzylthio)-6-fluoroquinolin-3-yl)((2-methoxyphenyl)amino)methyl)phosphonate (36)

Yellow solid, m.p = 201-203°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 1231 C-N, 2918 CH, 1613 C=C, 1148 P=O, 1077 P-O-C, 650 C-S, 2818 OCH$_3$, 3390 NH. $^1\text{H-NMR: (400 MHz, CDCl}_3$ $\delta$ (ppm) 8.57 (1H, s, NH), 8.16 (1H, s, Ar-H), 7.85 (2H, t, $J= 8.44$ Hz, Ar-H), 7.59 (1H, t, $J= 7.76$ Hz, Ar-H), 7.34 (3H, td, $J= 2.96$ Hz, Ar-H), 7.15 (2H, t, $J= 8.16$ Hz, Ar-H), 6.69-6.61 (2H, d, $J= 7.68$ Hz, Ar-H), 6.59-6.51 (2H, d, $J= 8.00$ Hz, Ar-H), 5.19 (1H, s, CH), 4.19 (3H, s, OCH$_3$), 4.03 (2H, s, CH$_2$), 4.01-3.97 (2H, m, $J= 3.84$ Hz, CH$_2$), 3.96-3.90
(2H, m, J= 3.76 Hz, CH2), 1.04 (3H, t, J= 2.52 Hz, CH3), 1.01 (3H, t, J= 2.60 Hz, CH3).

13C-NMR: (100 MHz, CDCl3) δ (ppm) 164.96, 163.05, 160.31, 150.51, 145.75, 142.81, 136.06, 132.58, 132.49, 131.23, 129.28, 127.94, 127.41, 126.80, 125.40, 124.09, 120.32, 117.08, 116.85, 103.83, 62.15, 61.52, 60.94, 53.63, 33.70, 13.86, 13.54. 31P-NMR: (400 MHz, CDCl3) δ (ppm) 20.27.

19F-NMR: (400 MHz, CDCl3) δ (ppm) -117.38.

Elemental Analysis: Anal. Calc. for C28H30FN2O4PS: C, 62.21; H, 5.59; N, 5.18; %. Found: C, 62.23; H, 5.60; N, 5.20; %.
5.1. Diethyl ((2-(benzylthio)quinolin-3-yl)(m-tolylamino)methyl)phosphonate (23)

![IR spectrum of 23](image1)

**Fig. 5.1.** IR spectrum of 23

![1H-NMR spectrum of 23](image2)

**Fig. 5.2.** $^1$H-NMR spectrum of 23
Fig. 5.3. $^{13}$C-NMR spectrum of 23

Fig. 5.4. $^{31}$P-NMR spectrum of 23
Fig. 5.5. HSQC spectrum of 23

Fig. 5.6. COSY spectrum of 23
**Fig. 5.7.** NOESY spectrum of 23

**Fig. 5.8.** HMBC spectrum of 23

271
5.2. Diethyl ((2-(benzylthio)quinolin-3-yl)(phenylamino)methyl)phosphonate (24)

Fig. 5.11. IR spectrum of 24

Fig. 5.12. $^1$H-NMR spectrum of 24
Fig. 5.13. $^{13}$C-NMR spectrum of 24

Fig. 5.14. $^{31}$P-NMR spectrum of 24
5.3. Diethyl ((2-(benzylthio)quinolin-3-yl)((2-methoxyphenyl)amino)methyl)phosphonate (25)

Fig. 5.15. IR spectrum of 25

Fig. 5.16. $^1$H-NMR spectrum of 25
Fig. 5.17. $^{13}$C-NMR spectrum of 25

Fig. 5.18. $^{31}$P-NMR spectrum of 25
5.4. Diethyl (2-(benzylthio)quinolin-3-yl)(o-tolylamino)methyl)phosphonate (26)

**Fig. 5.19.** IR spectrum of 26

**Fig. 5.20.** $^1$H-NMR spectrum of 26
Fig. 5.21. $^{13}$C-NMR spectrum of 26

Fig. 5.22. $^{31}$P-NMR spectrum of 26
5.5. Diethyl ((2-(benzylthio)quinolin-3-yl)((3-fluorophenyl)amino)methyl) phosphonate (27)

Fig. 5.23. IR spectrum of 27

Fig. 5.24. $^1$H-NMR spectrum of 27
Fig. 5.25. $^{13}$C-NMR spectrum of 27

Fig. 5.26. $^{31}$P-NMR spectrum of 27
5.6. Diethyl ((2-(benzylthio)quinolin-3-yl)((3-chloro-4-fluorophenyl)amino)methyl)phosphonate (28)

Fig. 5.27. IR spectrum of 28

Fig. 5.28. $^1$H-NMR spectrum of 28
Fig. 5.29. $^{13}$C-NMR spectrum of 28

Fig. 5.30. $^{31}$P-NMR spectrum of 28
5.7. Diethyl ((2-(benzylthio)quinolin-3-yl)((2-nitrophenyl)amino)methyl) phosphonate (29)

Fig. 5.31. IR spectrum of 29

Fig. 5.32. $^1$H-NMR spectrum of 29
Fig. 5.33. $^{13}$C-NMR spectrum of 29

Fig. 5.34. $^{31}$P-NMR spectrum of 29
5.8. Diethyl ((2-(benzylthio)quinolin-3-yl)((2,4-dimethylphenyl)amino)methyl) phosphonate (30)

Fig. 5.35. IR spectrum of 30

Fig. 5.36. $^1$H-NMR spectrum of 30
Fig. 5.37. $^{13}$C-NMR spectrum of 30

Fig. 5.38. $^{31}$P-NMR spectrum of 30
5.9. Diethyl ((2-(benzylthio)quinolin-3-yl)((4-methoxyphenyl)amino)methyl) phosphonate (31)

Fig. 5.39. IR spectrum of 31

Fig. 5.40. $^1$H-NMR spectrum of 31
Fig. 5.41. $^{13}$C-NMR spectrum of 31

Fig. 5.42. $^{31}$P-NMR spectrum of 31
5.10. Diethyl ((2-(benzylthio)quinolin-3-yl)((pyridin-2-ylmethyl)amino)methyl) phosphonate (32)

Fig. 5.43. IR spectrum of 32

Fig. 5.44. $^1$H-NMR spectrum of 32
Fig. 5.45. $^{13}$C-NMR spectrum of 32

Fig. 5.46. $^{31}$P-NMR spectrum of 32
5.11. Diethyl ((2-(benzylthio)quinolin-3-yl)((4-bromophenyl)amino)methyl)phosphonate (33)

Fig. 5.47. IR spectrum of 33

Fig. 5.48. $^1$H-NMR spectrum of 33
Fig. 5.49. $^{13}$C-NMR spectrum of 33

Fig. 5.50. $^{31}$P-NMR spectrum of 33
5.12. Diethyl ((2-(benzylthio)quinolin-3-yl)((4-chlorophenyl)amino)methyl) phosphonate (34)

Fig. 5.51. IR spectrum of 34

Fig. 5.52. $^1$H-NMR spectrum of 34
Fig. 5.53. $^{13}$C-NMR spectrum of 34

Fig. 5.54. $^{31}$P-NMR spectrum of 34
5.13. Diethyl ((2-(benzylthio)quinolin-3-yl)((3,4-dichlorophenyl)amino)methyl)phosphonate (35)

Fig. 5.55. IR spectrum of 35

Fig. 5.56. $^1$H-NMR spectrum of 35
Fig. 5.57. $^{13}$C-NMR spectrum of 35

Fig. 5.58. $^{31}$P-NMR spectrum of 35
5.14. Diethyl ((2-(benzylthio)-6-fluoroquinolin-3-yl)((2-methoxyphenyl)amino)methyl) phosphonate (36)

Fig. 5.59. IR spectrum of 36

Fig. 5.60. $^1$H-NMR spectrum of 36
Fig. 5.61. $^{13}$C-NMR spectrum of 36

Fig. 5.62. $^{19}$F-NMR spectrum of 36
Fig. 5.63. $^{31}$P-NMR spectrum of 36
Chapter-VI

Ultrasonicated synthesis of benzylthioquinolinyl-1,4-dihydropyridines by iodine-loaded boron nitride catalyst and their antimicrobial, antioxidant, toxicity assessment and molecular docking studies
Chapter Six

Ultrasonicated synthesis of benzylthioquinolinyl-1,4-dihydropyridines by iodine-loaded boron nitride catalyst and their antimicrobial, antioxidant, toxicity assessment and molecular docking studies

6.1. Abstract
A series of novel benzylthioquinolinyl-1,4-dihydropyridines (BTQ-DHPs) were synthesized with high yields in short reaction time by a four-component reaction of 2-(benzylthio)quinoline carbaldehyde, malononitrile, arylamines and dimethyl acetylene dicarboxylate in the presence of iodine-loaded boron nitride catalyst (I/BN). A total of 14 novel compounds were synthesized and characterized by FTIR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis. The novel heterogeneous catalyst was synthesised from iodine and boron nitride by simply stirring the mixture for three days. I/BN was analysed by XRD, SEM with EDX, TEM, TGA, DSC and FTIR. A total of 10 BTQ-DHPs were subjected to antibacterial, antioxidant and toxicity studies. Among them, seven BTQ-DHPs showed potential antibacterial activities against *Bacillus cereus*, *Staphylococcus aureus* and *Enterococcus faecalis* whilst four BTQ-DHPs showed antioxidant activity effectively. 10 BTQ-DHPs were evaluated using brine shrimp assay and it was observed that five BTQ-DHPs showed mortality rate less than 50 % (48 h). Furthermore, molecular docking was used to estimate the ligand-protein interactions based on Libdock score. The docked BTQ-DHPs showed Libdock scores ranging from 83.20 to 125.27 kcal/mol. The molecule 24 showed more potency toward *Staphylococcus aureus* gyrase by forming a strong ligand-protein interaction with a Libdock score of 125.27 kcal/mol. The advantages of the protocol used for the synthesis of BTQ-DHPs are the use of water solvent, an inexpensive catalyst with efficient recyclability, mild reaction conditions, and quick reaction time with high yields.

6.2. Introduction
High throughput screening is one of the main strategies used for drug discovery. This requires a good library of lead compounds. To satisfy such requirements, new synthetic
strategies with good efficiency in providing a massive amount of structurally diverse organic products is required. Multi-component reactions (MCRs) which employ three or more reactants to furnish products containing structure or substructure of all starting materials in one-pot is a powerful method for increasing productivity (Dömling, 2006; Dömling and Ugi, 2000; Nair et al., 2003; Wan and Liu, 2011). The Hantzsch reaction which provides 1,4-DHPs, is one of the earliest and now a well-known MCR.

The biological activities of 1,4-DHPs include anticonvulsant (Borowicz et al., 1997), antihypertensive, antianginal (Love et al., 1974; Bossert et al., 1981; Breitenbacher et al., 2000), antitumor (Boer and Gekeler, 1995), anti-inflammatory (Briukhanov et al., 1993), antitubercular (Sushilkumar and Devanand, 2002), analgesic (Gullapalli and Ramarao, 2002), and antithrombolytic (Sunkel et al., 1990; Ono and Kimura, 1981). This has increased the interest for the synthesis of 1,4-DHPs as synthetic targets. Interestingly, the combination of 1,4-DHPs and quinoline derivatives are notably an important class of bioactive molecules in the pharmaceutical field (Vijesh et al., 2011).

The quinoline scaffold is important in the synthesis of new molecules which provide medical benefits. A number of quinoline derivatives are known to possess antimicrobial, antitumor, antifungal, hypotensive, anti-HIV, analgesic and antiinflammatory properties. Quinoline and its analogues have recently been examined for their mode of action in the inhibition of tyrosine kinases, proteasome, tubulin polymerization, topoisomerase and DNA repair. Substitution of the group in a suitable position of a bioactive molecule is found to exert a profound pharmacological effect (Gasparotto et al., 2006). Although the structural core of quinolines can be prepared using various conventional methods, the development of new synthetic methods is important in organic chemistry. For example, quinoline-2-thiones have been investigated as synthetic intermediates (Alhaider et al., 1985; Segawa et al., 1992; Jinbo et al., 1993), as biologically active compounds (Joseph et al., 2002), as sulfur-nitrogen mixed donor ligands (Xavier, 1958; Nakano et al., 1977; Leeaphon et al., 1995) and as the protective group of thiols (Zhang and Matteucci, 1999). However, direct synthetic methods that involve construction of a quinoline scaffold with simultaneous introduction of a thio group are limited (Molina et al., 1989). Therefore in the current study, 2-(benzylthio)quinoline-3-carbaldehydes have been synthesized and used as a starting material for the synthesis of new bioactive 1,4-dihydropyridines.
Many catalysts have been used to synthesize 1,4-DHPs such as silica gel/NaHSO$_4$ (Chari and Syamasundar, 2005), AlCl$_3$$\cdot$6H$_2$O (Sharma et al., 2008), TMSI (Sabitha et al., 2003), HClO$_4$-SiO$_2$ (Maheswara et al., 2006), triphenylphosphine (Debache et al., 2009), ionic liquid (Li et al., 2006), 3,4,5-trifluorobenzeneboronic acid (Sridhar and Perumal, 2005), iodonitromethane (Sabitha et al., 2003), Sc(OTf)$_3$ (Donelson et al., 2006), HY-Zeolite (Das et al., 2006), CAN (Reddy and Raghu, 2008), Yb(OTf)$_3$ (Wang et al., 2005) and organocatalysts (Kumar and Maurya, 2007).

Yavari et al., 2010 showed a one-pot synthesis of highly functionalized 1,2-dihydropyridines (5) from alkyl isocyanide (1), acetylenic esters (2, 3) and a primary alkyl amine (4). The reaction proceeded smoothly in dichloromethane at room temperature and produced 4-(alkylamino)-1-alkyl(aryl)-1,2-dihydropyridine-2,3,5,6-tetracarboxylates with a 59-95 % yield. The main drawback of the reaction was its long reaction time of 10 h.

\[
\begin{align*}
\text{NH} & \equiv \text{C}^- \\
\text{COOEt} \quad \text{COOEt} & \quad \text{COOCH}_3 \\
\text{NH}_2 & \text{CH}_2\text{Cl}_2 \quad \text{EtOOCCOCH}_3 \\
\text{R} & \text{EtOOCCOCH}_3
\end{align*}
\]

\[R = 4-\text{OCH}_3, 2-\text{Cl}, 4-\text{Cl}, 4-\text{NO}_2\]

Sridharan et al., 2007 developed a one-pot synthesis using cinnamaldehyde (6), amines (7) and ethyl acetoacetate (8) with cerium ammonium nitrate (CAN) in ethanol, which produced 1,4-DHPs (9) with a 52-71% isolated yield. However, the recyclability of the catalyst was not elucidated.

\[
\begin{align*}
\text{NH}_2 & \quad \text{H}_3\text{C} \equiv \text{C} \quad \text{H}_3\text{C} \equiv \text{C} \\
\text{H}_2\text{CCH}_2\text{C} & \quad \text{H}_3\text{C} \quad \text{H}_3\text{C} \quad \text{H}_3\text{C} \\
\text{R} & \quad \text{H} \\
\text{Ethanol, rt} & \quad \text{CAN}
\end{align*}
\]

\[R = \text{H}, 3-\text{CH}_3, 3-\text{OCH}_3, 4-\text{F}, 4-\text{Cl}, 4-\text{CH}_3\]
Debache et al., 2009 synthesized 1,4-DHPs (12) by using aromatic aldehydes (10), ethyl acetoacetate (8) and ammonium acetate (11) in triphenylphosphine (PPh₃) with 72-94% yield. The disadvantage of the reaction was a long reaction time of 5 h.

\[
\begin{align*}
\text{CHO} & + \text{OCH}_2\text{CH}_3 \quad \text{NH}_4\text{OAc} \quad \text{PPh}_3 (20 \text{ mol} \%) \\
10 & + 8 & 11 \\
\rightarrow & \quad \text{EtO} \quad \text{O} \quad \text{OEt} \\
\text{EtO} & \quad \text{H}_3\text{C} & \quad \text{H}_3\text{C} \quad \text{N} \quad \text{CH}_3 \\
12 & + 10 & 8 & 11 \\
\end{align*}
\]

\( R = \text{H, 4-CH}_3, \text{4-OCH}_3, \text{4-Cl, 4-Br, 2-Furyl} \)

Sabitha et al., 2009 developed an efficient one-pot synthesis of 1,4-DHPs (14) from aromatic aldehydes (13), ethyl acetoacetate (8) and ammonium acetate (11) within 3-6 h at room temperature. CeCl₃•7H₂O was used as a catalyst. The maximum yield obtained was 91% but it has several drawbacks including long reaction time and non-recyclability of the catalyst.

\[
\begin{align*}
\text{CHO} & + \text{OCH}_2\text{CH}_3 \quad \text{NH}_4\text{OAc} \quad \text{CeCl}_3\cdot\text{7H}_2\text{O} \\
13 & + 8 & 11 \\
\rightarrow & \quad \text{EtO} \quad \text{O} \quad \text{OEt} \\
\text{EtO} & \quad \text{H}_3\text{C} & \quad \text{H}_3\text{C} \quad \text{N} \quad \text{CH}_3 \\
14 & + 13 & 8 & 11 \\
\end{align*}
\]

\( R = \text{H, 4-OCH}_3, \text{4-F, 4-Cl, 4-CH}_3, \text{4-NO}_2 \)

Arslan et al., 2009 synthesised 1,4-DHPs (17) in the presence of alumina-sulphuric acid (ASA) as a catalyst.

\[
\begin{align*}
\text{CHO} & + \text{O} \quad \text{NH}_4\text{OAc} \quad \text{ASA} \\
15 & + 16 & 11 \\
\rightarrow & \quad \text{O} \quad \text{OEt} \\
\text{O} & \quad \text{R} \quad \text{H} \quad \text{CH}_3 \\
17 & + 15 & 16 & 11 \\
\end{align*}
\]

\( R = \text{H, 4-Br, 4-NO}_2, 2-\text{Cl, 3-NO}_2, 4-\text{CH}_3, 4-\text{OH} \)
Aldehydes (15), 1,3-dicarbonyl compounds (16) and ammonium acetate (11) were used: the product yield was 82-95 %. The disadvantages included the non-reusability of the catalyst and duration of the reaction time of 2-5 h.

All of these syntheses required long reaction times, harsh reaction conditions, tedious work up procedure, unsatisfactory yield due to side products, and the use of large quantities of volatile organic solvents. Therefore, alternate protocols are being sought-after. Ultrasonic irradiation is increasingly being used as a non-traditional technique for accelerating organic reactions. Compared with traditional methods, the salient features and benefits of ultrasonic irradiation includes reduced reaction times, reduced energy consumption, enhanced selectivity and improved product yields (Celle and Stefani, 2009; Cravotto and Cintas, 2006). Often, the reactions under ultrasound irradiation are easier to work-up than those by conventional methods.

Insulating oxides such as SiO₂, Al₂O₃, silica-alumina and zeolites are materials commonly used as catalyst supports (Spivey, 1987). These oxides possess disadvantages such as low thermal conductivity, generates sintering of the assisted metal on hot spots, various acidic and basic sites and the hydrophilic surface of the catalyst with water at low temperatures. Various two dimensional nanomaterials have received considerable attention for heterogeneous catalysis. Among them, boron nitride (BN) has gained attention due to its high elastic modulus, high melting-point, excellent thermal conductivity and a large and direct band gap. Such properties can be of high value for substrates (Liu et al., 2014). The graphene like hexagonal boron nitride (h-BN) (Nag et al., 2010) is the most stable isomer. It has acid-base resistance, good thermal and electrical conductivity. Moreover, BN is hydrophobic therefore preventing moisture condensation on its surface. Activated BN exhibits an excellent adsorption performance for various metal ions such as Cr³⁺, Co²⁺, Ni²⁺, Ce³⁺, Pb²⁺, organic pollutants such as tetracycline, methyl orange and congo red and volatile organic compounds such as benzene (Li et al., 2013). BN contains an equal number of boron and nitrogen atoms, isoelectronic with carbon and is analogous to graphite. The strong B-N covalent bonds impart high mechanical, thermal and chemical stability. In view of the above, BN has gained interest in the field of heterogeneous catalysis over the past decade (Primo et al., 2015; Wang et al., 2011; Molla et al., 2013) thus can be used as catalyst support. One of the important features of BN is that it behaves as both a Lewis acid and a Lewis base.
Iodine, as a cheap, less toxic and readily available mild Lewis acid has been used to catalyse various organic transformations (Togo and Iida, 2006; Ren and Cai, 2007; Ren and Cai, 2007; Ren and Cai, 2008). To enhance the ease of separation and Lewis acidity, we incorporated iodine on BN. Due to these facts and interest in the synthesis of 1,4-DHPs with potential biological activities, a convenient ultrasonic-assisted synthesis of benzylthioquinoline-1,4-dihydropyridines (BTQ-DHPs) in the presence of I/BN, is reported.

6.3. Results and discussion

Iodine is used in several organic syntheses (Stowell and Widlanski, 1995; Kitagawa et al., 1995; Joseph et al., 1995), however several drawbacks exist such as its ease of vaporization and sublimation limits its wide applicability. Therefore, the development of a new catalyst was sought. This study presents the synthesis and characterisation of a new iodine-loaded boron nitride (I/BN) catalyst and its application in the synthesis of new quinolone-based 1,4 DHPs. Briefly, BN was added to a solution of I$_2$ and the mixture stirred at room temperature for three days. The reaction mixture was filtered, washed with THF and dried to produce a white powder with 98% yield (Scheme 1). I/BN was characterized by XRD, SEM, EDX, TEM, TGA, DSC and FTIR.

**Scheme 1.** Synthesis of iodine-loaded boron nitride (I/BN)
The diffraction peaks (Fig. 1) which were observed at approximately 41.8°, 50°, 55° and 75.2° were assigned to the crystal planes (101), (200), (211) and (204) of Bragg’s reflection for iodine (Hou et al., 2011). The diffraction peaks indicated the anatase crystallite structure. The average crystallite size calculated from the full width at half maximum of the main peak (101), based on the Debye-Scherrer formula \(d = 0.9 \lambda/\beta_{1/2}\cos\theta\), was approximately 160 nm, and it was shown to be consistent with the TEM image (Fig. 4). The Bragg’s reflection which was observed at approximately 26.3°, 43.4° and 81.8° corresponded to the crystal planes (002), (102) and (004), respectively. These peaks confirmed the hexagonal crystal structure of boron nitride (Trivedi et al., 2015).

The surface and morphology of I/BN is shown in Fig. 2 (A-D). SEM images, at different scales, showed good spherical morphology with little agglomeration and smooth featureless surfaces (Fig. 2A-B). The spheres had small diameters of approximately 166 nm. The sponge like appearance observed at the surface in the range of 1-2 µm (Fig. 2C and 2D) was probably due to the incorporation of iodine: a similar observation was reported in the literature (Lin et al., 2015). Fig. 2C and 2D indicated that I/BN was agglomerated and consisted of irregular shapes and plate-like crystal structures. The
dimensions of these crystals were about 70 and 180 nm and the thickness of sheet was about 25 nm (Fig. 4A-E, See: histogram). Also, the average crystalline size calculated from Debye-Scherrer’s formula (Scherrer, 1918) was ~24 nm, thus the observed SEM images suggested that h-BN crystals consist of many small crystallites.

**Fig. 2.** Scanning electron microscopy images of I/BN

The EDX spectrum (Fig. 3) acquired from the SEM images revealed that the elements iodine, boron, nitrogen, carbon and gold were present.
Fig. 3. The energy dispersive X-ray spectrum and elemental mapping of I/BN

The iodine peaks were obtained at 2.75, 3.95, 4.20, 4.35, 4.40 and 4.55. The appearance of gold peaks was due to the coating of the sample with conducting material gold (Au) to reflect electrons (sputter coating).

The particle size and the surface morphology of I/BN was observed as presented in Fig. 4 (A-E). The TEM images showed spindle-like anatase nanocrystals (Fig. 4B-D) which confirmed iodine in the samples (Wen et al., 2007). The anatase nanocrystals were extended probably due to the incorporation of iodine atoms or by formation of lattice vacancies related to the iodine. Also, irregular and spherical morphologies of h-BN material were observed. The formation of particles with an average size of 166 nm (size variation: ± 19 nm by ImageJ, see: histogram) was observed. The closely packed
aggregated particles arose due to the presence of cavities and the movement of iodine atoms toward the interior sphere of the h-BN material (Wang et al., 2009).

**Fig. 4.** Transmission electron microscopy images of I/BN

The TGA curve (Fig. 5) showed the decomposition of I/BN at the temperature range of 25°C to 1000°C. The first decomposition of 1.8 % mass loss of the sample was observed at 80°C-90°C which was due to the loss of absorbed water. The catalyst appeared stabilized from 98°C to 300°C. Three continuous decomposition peaks were observed at 310°C, 575°C and 698°C. In the temperature range of 310°C-620°C, there was a mass loss
of approximately 35 % which was due to the thermal degradation of the iodine complex, as reported by Danilovas et al., 2014. During this stage, it is suggested that iodine was released from the complex which further induced thermochemical degradation of the boron nitride material.

**Fig. 5.** Thermogravimetric analysis curve of I/BN

The decomposition and subsequent oxidation was observed at 698°C due to BN oxidation and the subsequent formation of boron trioxide (B$_3$O$_3$) on its surface. As the catalyst decomposed, boron reacted with atmospheric oxygen and produced B$_2$O$_3$ while nitrogen gas (N$_2$) was released (Oda and Yoshio, 1993). The process can be explained by the following chemical reaction:

$$2\text{BN} + \frac{3}{2}\text{O}_2 \rightarrow \text{B}_2\text{O}_3 + \text{N}_2$$

A mass loss of 16 % was observed between the temperature ranges of 698°C -1000°C which was due to the loss or degradation of h-BN.

The DSC curve (Fig. 6) of I/BN provided the heat flow inside the furnace as a function of temperature, arising from a series of physical and chemical procedures such as decomposition and oxidation. A broad endothermic effect in the temperature ranges of
500°C - 90°C was observed which might be connected with the weight decrease and was assigned to partial breaking of B-I bonds (Marinoiu et al., 2017). A small exothermic peak appeared at 92°C which could be associated with atmospheric moisture.

![Differential scanning calorimetry curve of I/BN](image)

**Fig. 6.** Differential scanning calorimetry curve of I/BN

![Fourier transform infrared spectrum of I/BN](image)

**Fig. 7.** Fourier transform infrared spectrum of I/BN

Fig. 7 shows the FTIR spectrum of I/BN. The broad absorption peaks at 3284 cm$^{-1}$ and 2194 cm$^{-1}$ corresponded to the hydroxyl group (O-H) and B-H, respectively. Both peaks
were recognized due to the absorbance of water molecules from atmospheric moisture. The peaks at 1648 cm\(^{-1}\) and 829 cm\(^{-1}\) was assigned to N-B-N and B-N-B, respectively (Muthu et al., 2016). The interaction of iodine with h-BN was confirmed by the B-I peak observed at 502 cm\(^{-1}\) (Hassanzadeh and Andrews, 1993) (Fig. 7).

After the successful characterization of I/BN, the heterogeneous catalytic activity was investigated by a one-pot four-component reaction. The starting compound chloroformylquinoline (CFQ) was synthesized by using the Vilsmeier-Haack reaction as per the procedure (Srivastava and Singh, 2005).

![Scheme 2](image)

**Scheme 2.** Synthesis of benzylthioquinoline-1,4-dihydropyridines (22-35) via a four-component condensation reaction under ultrasonication by using I/BN

CFQ was used to synthesize the starting material 2-(benzylthio)quinoline-3-carbaldehyde (BTQC) (18) via reaction with 2-benzylmercaptan for 2-3 h in the presence of NaH, in DMSO, at 90°C. The mixture was cooled and then separated by a dichloromethane/water mixture (1:3) followed by column chromatography to produce BTQC. In a preliminary investigation, an equimolar mixture (1 mmol) of 18, malononitrile (19), o-anisidine and dimethyl acetylene dicarboxylate (DMAD) (21) was reacted with triethylamine as a catalyst, in ethanol at ambient temperature for 24 h. The
product was purified by column chromatography. The yield of benzylthioquinoline-1,4-dihydropyridines (BTQ-DHPs) (22) was 40 %. When the same reaction was conducted in the presence of I/BN, 53 % yield was obtained within 24 h. The reaction time was too long and therefore ultrasonic irradiation was investigated; the reaction time was reduced to 10 minutes with 71 % yield. The percentage yield increased to 97 %, when the reaction was conducted in water for 3 minutes (Scheme 2).

22 was purified by column chromatography (eluents: ethyl acetate: petroleum ether, 25 %) and characterized by FTIR, $^1$H-NMR, $^{13}$C-NMR, HSQC, HMBC and elemental analysis. The IR spectrum (Fig. 6.1, Appendix VI) of 22 showed stretching frequencies (cm$^{-1}$) at 2187 for C≡N, 1614 for C=N, 1277 for C-N, 2918 for CH, 1652 for C=C, 703 for C-S, 1736 for C=O, 2890 for OCH$_3$ and 3308 for NH. The $^1$H-NMR spectrum (Fig. 6.2) of 22 showed three singlets at $\delta$ 5.03 for (C4”), $\delta$ 5.58 for (C14) and $\delta$ 8.19 for (C4).

Fig. 8. Selected $^1$H and $^{13}$C-NMR chemical shifts for 22

The benzyl thio group proton CH$_2$ (C2’) showed a triplet at $\delta$ 4.73 for one proton with a coupling constant value, $J$=13.6 Hz. Methoxy group, OCH$_3$ (C10) was identified as a triplet at $\delta$ 2.49 and the other two OCH$_3$ (C7” and C12) groups showed broad singlets at
δ 3.96 and 3.82. The $^{13}$C-NMR spectrum (Fig. 6.3) showed two carbonyl groups (C9 and C11) at δ 164.68. The peak observed at δ 45.75 attributed to CH (C4”). The carbonitile (C13) carbon showed a peak at δ 112.83. Methoxy carbons (C10, C12 and C7”) showed peaks at δ 52.29, 51.63 and 56.47, respectively. The structure was further confirmed by 2D NMR spectral studies. The selected $^1$H and $^{13}$C-NMR chemical shift for 22 is showed in Fig. 8.

The $^{13}$C, $^1$H-COSY correlation of carbon signals at δ 129.14, 128.21, 127.37, 127.22, 125.84, 123.20, 120.68, 112.83, 59.12, 56.47, 51.63 and 33.76 were assigned to C6’, C5, C4’, C8’, C4”, C3”, C5”, C13, C7”, C10 and C2”. The carbon signal at δ 45.75 was due to CH (C4”’) carbon which is shown (Fig. 6.4).

![Fig. 9. HMBC correlations and chemical shifts of 22](image-url)
The HMBC correlation of protons assigned to the corresponding carbons is showed in Fig. 9. The HMBC spectrum (Fig. 6.5) of 22 showed the long-range correlations as follows: The C4”-H of 22 coupled with pyridinyl carbons (C5”’) at δ 59.12 and C2” at δ 156.80, quaternary carbon C1” at δ 139.73, quinolinyl carbons C5 at δ 128.21, C4a at δ 146.26 and C8a at δ 151.30, carbonyl carbons (C9 and C11) at δ 164.68. This correlation of C4”-H to the quaternary carbon (C1”), quinolinyl carbons (C4a and C8a), pyridinyl carbons (C5”’ and C2”’) and carbons (C9) and (C11) of carbonyl group indicated that the four groups were attached to C4”” (CH). Thus it was evident that the four different moieties were bonded to a common carbon (CH) and hence added valuable information to 22.

The C2’-H proton was coupled with the benzyl mercaptan group carbons, C8’ at δ 127.22 and C7’ at δ 129.88. The methoxy group C7”-H proton was coupled with C2’” at δ 156.80 and C6” at δ 157.13. The C14-H proton was coupled with pyridinyl carbon C5”’ at δ 59.12. The benzyl mercaptan ring C5’-H proton was coupled with carbons C2’ at δ 33.76, C4’ at δ 127.32 and C7’ at δ 56.47. The proton of C3’-H was coupled with the same o-anisidine ring carbon, C1” at δ 139.73 and C5” at δ 59.12. The C4-H proton was coupled with C2’ at δ 33.76, C4a at δ 146.26 and C2’” at δ 156.80. The C2’’-H was correlated with o-anisidine ring carbon C5” at δ 120.68 and thus the selected 1H, 13C-NMR and HMBC chemical shifts of the compound 22 are shown in Table 1.

**Table 1. HMBC correlation and chemical shifts**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Protons</th>
<th>Correlated carbons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C4”-H (s, 1H) at δ 5.03 ppm</td>
<td>C5”” (59.12), C5 (128.21), C1”” (139.73), C4a (146.26), C8a (151.30), C2’” (156.80), C9 (164.68) and C11 (164.68)</td>
</tr>
<tr>
<td>2</td>
<td>C2’-H (d, 2H, J=13.56 Hz) at δ 4.73 and 4.57 ppm</td>
<td>C8’ (127.22) and C7’ (129.88)</td>
</tr>
<tr>
<td>3</td>
<td>C7”-H (s, 3H) at δ 3.96 ppm</td>
<td>C2’” (156.80) and C6” (157.13)</td>
</tr>
<tr>
<td>4</td>
<td>C14-H (s 2H) at δ 5.58 ppm</td>
<td>C5”” (59.12)</td>
</tr>
<tr>
<td>5</td>
<td>C5’-H (t, 1H, J=7.6 Hz) at δ 7.54 ppm</td>
<td>C2’ (33.76), C4’ (127.37) and C7’ (56.47)</td>
</tr>
<tr>
<td>6</td>
<td>C3’-H (t, 1H, J=7.56 Hz) at δ 7.28 ppm</td>
<td>C1”” (139.73) and C5” (59.12)</td>
</tr>
<tr>
<td>7</td>
<td>C4-H (s, 1H) at δ 8.19 ppm</td>
<td>C2’ (33.76), C4a (146.26) and C2’” (156.80)</td>
</tr>
<tr>
<td>8</td>
<td>C2’’-H (t, 1H, J=7.6 Hz) at δ 7.05 ppm</td>
<td>C5” (120.68)</td>
</tr>
</tbody>
</table>
Based on the above spectral details and elemental analysis results: Anal. Calc. for C$_{33}$H$_{28}$N$_{4}$O$_{5}$S: C, 66.88; H, 4.76; N, 9.45; %. Found: C, 66.89; H, 4.78; N, 9.47; %, the structure was confirmed as dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(2-methoxyphenyl)-1,4-dihydropyridine-2,3-dicarboxylate (22).

To determine the optimal reaction medium, different solvents were selected and the results are summarized in Table 2. Among the solvents, the reaction in water gave best yield (Table 2, entry 5). The yield of 22 decreased to 62 % when the solvent was changed. The low yield was probably due to unreacted substrate (Table 2, entry 1). The maximum yield of 97 % was obtained when the reaction was carried out in water with 3 minutes reaction time.

**Table 2.** The effect of solvents

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time (min)</th>
<th>Yield (%) b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetonitrile</td>
<td>15</td>
<td>62</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>12</td>
<td>77</td>
</tr>
<tr>
<td>3</td>
<td>Toluene</td>
<td>13</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>Methanol</td>
<td>10</td>
<td>76</td>
</tr>
<tr>
<td>5</td>
<td>Water</td>
<td>3</td>
<td>97</td>
</tr>
</tbody>
</table>

b Isolated yields

To determine the optimum mole % of I/BN, the reaction was conducted with different mole percentages: 5 mol %, 10 mol %, 15 mol % and 20 mol % (Table 3). It was observed that a trace amount of dihydropyridine was obtained when 25 mol % of h-BN used (Table 3, entry 1). Among the various mole percentages, the best yield was with 10 mol % of I/BN (Table 3, entry 3).

**Table 3.** Catalyst optimization

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Mol %</th>
<th>Time (min)</th>
<th>Yield (%) b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BN</td>
<td>25</td>
<td>20</td>
<td>Trace</td>
</tr>
<tr>
<td>2</td>
<td>I/BN</td>
<td>5</td>
<td>8</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>I/BN</td>
<td>10</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>4</td>
<td>I/BN</td>
<td>15</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>I/BN</td>
<td>20</td>
<td>10</td>
<td>90</td>
</tr>
</tbody>
</table>

b Isolated yields
Water has unique physical and chemical properties such as amphiphilicity and hydrogen bonding capability which influence the reactivity of chemicals in addition to the selectivity of reactions (Hayashi et al., 2008; Lindström et al., 2002; Head-Gordon and Hura, 2002; Bellissent and Dore, 1994). The use of hexagonal boron nitride as a catalyst in water solvent requires a basic understanding of the interaction between water and the h-BN surface and is important in understanding the water-BN interface. Cheng et al., 2017 reported that the h-BN showed a strong interaction between O-H bonds of water molecule at 3420 cm$^{-1}$ in the ligand free C-C coupling reaction using h-BN supported palladium (II) as a catalyst. The reactions had high efficiencies with excellent yields when conducted in water (Cheng et al., 2017).

The recyclability of a catalyst is usually conducted to determine cost effectiveness. I/BN was recycled for the model reaction (Fig. 10). Briefly, after a reaction, I/BN was collected after a simple filtration of the reaction content. The filtered I/BN was washed with a CHCl$_3$: MeOH (1:1) combination followed by acetone. The solid was dried in an oven for 1-2 h and subsequently employed for five successive cycles on the model reaction. There was a loss of only 12% in catalytic activity after five cycles hence the catalyst is good for possible scale-up.

**Fig. 10.** Recyclability of the catalyst I/BN

Furthermore, the efficiency of I/BN was compared with previously reported catalysts and commercially available catalysts (Table 4).
Table 4. A comparison of catalyst for the synthesis of dihydropyridines

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Condition</th>
<th>Time</th>
<th>Yield (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Silica gel/NaHSO₄</td>
<td>CH₃CN/Stirring</td>
<td>6 h</td>
<td>85</td>
<td>Chari et al., 2005</td>
</tr>
<tr>
<td>2</td>
<td>AlCl₃·6H₂O</td>
<td>Heating (60°C)</td>
<td>1 h</td>
<td>83</td>
<td>Sharma, et al., 2008</td>
</tr>
<tr>
<td>3</td>
<td>TMSI/NaI</td>
<td>CH₃CN/Stirring</td>
<td>2 h</td>
<td>85</td>
<td>Sabitha et al., 2003</td>
</tr>
<tr>
<td>4</td>
<td>HClO₄·SiO₂</td>
<td>Stirring</td>
<td>20 min</td>
<td>95</td>
<td>Maheswara et al., 2006</td>
</tr>
<tr>
<td>5</td>
<td>PPh₃</td>
<td>EtOH/Reflux</td>
<td>5 h</td>
<td>72</td>
<td>Debache et al., 2009</td>
</tr>
<tr>
<td>6</td>
<td>F₃C₆H₂COOH</td>
<td>Stirring</td>
<td>4 h</td>
<td>90</td>
<td>Sridhar et al., 2005</td>
</tr>
<tr>
<td>7</td>
<td>I/BN</td>
<td>Sonication</td>
<td>3 min</td>
<td>97</td>
<td>Present work</td>
</tr>
</tbody>
</table>

The reaction time was long and the percentage yield was less for various catalysts (Table 4). These catalysts (Table 4, entries 7-14) produced 25-39 % yield making them less effective. The procedure for the synthesis of 22 provided a way to compare the effectiveness of I/BN with other reported catalysts and 97 % yield was observed in the current study, in a relatively shorter reaction time of 3 minutes.

Special efforts were made to evaluate and compare the conventional techniques with the ultrasonic irradiation technique: the results are shown in Table 5. Without sonication, 22 was obtained with 51 % yield by stirring at room temperature for 24 h. When the same mole ratio of the catalyst was used under reflux conditions for 24 h, 63 % yield was obtained (Table 5, entry 2). The effect of ultrasonic irradiation with different power was also investigated (Table 5, entries 3-6). It was clearly observed that the ultrasonic irradiation power of 70 W afforded the best yield (97 %) after 3 minutes (Table 5, entry 5).

Table 5. Effects of sonication

<table>
<thead>
<tr>
<th>Entry</th>
<th>Power (W)</th>
<th>Time (min)</th>
<th>Yield (%) b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Without sonication (r.t)</td>
<td>24 h</td>
<td>51</td>
</tr>
<tr>
<td>2</td>
<td>Without sonication (110 ℃)</td>
<td>24 h</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>15</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>10</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>6</td>
<td>80</td>
<td>12</td>
<td>90</td>
</tr>
</tbody>
</table>

b Isolated yields

Having established the framework of BTQ-DHP in a one-pot fashion, the reaction scope was profiled for other derivatives. The reaction proceeded smoothly with different
anilines. These compounds were characterised by FTIR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis (Appendix VI). A chemical library of fused benzylthioquinoline-1,4-dihydropyridines (Table 6) was obtained.

Table 6. Synthesis of BTQ-DHPs by I/BN under ultrasonication

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehyde</th>
<th>Amine</th>
<th>Product</th>
<th>Product</th>
<th>Time (min)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C$_2$H$_5$NOS</td>
<td>C$_2$H$_5$NO</td>
<td>C$<em>{33}$H$</em>{26}$N$_4$O$_5$S</td>
<td>22</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>2</td>
<td>C$_2$H$_5$NOS</td>
<td>C$_6$H$_5$FN</td>
<td>C$<em>{32}$H$</em>{25}$FN$_4$O$_3$S</td>
<td>23</td>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>C$_2$H$_5$NOS</td>
<td>C$_6$H$_5$N$_2$O$_2$</td>
<td>C$<em>{33}$H$</em>{26}$N$_4$O$_5$S</td>
<td>24</td>
<td>8</td>
<td>88</td>
</tr>
<tr>
<td>4</td>
<td>C$_2$H$_5$NOS</td>
<td>C$_6$H$_5$N</td>
<td>C$<em>{33}$H$</em>{26}$N$_4$O$_5$S</td>
<td>25</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>5</td>
<td>C$_2$H$_5$NOS</td>
<td>C$_6$H$_5$N</td>
<td>C$<em>{33}$H$</em>{26}$N$_4$O$_5$S</td>
<td>26</td>
<td>5</td>
<td>96</td>
</tr>
<tr>
<td>6</td>
<td>C$_2$H$_5$NOS</td>
<td>C$_6$H$_5$N</td>
<td>C$<em>{33}$H$</em>{26}$N$_4$O$_5$S</td>
<td>27</td>
<td>4</td>
<td>95</td>
</tr>
<tr>
<td>7</td>
<td>C$_2$H$_5$NOS</td>
<td>C$_6$H$_5$N$_2$O$_2$</td>
<td>C$<em>{33}$H$</em>{26}$N$_4$O$_5$S</td>
<td>28</td>
<td>5</td>
<td>89</td>
</tr>
<tr>
<td>8</td>
<td>C$_2$H$_5$NOS</td>
<td>C$_7$H$_9$NO</td>
<td>C$<em>{33}$H$</em>{26}$N$_4$O$_5$S</td>
<td>29</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>9</td>
<td>C$_2$H$_5$NOS</td>
<td>C$_6$H$_5$N$_2$</td>
<td>C$<em>{33}$H$</em>{30}$N$_4$O$_5$S</td>
<td>30</td>
<td>4</td>
<td>93</td>
</tr>
<tr>
<td>10</td>
<td>C$_2$H$_5$NOS</td>
<td>C$_6$H$_5$N$_2$</td>
<td>C$<em>{33}$H$</em>{26}$N$_4$O$_5$S</td>
<td>31</td>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td>11</td>
<td>C$_2$H$_5$NOS</td>
<td>C$_6$H$_5$N$_4$</td>
<td>C$<em>{33}$H$</em>{33}$N$_4$O$_5$S</td>
<td>32</td>
<td>6</td>
<td>90</td>
</tr>
<tr>
<td>12</td>
<td>C$_2$H$_5$NOS</td>
<td>C$_6$H$_5$Cl$_2$N</td>
<td>C$<em>{32}$H$</em>{32}$Cl$_2$N$_2$O$_5$S</td>
<td>33</td>
<td>8</td>
<td>88</td>
</tr>
<tr>
<td>13</td>
<td>C$_2$H$_5$NOS</td>
<td>C$_6$H$_5$BrN</td>
<td>C$<em>{32}$H$</em>{32}$BrN$_4$O$_5$S</td>
<td>34</td>
<td>8</td>
<td>87</td>
</tr>
<tr>
<td>14</td>
<td>C$_2$H$_5$FNOS</td>
<td>C$_4$H$_5$N</td>
<td>C$<em>{34}$H$</em>{34}$FN$_4$O$_5$S</td>
<td>35</td>
<td>6</td>
<td>92</td>
</tr>
</tbody>
</table>

* Isolated yields

It was determined that the presence of an electron donating group in the amine enabled a faster reaction with higher product yields. The presence of electron withdrawing groups slowed the reaction. Thus the one-pot multi-component synthesis of BTQ-DHPs (22-35) was successful.

A plausible mechanism is proposed for the formation of BTQ-DHPs (Scheme 3). The isoelectronic nature of boron nitride increased the electrophilicity of the carbonyl carbon of aldehyde groups (Ruifang *et al*., 2016). In the first transformation, aryl aldehyde 1 and malononitrile 2 forms a Knoevenagel condensed product 5a by removal of a water molecule. Thereafter, the catalyst promoted the formation of an intermediate 5b with a 1:1 interaction between $o$-anisidine 3 and dimethyl acetylene dicarboxylate 4. The intermediate 5b attacked the Knoevenagel adduct 5a obtained from the initial step and produced a 1,5-dipolar intermediate 6. The subsequently generated intermediate 7 was transformed into 8 and finally the corresponding product 9 was obtained presumably upon ring closure and a tautomerization processes.
Antibacterial resistance is a challenge that is associated with high morbidity and mortality (Wesgate et al., 2016). Multidrug resistance patterns in Gram-positive and Gram-negative bacteria are difficult to treat and may even be untreatable with conventional antibiotics. There is currently a shortage of effective therapies, lack of successful prevention measures, and only a few new antibiotics available, which require
development of treatment options and alternative antibacterial therapies (Laxminarayan et al., 2013). Dihydropyridines were involved in multidrug resistance and can present challenges for infection control.

Fig. 11. Antibacterial activity of BTQ-DHPs

1 = 22; 2 = 23; 3 = 24; 4 = 25; 5 = 26; 6 = 27; 7 = 28; 8 = 29; 9 = 30; 10 = 31.
In this present study, a total of 10 benzylthioquinolinyl dihydropyridines (BTQ-DHPs) were screened with pathogenic strains of Gram-positive and Gram-negative bacteria.

The antibacterial activity of benzylthioquinolinyl dihydropyridine derivatives was evaluated by determining the zone of inhibition against a range of bacteria (Bacillus cereus (B. cereus), Staphylococcus aureus (S. aureus), Escherichia coli (E. coli) and Enterococcus faecalis (E. faecalis)) (Fig. 11). The present investigation focused on Gram-positive and Gram-negative bacteria which showed that compounds 24, 27, 28, 29, 30 and 31 had preferential activity towards Gram-positive species (Fig. 11; Table 7). Moreover, compounds 28 and 30 showed activity against S. aureus only. Among BTQ-DHPs, compound 24 showed potential activity against E. faecalis. There were no potential activities obtained against E. coli (Fig. 11; Table 7). The zone of inhibition by BTQ-DHPs is mentioned in Table 7. BTQ-DHPs containing sulfur-based quinoline as the core structure with an amine and nitro group (24), methyl groups (27 and 30), nitro group (28), methoxy group (29) and pyridinyl group (31) showed potential against Gram-positive species.

Table 7. Antibacterial activity of BTQ-DHP derivatives

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zone of inhibition by benzylthioquinolinyl dihydropyridine (BTQ-DHP) derivatives</th>
<th>Ciprofloxacin (Positive control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td>Ciprofloxacin (Positive control)</td>
<td></td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>0 0 0 14 ± 0.4 0 15 ± 0.3 0 14 ± 0.3</td>
<td>27 ± 0.3</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0 0 0 12 ± 0.3 0 20 ± 0.3 19 ± 0.4 22 ± 0.4 10 ± 0.3</td>
<td>25 ± 0.4</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0 0 0 0 0 0 0 0 0</td>
<td>25 ± 0.3</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>0 0 12 ± 0.3 0 0 0 0 0 0 0 24 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

The potential activity of BTQ-DHPs against S. aureus might be due to the presence of a methyl group (27), methoxy group (29) and pyridinyl group with a benzylthioquinoline substrate (31). The presence of an amine and nitro group (24) containing benzylthioquinolinyl-1,4-dihydropyridine showed preferential activity towards E. faecalis.

Antioxidants are considered important nutraceuticals due to many health benefits (Valko et al., 2007). The antioxidant activity using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay was investigated. The scavenging assay was used for initial screening of
the compounds for their antioxidant activity. A total of 10 BTQ-DHPs were tested. Among them, BTQ-DHPs containing sulfur-based quinoline as core structure with a methoxy group (22), amine and nitro group (24), nitro group (28) and pyridinyl group (31) showed significant DPPH scavenging activities.

Brine shrimp is used for preliminary assessment of toxicity (Rajabi et al., 2015). The toxicity was assessed for all BTQPs at different time intervals (24 h and 48 h), which showed activity towards Gram-positive or Gram-negative species. We found that among all compounds tested, 22, 24, 27, 28 and 31 were less toxic, having mortality rates below 50% (Fig. 12), which suggested that these compounds are safe for further biological applications (Meyer et al., 1982). BTQ-DHPs containing sulfur-based quinoline as core structure with a methoxy group (22), amine and nitro group (24), methyl group (27), nitro group (28) and pyridinyl group (31) showed mortality rate below than 50%.

Molecular docking is one of the computational techniques used to identify the ligand-protein interaction. In this study, molecular docking was used for benzylthioquinolinyl-1,4-dihydropyridines to predict the affinity toward S. aureus gyrase. The recently reported crystal structure of S. aureus gyrase was employed to conduct molecular

![Graph](image)

**Fig. 12.** Toxicity of BTQ-DHPs was assessed using *Artemia salina* (brine shrimp)

1= 22; 2= 23; 3= 24; 4= 25; 5= 26; 6= 27; 7= 28; 8= 29; 9= 30; 10= 31.

Molecular docking is one of the computational techniques used to identify the ligand-protein interaction. In this study, molecular docking was used for benzylthioquinolinyl-1,4-dihydropyridines to predict the affinity toward S. aureus gyrase. The recently reported crystal structure of S. aureus gyrase was employed to conduct molecular
docking (PDB ID: 4PLB) (Singh et al., 2014). To perform molecular docking, Discovery Studio software was employed (Biovia, 2015). Initially, the ligands were constructed in Chem 3D Biodraw software and then minimized by MM2 method. The minimized 3D structure of the ligands was prepared for the ionization state, stereoisomers, conformation generation etc. in Discovery Studio software. Furthermore, the crystal structure of *S. aureus* gyrase was also prepared in Chimera software (Pettersen et al., 2004). During the preparation, the structure was fixed by adding missing hydrogen, removing water molecules etc. Then, molecular docking was conducted in Discovery Studio software using the Libdock module.

![Figure 13](image)

**Fig. 13.** Ligand-protein bonding interaction of 24 with *Staphylococcus aureus* gyrase enzyme.

The ligands were docked at 15Å sphere which was generated around the bound ligand of *S. aureus* gyrase. The results of molecular docking were analysed to estimate the ligand-protein interactions based on Libdock score.
Table 8. Molecular docking scores of BTQ-DHPs with *Staphylococcus aureus* gyrase

<table>
<thead>
<tr>
<th>Entry</th>
<th>Molecule</th>
<th>Absolute Energy</th>
<th>LibDock Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>132.43</td>
<td>123.58</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>115.564</td>
<td>120.31</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>117.96</td>
<td>125.27</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>121.08</td>
<td>116.75</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>126.74</td>
<td>108.90</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>124.30</td>
<td>106.04</td>
</tr>
<tr>
<td>7</td>
<td>28</td>
<td>130.25</td>
<td>83.20</td>
</tr>
<tr>
<td>8</td>
<td>29</td>
<td>119.45</td>
<td>108.7</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>118.99</td>
<td>118.07</td>
</tr>
<tr>
<td>10</td>
<td>31</td>
<td>112.467</td>
<td>110.52</td>
</tr>
<tr>
<td>11</td>
<td>Reference Ligand AM8191</td>
<td>80.31</td>
<td>193.142</td>
</tr>
</tbody>
</table>

The docked benzylthioquinolinyl-1,4-dihydropyridines have shown Libdock scores ranging from 83.20 to 125.27 kcal/mol. The molecule 24 showed more potency among the series toward *S. aureus* gyrase by forming a strong ligand-protein interaction with the Libdock score of 125.27 kcal/mol (Fig. 13). Molecular docking scores of benzylthioquinolinyl-1,4-dihydropyridines toward *S. aureus* gyrase is mentioned in Table 8.

6.4. Conclusion

In conclusion, a novel, clean and efficient procedure for the synthesis of benzylthioquinoline-1,4-dihydropyridines via a one-pot four-component condensation reaction was developed. The product yields were excellent when iodine-loaded boron nitride (I/BN) was used as a catalyst. The catalyst was characterized by XRD, SEM with EDX, TEM, TGA, DSC and FTIR analysis. A total of 14 novel BTQ-DHPs were synthesised and characterized by FTIR, ¹H-NMR, ¹³C-NMR and elemental analysis. 10 BTQ-DHP derivatives were evaluated for antibacterial and antioxidant activities: Compounds 24, 26, 27, 28, 29, 30 and 31 showed positive results for antibacterial activity and 22, 24, 28 and 31 showed positive results for antioxidant activity respectively. Since these BTQ-DHPs showed antibacterial and antioxidant activity and are also safe for biological studies, they could be further investigated for pharmaceutical applications. A series of benzylthioquinolinyl-1,4-dihydropyridines were investigated by the molecular docking approach to estimate their biological importance. Among them, 24 showed stronger potency toward *S. aureus* gyrase. The procedure established in the study has
several advantages including high efficiency, recyclable performance, short reaction times, high yields and using water as solvent, which has the potential application in green synthesis.

6.5. Experimental

Typical procedure for the preparation of iodine-loaded boron nitride

To a solution of iodine (0.10 g, 0.8 mmol), in THF (50 mL), boron nitride (2.66 g, 0.1 mol) was added and the suspension was stirred at room temperature for 3 days. The mixture was filtered and the resultant powder was washed with THF repeatedly and subsequently dried in furnace at 400°C. The iodine-loaded boron nitride I/BN was obtained as a white powder mass of 2.750 g, 98 % yield.

General procedure for the ultrasonicated synthesis of benzylthioquinoline-1,4-dihydropyridines

To a solution of 18 (1 mmol) and 19 (1 mmol) in water (15 mL), I/BN (10 mol %) was added and the reaction mixture was sonicated in an ultrasonic apparatus with 70 W power at room temperature for 1 minute. After the addition of 20 (1 mmol) and 21 (1 mmol), the reaction mixture was sonicated for 2 minutes. The progress of the reaction was monitored by TLC. Following completion, I/BN catalyst was isolated by simple filtration. The residue was purified by column chromatography (eluent: ethyl acetate: petroleum ether, 25 %) followed by EtOAc: H2O (2:1) separation. Thereafter, recrystallization of the product was performed at 5:1 EtOAc: MeOH to yield the pure desired product.

Bacterial strains

The antibacterial activity of each synthesized compound was assessed using four bacterial strains (Bacillus cereus, Staphylococcus aureus, Escherichia coli and Enterococcus faecalis) causing diseases. The bacterial strains were provided from the culture collection of Department of Biotechnology and Food Technology, Durban University of Technology, South Africa.
**Inoculum preparation**

Each bacterial strain was sub-cultured overnight at 37 °C on a Mueller-Hilton agar plate. Further, bacterial cultures were grown in Mueller-Hilton broth at 37 °C, 200 rpm in order to attain the viable count of approximately 10^8 cfu/mL.

**Antibacterial activity**

The agar well diffusion method was used to evaluate the antibacterial activity of each compound. Hundred microliter of ~10^8 cfu/mL bacterial suspension was plated on Mueller-Hinton Agar plates. A well of 6 mm diameter was made using a sterile cork borer and 30 μL of each compound (3 mg/ml) was added in each well and kept at 37 °C for 16 h. The assays were carried out in triplicate. Ciprofloxacin (3 mg/ml) was used as positive control and DMSO (100%) as a negative control.

**Antioxidant assays of BTQ-DHP derivatives**

The antioxidant activity of BTQ-DHPs were determined by the decolourization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH). Hundred microliter of each compound was added separately to 1 mL of 0.1 mM DPPH solution, and the colour change was observed at regular intervals. Rutin hydrate was used as the positive control and methanol (95%) as negative control.

**Toxicity assessment**

The brine shrimp larvae (*Artemia salina*) were hatched in sea water for 24-48 h prior to being used. An aliquot of 5 mL sea water containing ten brine shrimp was added to each vial and supplemented with different derivatives of BTQ-DHP. Derivative solution volumes of 300 μg were used in individual vials. Brine shrimp death was observed at regular intervals (24 h and 48 h) in order to determine the toxic nature of each compound.
References


[16] (a) Xavier, J. F. 1958. 2-Mercaptoquinoline (thiocarbostyril) as an analytical reagent for copper and palladium. Fresenius’ Zeitschrift für analytische Chemie,


[34] Debache, A., Ghalem, W., Boulcina, R., Belfaitah, A., Rhouati, S., Carboni, B. 2009. An efficient one-step synthesis of 1, 4-dihydropyridines via a


6.1. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(2-methoxyphenyl)-1,4-dihydropyridine-2,3-dicarboxylate (22)

Pale yellow solid, m.p = 201-203°C; IR νmax (cm⁻¹): 2187 C≡N, 1614 C≡N, 1277 C-N, 2918 CH, 1652 C=C, 703 C-S, 1736 C=O, 2890 OCH₃, 3308 NH. ¹H-NMR: (400 MHz, CDCl₃) δ (ppm) 8.19 (1H, s, Ar-H), 8.03 (1H, d, J=7.76 Hz, Ar-H), 7.94 (2H, t, J=8.44 Hz, Ar-H), 7.81 (1H, d, J=8.04 Hz, Ar-H), 7.73 (2H, d, J=7.04 Hz, Ar-H), 7.54 (3H, t, J=7.6 Hz, Ar-H), 7.28 (3H, t, J=7.56 Hz, Ar-H), 7.05 (1H, d, J=7.6 Hz, Ar-H), 5.58 (2H, brs, NH₂), 5.03 (1H, s, CH), 4.73 (1H, t, J=13.56 Hz, CH₂), 4.57 (1H, t, J=13.6 Hz, CH₂), 7.54 (3H, t, J=7.6 Hz, Ar-H), 7.28 (3H, t, J=7.56 Hz, Ar-H), 7.05 (1H, d, J=7.6 Hz, Ar-H), 5.58 (2H, brs, NH₂), 5.03 (1H, s, CH), 4.73 (1H, t, J=13.56 Hz, CH₂), 4.57 (1H, t, J=13.6 Hz, CH₂), 7.54 (3H, t, J=7.6 Hz, Ar-H), 7.28 (3H, t, J=7.56 Hz, Ar-H), 7.05 (1H, d, J=7.6 Hz, Ar-H), 5.58 (2H, brs, NH₂), 5.03 (1H, s, CH), 4.73 (1H, t, J=13.56 Hz, CH₂), 4.57 (1H, t, J=13.6 Hz, CH₂). ¹C-NMR: (100 MHz, CDCl₃) δ (ppm) 164.88, 164.68, 162.73, 157.13, 156.80, 151.30, 146.26, 139.73, 138.32, 135.15, 134.67, 131.99, 131.70, 129.88, 129.14, 128.21, 127.37, 127.22, 126.81, 126.02, 125.84, 123.20, 120.68, 112.83, 103.57, 59.12, 56.47, 52.29, 51.63, 45.75, 45.75, 33.76. Elemental Analysis: Anal. Calc. for C₃₃H₂₈N₄O₅S: C, 66.88; H, 4.76; N, 9.45 %. Found: C, 66.89; H, 4.78; N, 9.47 %.

6.2. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(3-fluorophenyl)-1,4-dihydropyridine-2,3-dicarboxylate (23)

Brown solid, m.p = 213-215°C; IR νmax (cm⁻¹): 2163 C≡N, 1219 C≡N, 1360 C-N, 2970 CH, 1614 C=C, 705 C-S, 1738 C=O, 2920 OCH₃, 1091 C-F, 3448 NH. ¹H-NMR: (400 MHz, DMSO-d₆) δ (ppm) 8.91 (1H, s, Ar-H), 8.31 (2H, d, J= 7.68 Hz, Ar-H), 8.10 (1H, d, J= 7.32 Hz, Ar-H), 8.00 (1H, d, J= 8.32 Hz, Ar-H), 7.91 (1H, td, J= 1.36 Hz, Ar-H), 7.61 (1H, td, J= 7.04 Hz, Ar-H), 7.49 (2H, d, J= 7.16 Hz, Ar-H), 7.42 (2H, s, NH₂), 7.28 (2H, t, J= 7.32 Hz, Ar-H), 7.19 (1H, t, J= 4.20 Hz, Ar-H), 6.68 (2H, d, J=7.08 Hz, Ar-H), 5.89 (1H, s, CH), 4.54 (2H, s, CH₂), 4.31 (3H, s, OCH₃), 4.19 (3H, s, OCH₃). ¹C-NMR: (100 MHz, CDCl₃) δ (ppm) 159.89, 159.85, 153.12, 150.74, 145.87, 136.85, 136.79,
6.3. Dimethyl 6-amino-1-(2-amino-4-nitrophenyl)-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1,4-dihydropyridine-2,3-dicarboxylate (24)

Dark yellow, m.p = 231-233°C ; IR ν_max (cm⁻¹): 2155 C≡N, 1642 C=N, 1232 C-N, 3092 CH, 1670 C=C, 659 C-S, 1747 C=O, 2949 OCH₃, 3373 NH. \(^1\)H-NMR: (400 MHz, DMSO-d₆) δ (ppm) 11.06 (2H, brs, NH₂), 9.39 (2H, d, J= 7.32 Hz, Ar-H), 9.05 (1H, d, J= 7.04 Hz, Ar-H), 8.90 (1H, d, J= 7.08 Hz, Ar-H), 8.50 (1H, s, Ar-H), 8.09 (1H, s, Ar-H), 7.97 (1H, dd, J= 7.68 Hz, Ar-H), 7.85 (2H, s, NH₂), 7.54 (1H, t, J= 6.76 Hz, Ar-H), 7.24 (1H, s, Ar-H), 7.14 (1H, d, J= 8.44 Hz, Ar-H), 6.78 (2H, dd, J= 6.44 Hz, Ar-H), 6.48 (1H, t, J= 7.00 Hz, Ar-H), 5.57 (1H, s, CH), 4.63 (2H, s, CH₂), 3.69 (3H, s, OCH₃), 2.49 (3H, s, OCH₃). \(^{13}\)C-NMR: (100 MHz, CDCl₃) δ (ppm) 159.88, 159.84, 145.96, 145.94, 144.53, 144.39, 136.74, 136.68, 129.71, 129.11, 127.70, 126.87, 125.16, 125.13, 124.32, 123.21, 120.66, 120.64, 114.76, 77.36, 76.72, 63.60, 63.53, 63.40, 63.32, 53.91, 49.68, 48.15, 16.48, 16.43, 16.16, 16.11. Elemental Analysis: Anal. Calc. for C₃₂H₂₆N₆O₆S: C, 61.73; H, 4.21; N, 13.50; %. Found: C, 61.53; H, 4.23; N, 13.52; %.

6.4. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-phenyl-1,4-dihydropyridine-2,3-dicarboxylate (25)

Brown solid, m.p = 220-222°C ; IR ν_max (cm⁻¹): 2155 C≡N, 1585 C≡N, 1329 C-N, 2970 CH, 1615 C=C, 657 C-S, 1738 C=O, 2790 OCH₃, 3463 NH. \(^1\)H-NMR: (400 MHz, CDCl₃) δ (ppm) 8.39 (1H, s, Ar-H), 7.87 (1H, t, J= 8.24 Hz, Ar-H), 7.67 (1H, d, J= 8.08 Hz, Ar-H), 7.46 (1H, d, J= 6.80 Hz, Ar-H), 7.36 (2H, t, J= 6.44 Hz, Ar-H), 7.29 (1H, t, J= 7.80 Hz, Ar-H), 7.22 (1H, d, J= 2.04 Hz, Ar-H), 7.19 (2H, d, J= 7.72 Hz, Ar-H), 7.16
(1H, d, J= 8.32 Hz, Ar-H), 7.13 (1H, d, J= 7.04 Hz, Ar-H), 6.99 (1H, t, J= 7.44 Hz, Ar-H), 6.80 (2H, d, J= 7.76 Hz, Ar-H), 6.63 (2H, s, NH2), 5.30 (1H, s, CH), 4.23 (2H, s, CH2), 3.64 (3H, s, OCH3), 3.59 (3H, s, OCH3).

13C-NMR: (100 MHz, CDCl3) δ (ppm) 150.21, 150.15, 147.00, 146.98, 145.30, 145.16, 139.30, 137.83, 137.78, 130.78, 129.29, 129.12, 129.11, 128.06, 127.90, 127.32, 127.28, 119.92, 114.47, 110.60, 63.99, 63.92, 63.57, 63.49, 52.58, 51.06, 21.55, 16.49, 16.44, 16.14, 16.08. Elemental Analysis: Anal. Calc. for C32H26N4O4S: C, 68.31; H, 4.66; N, 9.96; %. Found: C, 68.33; H, 4.67; N, 9.98; %.

6.5. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(m-tolyl)-1,4-dihydropyridine-2,3-dicarboxylate (26)

Brown solid, m.p = 230-232°C ; IR νmax (cm⁻¹): 2187 C≡N, 1581 C=N, 1248 C-N, 2980 CH, 1738 C=C, 619 C-S, 1687 C=O, 2804 OCH3, 3318 NH. 1H-NMR: (400 MHz, CDCl3) δ (ppm) 8.61 (1H, d, J= 8.32 Hz, Ar-H), 8.43 (1H, d, J= 6.44 Hz, Ar-H), 8.21 (1H, s, Ar-H), 7.86 (1H, d, J= 8.20 Hz, Ar-H), 7.65 (2H, d, J= 7.56 Hz, Ar-H), 7.58 (1H, t, J= 8.28 Hz, Ar-H), 7.38 (2H, d, J= 7.08 Hz, Ar-H), 7.16 (3H, t, J= 5.40 Hz, Ar-H), 7.11 (1H, t, J= 7.00 Hz, Ar-H), 6.99 (1H, d, J= 6.24 Hz, Ar-H), 6.60 (2H, s, NH2), 5.13 (1H, s, CH), 4.56 (2H, s, CH2), 3.99 (3H, s, OCH3), 3.56 (3H, s, OCH3), 2.46 (3H, s, CH3). 13C-NMR: (100 MHz, CDCl3) δ (ppm) 169.73, 164.64, 150.63, 150.39, 148.30, 147.73, 137.29, 136.26, 129.32, 129.23, 129.17, 128.79, 128.56, 128.40, 127.35, 124.59, 124.47, 124.35, 120.98, 120.56, 120.47, 120.43, 119.90, 118.17, 114.90, 92.37, 53.34, 52.70, 52.56, 52.34, 51.70, 14.28, 13.61. Elemental Analysis: Anal. Calc. for C33H28N4O4S: C, 68.73; H, 4.89; N, 9.72; %. Found: C, 68.74; H, 4.90; N, 9.71; %.
6.6. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(p-tolyl)-1,4-dihydropyridine-2,3-dicarboxylate (27)

Pale yellow solid, m.p = 237-239°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 2132 C≡N, 1589 C≡N, 1217 C-N, 2970 CH, 1625 C=C, 572 C=S, 1738 C=O, 2948 OCH$_3$, 3416 NH. $^1$H-NMR: (400 MHz, DMSO-d$_6$) $\delta$ (ppm) 7.95 (1H, s, Ar-H), 7.66 (2H, d, J = 7.76 Hz, Ar-H), 7.48 (2H, t, J = 4.08 Hz, Ar-H), 7.31 (2H, d, J = 8.24 Hz, Ar-H), 7.13 (2H, t, J = 7.52 Hz, Ar-H), 6.84-6.80 (2H, m, J = 8.12 Hz, Ar-H), 6.59 (2H, td, J = 6.28 Hz, Ar-H), 6.44 (1H, dd, J = 6.60 Hz, Ar-H), 5.91 (2H, s, NH$_2$), 5.24 (1H, s, CH), 4.14-4.07 (2H, m, J = 7.20 Hz, CH$_2$), 3.83 (3H, s, OCH$_3$), 3.74 (3H, s, OCH$_3$), 2.40 (3H, s, CH$_3$). $^{13}$C-NMR: (100 MHz, CDCl$_3$) $\delta$ (ppm) 195.80, 195.77, 161.31, 160.72, 160.30, 160.24, 159.29, 159.24, 146.56, 145.93, 145.80, 136.83, 136.60, 129.96, 129.86, 128.54, 126.81, 124.58, 122.17, 119.74, 119.70, 119.63, 115.87, 113.84, 113.60, 53.50, 53.24, 49.93, 40.09, 39.88, 39.81, 31.77, 31.64, 28.57, 28.52, 26.39, 26.31. Elemental Analysis: Anal. Calc. for C$_{33}$H$_{28}$N$_4$O$_4$S: C, 68.73; H, 4.89; N, 9.72; %. Found: C, 68.75; H, 4.90; N, 9.73; %.

6.7. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(2-nitrophenyl)-1,4-dihydropyridine-2,3-dicarboxylate (28)

Yellow solid, m.p = 194-196°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 2163 C≡N, 1556 C≡N, 1224 C-N, 2979 CH, 1590 C=C, 701 C-S, 1739 C=O, 2832 OCH$_3$, 3308 NH. $^1$H-NMR: (400 MHz, DMSO-d$_6$) $\delta$ (ppm) 8.09 (1H, s, Ar-H), 7.57 (1H, d, J = 7.76 Hz, Ar-H), 7.47 (1H, t, J = 7.68 Hz, Ar-H), 7.31 (2H, d, J = 8.24 Hz, Ar-H), 7.15 (2H, t, J = 7.56 Hz, Ar-H), 7.03 (3H, t, J = 7.48 Hz, Ar-H), 6.70 (2H, d, J = 7.88 Hz, Ar-H), 6.58 (2H, t, J = 6.36 Hz, Ar-H), 6.31 (2H, brs, NH$_2$), 5.32 (1H, s, CH), 4.14-4.07 (2H, m, J = 7.08 Hz, CH$_2$), 3.89 (3H, s, OCH$_3$), 3.19 (3H, s, OCH$_3$). $^{13}$C-NMR: (100 MHz, DMSO-d$_6$) $\delta$ (ppm) 176.01, 170.29, 144.93, 142.85, 139.04, 136.54, 135.73, 133.99, 131.00,
6.8. Dimethyl 6-amino-4-(2-benzylthio)quinolin-3-yl)-5-cyano-1-(4-methoxy phenyl)-1,4-dihydropyridine-2,3-dicarboxylate (29)

Brown solid, m.p = 220-222°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 2253 C≡N, 1588 C≡N, 1092 C≡N, 2970 CH, 1614 C=C, 702 C=S, 1709 C=O, 2918 OCH$_3$, 3363 NH. $^1$H-NMR: (400 MHz, CDCl$_3$) $\delta$ (ppm) 8.15 (1H, s, Ar-H), 7.83 (1H, d, J = 8.36 Hz, Ar-H), 7.63 (2H, d, J = 8.04 Hz, Ar-H), 7.57 (2H, t, J = 7.52 Hz, Ar-H), 7.31 (2H, t, J = 7.48 Hz, Ar-H), 6.82 (2H, t, J = 8.80 Hz, Ar-H), 6.66 (2H, dd, J = 2.76 Hz, Ar-H), 6.43 (2H, dt, J = 3.20 Hz, Ar-H), 6.19 (2H, s, NH$_2$), 5.28 (1H, s, CH), 4.19 (3H, s, OCH$_3$), 3.89 (3H, s, OCH$_3$), 3.75-3.65 (2H, m, J = 7.20 Hz, CH$_2$), 3.48 (3H, s, OCH$_3$). $^{13}$C-NMR: (100 MHz, CDCl$_3$) $\delta$ (ppm) 160.01, 145.90, 145.75, 139.04, 136.84, 136.78, 129.59, 129.13, 127.76, 126.74, 125.26, 125.23, 124.22, 121.36, 121.34, 119.50, 114.54, 110.55, 63.55, 63.49, 63.25, 63.18, 63.12, 53.95, 49.45, 47.93, 21.57, 16.50, 16.44, 16.18, 16.12. Elemental Analysis: Anal. Calc. for C$_{32}$H$_{25}$N$_5$O$_6$S: C, 63.25; H, 4.15; N, 11.53%; Found: C, 63.27; H, 4.17; N, 11.54%. %.

6.9. Dimethyl 6-amino-4-(2-benzylthio)quinolin-3-yl)-5-cyano-1-(2,4-dimethyl phenyl)-1,4-dihydropyridine-2,3-dicarboxylate (30)

Yellow solid, m.p = 232-234°C; IR $\nu_{\text{max}}$ (cm$^{-1}$): 2172 C≡N, 1603 C≡N, 1216 C≡N, 2951 CH, 1666 C=C, 679 C=S, 1740 C=O, 2840 OCH$_3$, 3017 NH. $^1$H-NMR: (400 MHz, DMSO-d$_6$) $\delta$ (ppm) 8.36 (2H, s, NH$_2$), 7.53 (1H, s, Ar-H), 7.25 (3H, t, J = 8.32 Hz, Ar-H), 7.00 (2H, q, J = 8.04 Hz, Ar-H), 6.38 (1H, dd, 1.28 Hz, Ar-H), 6.36 (1H, dd, J = 0.76 Hz, Ar-H), 6.33 (1H, t, J = 2.32 Hz, Ar-H), 6.30 (2H, t, J = 2.20 Hz, Ar-H), 6.25 (1H, d, J = 2.48 Hz, Ar-H), 6.23 (1H, d, J = 2.48 Hz, Ar-H), 5.17 (1H, s, CH), 3.99-3.87 (2H, m, J = 6.32 Hz, CH$_2$), 3.69 (3H, s, OCH$_3$), 2.50 (3H, s, OCH$_3$), 1.21 (3H, t, J = 5.16 Hz, CH$_3$),
1.20 (3H, t, J = 1.47 Hz, CH₃). ¹³C-NMR: (100 MHz, DMSO-d₆) δ (ppm) 164.44, 162.07, 150.42, 150.32, 138.37, 130.36, 130.22, 130.12, 129.33, 129.16, 129.04, 128.36, 128.23, 127.74, 127.26, 126.92, 110.09, 102.00, 101.79, 100.30, 100.06, 63.08, 60.08, 60.03, 55.99, 40.05, 33.75, 18.49, 16.18, 16.11, 15.86. Elemental Analysis: Anal. Calc. for C₃₄H₃₀N₄O₄S: C, 69.13; H, 5.12; N, 9.49; %. Found: C, 69.15; H, 5.14; N, 9.48; %.

6.10. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(pyridin-2-ylmethyl)-1,4-dihydropyridine-2,3-dicarboxylate (31)

Yellow solid, m.p = 198-200°C ; IR νmax (cm⁻¹): 2252 C≡N, 1615 C≡N, 1229 C=N, 2970 CH, 1615 C=C, 708 C=S, 1738 C=O, 2815 OCH₃, 3305 NH. ¹H-NMR: (400 MHz, CDCl₃) δ (ppm) 8.10 (1H, s, Ar-H), 7.88 (2H, d, J= 7.36 Hz, Ar-H), 7.69 (2H, d, J= 7.96 Hz, Ar-H), 7.53 (2H, t, J= 7.40 Hz, Ar-H), 7.40 (3H, t, J= 6.36 Hz, Ar-H), 7.18 (1H, t, J= 7.76 Hz, Ar-H), 7.10 (1H, t, J= 7.04 Hz, Ar-H), 6.97 (1H, t, J= 7.48 Hz, Ar-H), 6.53 (1H, d, J= 7.64 Hz, Ar-H), 6.24 (2H, s, NH₂), 4.96 (1H, s, CH), 4.63 (2H, s, CH₂), 4.22-4.08 (2H, m, J= 5.64 Hz, CH₂), 3.87 (3H, s, OCH₃), 3.73 (3H, s, OCH₃). ¹³C-NMR: (100 MHz, CDCl₃) δ (ppm) 160.07, 160.02, 147.37, 145.93, 145.91, 136.75, 136.70, 135.82, 135.68, 129.50, 127.76, 126.79, 125.32, 125.29, 124.13, 121.39, 121.37, 121.08, 117.82, 110.83, 109.64, 63.55, 63.49, 63.17, 63.10, 55.50, 53.86, 49.29, 47.76, 16.48, 16.43, 16.21, 16.15. Elemental Analysis: Anal. Calc. for C₃₂H₂₇N₅O₄S: C, 66.54; H, 4.71; N, 12.12; %. Found: C, 66.55; H, 4.72; N, 12.14; %.

6.11. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-((2-(piperazin-1-yl)ethyl)amino)-1,4-dihydropyridine-2,3-dicarboxylate (32)

Brown solid, m.p = 218-220°C ; IR νmax (cm⁻¹): 2200 C≡N, 1574 C≡N, 1217 C=N, 2970 CH, 1666 C=C, 695 C=S, 1756 C=O, 2825 OCH₃, 3288 NH. ¹H-NMR: (400 MHz, CDCl₃) δ (ppm) 8.17 (2H, brs, NH₂), 7.94 (1H, d, J= 8.32 Hz, Ar-H), 7.75 (1H, s, Ar-H),
7.66 (3H, td, J = 8.56 Hz, Ar-H), 7.40 (2H, t, J = 7.16 Hz, Ar-H), 7.19 (2H, t, J = 6.92 Hz, Ar-H), 6.61 (1H, s, Ar-H), 5.30 (2H, s, CH₂), 5.06 (1H, s, CH), 4.68 (1H, s, NH), 3.82 (2H, s, CH₂), 3.79 (2H, s, CH₂), 3.72 (2H, s, CH₂), 3.62 (3H, s, OCH₃), 5.38 (2H, s, CH₂), 3.54 (3H, s, OCH₃), 3.39-3.34 (2H, m, J = 5.92 Hz, CH₂), 3.11-3.09 (2H, m, J = 4.96 Hz, CH₂), 2.41 (1H, s, NH).

13C-NMR: (100 MHz, CDCl₃) δ (ppm) 152.34, 150.84, 147.92, 147.85, 145.68, 137.63, 137.53, 135.64, 130.65, 129.89, 129.29, 128.99, 128.53, 128.46, 128.29, 127.95, 127.91, 127.64, 127.45, 127.25, 127.18, 126.00, 125.89, 125.77, 52.38, 51.84, 50.93, 50.76, 47.09, 46.67, 43.57, 41.51, 37.20, 23.39, 21.42. Elemental Analysis: Anal. Calc. for C₃₂H₃₅N₇O₄S: C, 62.62; H, 5.75; N, 15.98; %. Found: C, 62.64; H, 5.77; N, 15.99; %.

6.12. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(3,4-dichlorophenyl)-1,4-dihydropyridine-2,3-dicarboxylate (33)

Dark yellow solid, m.p = 211-213°C; IR νmax (cm⁻¹): 2280 C≡N, 1613 C=N, 1232 C-N, 2917 CH, 1672 C=C, 705 C=S, 1737 C=O, 2829 OCH₃, 789 C-Cl, 1737 C=O, 2829 OCH₃, 789 C-Cl, 1737 C=O, 2829 OCH₃, 789 C-Cl.

1H-NMR: (400 MHz, CDCl₃) δ (ppm) 8.59 (2H, brs, NH₂), 8.30 (1H, s, Ar-H), 8.09 (1H, d, J = 8.48 Hz, Ar-H), 7.74 (1H, t, J = 8.12 Hz, Ar-H), 7.70 (1H, d, J = 7.64 Hz, Ar-H), 7.49 (2H, d, J = 7.16 Hz, Ar-H), 7.45 (1H, t, J = 7.56 Hz, Ar-H), 7.31 (1H, d, J = 3.46 Hz, Ar-H), 7.27 (1H, t, J = 4.44 Hz, Ar-H), 7.24 (1H, d, J = 7.40 Hz, Ar-H), 6.59 (1H, d, J = 8.96 Hz, Ar-H), 6.53 (1H, d, J = 8.96 Hz, Ar-H), 6.21 (1H, s, Ar-H), 5.25 (1H, s, CH), 4.28-4.21 (2H, m, J = 7.20 Hz, CH₂), 3.91 (3H, s, OCH₃), 3.65 (3H, s, OCH₃). 13C-NMR: (100 MHz, CDCl₃) δ (ppm) 197.13, 170.30, 153.65, 147.10, 144.88, 139.77, 137.06, 134.20, 127.85, 127.63, 127.22, 126.78, 124.47, 123.97, 119.07, 111.93, 110.90, 106.56, 34.51, 32.34, 32.11, 31.42, 30.90, 30.18, 29.68, 29.45, 29.39, 28.34, 28.21, 28.10, 27.03. Elemental Analysis: Anal. Calc. for C₃₂H₂₄Cl₂N₄O₄S: C, 60.86; H, 3.83; N, 8.87; %. Found: C, 60.88; H, 3.84; N, 8.89; %.
6.13. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-1-(2-bromophenyl)-5-cyano-1,4-dihydropyridine-2,3-dicarboxylate (34)

Brown solid, m.p = 203-205°C ; IR $\nu_{\text{max}}$ (cm$^{-1}$): 2157 C≡N, 1567 C≡N, 1233 C-N, 2985 CH, 1614 C=C, 704 C-S, 1729 C=O, 2817 OCH$_3$, 572 C-Br, 3385 NH. $^1$H-NMR: (400 MHz, CDCl$_3$) $\delta$ (ppm) 8.15 (1H, d, J= 6.32 Hz, Ar-H), 8.00 (1H, s, Ar-H), 7.84 (2H, d, J= 8.40 Hz, Ar-H), 7.65 (2H, brs, NH$_2$), 7.56 (2H, t, J= 7.20 Hz, Ar-H), 7.31 (2H, t, J= 7.08 Hz, Ar-H). 13C-NMR: (100 MHz, CDCl$_3$) $\delta$ (ppm) 159.89, 159.84, 145.73, 142.96, 142.82, 136.88, 136.82, 132.54, 129.80, 128.48, 127.81, 126.67, 125.16, 125.14, 124.39, 120.66, 120.63, 119.19, 112.34, 110.51, 63.74, 63.68, 63.38, 63.31, 54.19, 49.93, 48.40, 16.52, 16.46, 16.26, 16.20. Elemental Analysis: Anal. Calc. for C$_{32}$H$_{25}$BrN$_4$O$_4$S: C, 59.91; H, 3.93; N, 8.73 %. Found: C, 59.93; H, 3.95; N, 8.74 %.

6.14. Dimethyl 6-amino-4-(2-(benzylthio)-6-fluoroquinolin-3-yl)-5-cyano-1-(2,4-dimethylphenyl)-1,4-dihydropyridine-2,3-dicarboxylate (35)

Yellow solid, m.p = 240-242°C ; IR $\nu_{\text{max}}$ (cm$^{-1}$): 2292 C≡N, 1610 C≡N, 1218 C-N, 2918 CH, 1671 C=C, 684 C-S, 1738 C=O, 2851 OCH$_3$, 1360 C-F, 3351 NH. $^1$H-NMR: (400 MHz, CDCl$_3$) $\delta$ (ppm) 9.51 (2H, s, NH$_2$), 7.86 (1H, s, Ar-H), 7.74 (1H, t, J= 8.00 Hz, Ar-H), 7.48 (2H, t, J= 7.00 Hz, Ar-H), 7.30 (1H, t, J= 7.04 Hz, Ar-H), 6.89 (2H, d, J= 8.80 Hz, Ar-H), 6.67 (2H, d, J= 8.00 Hz, Ar-H), 6.65 (2H, d, J= 7.32 Hz, Ar-H), 6.41 (1H, s, Ar-H), 5.34 (1H, s, CH), 4.71-4.65 (2H, m, J= 6.92 Hz, CH$_2$), 3.73 (3H, s, OCH$_3$), 3.65 (3H, s, OCH$_3$), 2.43 (3H, t, J= 3.42 Hz, CH$_3$), 2.26 (3H, t, J= 2.08 Hz, CH$_3$). 13C-NMR: (100 MHz, CDCl$_3$) $\delta$ (ppm) 149.34, 147.88, 137.74, 136.35, 136.29, 134.55, 133.39, 132.47, 131.45, 131.38, 131.34, 131.21, 131.17, 130.56, 130.37, 129.34, 128.44, 127.89, 127.82, 127.79, 127.31, 127.12,
126.98, 126.89, 126.81, 125.81, 125.55, 91.68, 34.98, 20.97, 20.79, 20.48, 17.95, 17.76, 17.26. 19F-NMR: (400 MHz, CDCl3) δ (ppm) -118.37. Elemental Analysis: Anal. Calc. for C34H29FN4O4S: C, 67.09; H, 4.80; N, 9.20; %. Found: C, 67.08; H, 4.82; N, 9.21; %. 
6.1. Dimethyl 6- amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(2-methoxyphenyl)-1,4-dihydropyridine-2,3-dicarboxylate (22)
Fig. 6.3. $^{13}$C-NMR spectrum of 22

Fig. 6.4. HSQC spectrum of 22
Fig. 6.5. HMBC spectrum of 22

6.2. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(3-fluorophenyl)-1,4-dihydropyridine-2,3-dicarboxylate (23)

Fig. 6.6. IR spectrum of 23
Fig. 6.7. $^1$H-NMR spectrum of 23

Fig. 6.8. $^{13}$C-NMR spectrum of 23
6.3. Dimethyl 6-amino-1-(2-amino-4-nitrophenyl)-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1,4-dihydropyridine-2,3-dicarboxylate (24)

Fig. 6.9. IR spectrum of 24

Fig. 6.10. $^1$H-NMR spectrum of 24
6.4. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-phenyl-1,4-dihydropyridine-2,3-dicarboxylate (25)

Fig. 6.11. $^{13}$C-NMR spectrum of 24

Fig. 6.12. IR spectrum of 25
Fig. 6.13. $^1$H-NMR spectrum of 25

Fig. 6.14. $^{13}$C-NMR spectrum of 25
6.5. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(m-tolyl)-1,4-dihydropyridine-2,3-dicarboxylate (26)

Fig. 6.15. IR spectrum of 26

Fig. 6.16. $^1$H-NMR spectrum of 26
6.6. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(p-tolyl)-1,4-dihydropyridine-2,3-dicarboxylate (27)
Fig. 6.19. $^1$H-NMR spectrum of 27

Fig. 6.20. $^{13}$C-NMR spectrum of 27
6.7. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(2-nitrophenyl)-1,4-dihydropyridine-2,3-dicarboxylate (28)

Fig. 6.21. IR spectrum of 28

Fig. 6.22. $^1$H-NMR spectrum of 28
Fig. 6.23. $^{13}$C-NMR spectrum of 28

6.8. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(4-methoxyphenyl)-1,4-dihydropyridine-2,3-dicarboxylate (29)

Fig. 6.24. IR spectrum of 29
Fig. 6.25. $^1$H-NMR spectrum of 29

Fig. 6.26. $^{13}$C-NMR spectrum of 29
6.9. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(2,4-
dimethylphenyl)-1,4-dihydropyridine-2,3-dicarboxylate (30)

Fig. 6.27. IR spectrum of 30

![IR Spectrum](image1.png)

Fig. 6.28. $^1$H-NMR spectrum of 30

![$^1$H-NMR Spectrum](image2.png)
6.10. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(pyridin-2-ylmethyl)-1,4-dihydropyridine-2,3-dicarboxylate (31)

Fig. 6.29. $^{13}\text{C}$-NMR spectrum of 30

Fig. 6.30. IR spectrum of 31
Fig. 6.31. $^1$H-NMR spectrum of 31

Fig. 6.32. $^{13}$C-NMR spectrum of 31
6.11. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-((2-(piperazin-1-yl)ethyl)amino)-1,4-dihydropyridine-2,3-dicarboxylate (32)

Fig. 6.33. IR spectrum of 32

Fig. 6.34. $^1$H-NMR spectrum of 32
Fig. 6.35. $^{13}$C-NMR spectrum of 32

6.12. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-5-cyano-1-(3,4-dichlorophenyl)-1,4-dihydropyridine-2,3-dicarboxylate (33)

Fig. 6.36. IR spectrum of 33
Fig. 6.37. $^1$H-NMR spectrum of 33

Fig. 6.38. $^{13}$C-NMR spectrum of 33
6.13. Dimethyl 6-amino-4-(2-(benzylthio)quinolin-3-yl)-1-(2-bromophenyl)-5-cyano-1,4-dihydropyridine-2,3-dicarboxylate (34)

**Fig. 6.39.** IR spectrum of 34

**Fig. 6.40.** $^1$H-NMR spectrum of 34
Fig. 6.41. $^{13}$C-NMR spectrum of 34

6.14. Dimethyl 6-amino-4-(2-(benzylthio)-6-fluoroquinolin-3-yl)-5-cyano-1-(2,4-dimethylphenyl)-1,4-dihydropyridine-2,3-dicarboxylate (35)

Fig. 6.42. IR spectrum of 35
Fig. 6.43. $^1$H-NMR spectrum of 35

Fig. 6.44. $^{13}$C-NMR spectrum of 35
Fig. 6.45. $^{19}$F-NMR spectrum of 35
Chapter-VII

Calcium loaded boron nitride material as a new catalyst for the synthesis of 2-amino-4H-pyran-3-carbonitriles and anti-bacterial studies
Chapter Seven

Calcium loaded boron nitride material as a new catalyst for the synthesis of 2-amino-4H-pyran-3-carbonitriles and antibacterial studies

7.1. Abstract

A new calcium-loaded boron nitride catalyst (Ca/BN) was prepared and characterized fully by XRD, SEM with EDX, Raman spectroscopy, BET, DSC-TGA and FTIR. Then Ca/BN was used in a one-pot multi-component reaction to synthesize 2-amino-4H-pyran-3-carbonitrile derivatives (APCs): high yields were obtained. This transformation transpired via a Knoevenagel condensation, Michael addition and intra-molecular cyclization. The APCs were characterised by FTIR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis. Furthermore, Ca/BN was easily separated from the reaction mixture and re-used more than five times with only 10% loss of activity. A total of five APCs were subjected to antibacterial studies against Staphylococcus aureus (S. aureus), Escherichia coli (E. coli) and Pseudomonas aeruginosa (P. aeruginosa). The minimal inhibitory concentration (MIC) value for the synthesized compounds were obtained. Among them, 29 showed MIC values of 128, 16 and 4 µg/mL towards E.coli, P. aeruginosa and S. aureus, respectively. The methodology used for the synthesis of APCs offers several advantages such as excellent product yields, use of inexpensive solvent and a relatively short reaction time.

7.2. Introduction

One-pot multi-component reactions (MCRs) are simple and efficient synthetic routes for producing diverse heterocycles. These reactions are straight-forward one-step transformations which offer significant advantages over conventional linear type syntheses due to their flexible, convergent and atom efficient nature. Thus, the development of MCRs has attracted considerable attention as an ideal synthesis protocol due to their efficiency, facile implementation and generally high product yield. These reactions are being used to produce elaborate, biologically active compounds hence are an important area of research in organic, combinatorial and medicinal chemistry. The
common MCRs procedure for the synthesis of 2-amino-4H-pyran-3-carbonitrile derivatives (APCs) employs a three-component mixture of cyclic 1,3-diketones, aldehydes and malononitrile and is performed under a variety of reaction conditions.

Catalysts such as piperidine/ammonium acetate (Hassanien et al., 1999) and triethylamine (Shestopalov et al., 2003) were reported with yields of 70-85%. However, aqueous solutions of alkyl ammonium salts (Balalaie et al., 2006) and (S)-proline (Fan et al., 2004) resulted in higher yields of 75-95% but, long reaction times of up to 10 h was a major disadvantage. Other reagents such as benzyltriethylammonium chloride (TEBA) (Shi et al., 2003), NaBr (Devi and Bhuyan, 2004), amino functionalized ionic liquid (Peng and Song, 2007), ammonium chloride (Dabiri et al., 2009), ethylenediamine diacetate (Hari and Lee, 2010), surfactant metal carboxylates (Wang et al., 2010) and β-cyclodextrin (Sridhar et al., 2009) were reported.

Insulating oxides such as SiO₂, Al₂O₃, silica-alumina and various zeolites are materials commonly used as catalyst supports (Spivey, 1987). These oxides possess low thermal conductivity, generating sintering of the assisted metal on hot spots, various acidic and basic sites and the coverage of the catalyst with water at low temperature due to its hydrophilic surface. Various two-dimensional (2D) nanomaterials have received considerable attention for developments as heterogeneous catalysis. Among them, boron nitride (BN) is showing potential because of its high elastic modulus, high melting-point, excellent thermal conductivity and a large and direct band gap. Such properties can be of great value for ultraviolet-light emitters, advanced ceramic composites, electrical insulators, solid lubricants and ideal substrates (Liu et al., 2014) therefore in this study BN was used as a catalyst support.

The graphene-like hexagonal BN (Nag et al., 2010) is the most stable isomer. A giant planar network of hexagonal boron nitride (hBN) provides acid and base resistance, good thermal and electrical conductivity and chemical inertness. BN is hydrophobic preventing the condensation of moisture on its surface. Activated BN exhibits excellent adsorption for various metal ions such as Cr³⁺, Co²⁺, Ni²⁺, Ce³⁺ and Pb²⁺ and for organic pollutants such as tetracycline, methyl orange and Congo red in water, as well as volatile organic compounds such as benzene in air (Li et al., 2013).
Wang et al., 2004 reported the synthesis of a series of 2-cyano-3-aryl-3-(3,4-dihydro-1(2H)-naphthalene-one-2-yl) propionitrile derivatives from malononitrile, 2-aryl methyl-idene-3,4-dihydro-1(2H)-naphthalenone and 2,6-biaryl methylidene cyclohexanone. This reaction used KF-Al$_2$O$_3$ as a catalyst under reflux for 10-14 h. The yield was in the range of 68 to 93%.

\[
\begin{align*}
\text{Ar} & = C_6H_5, 4-CH_3C_6H_5, 4-ClC_6H_4, 2,4-ClC_6H_4 \\
\end{align*}
\]

Zhou, 2003 synthesized 3-cyano-2-methoxylpyridine and 2-amino-3-cyano-4H-pyran derivatives by a one-pot reaction of 2,6-bisarylidene cyclohexanone with malononitrile in sodium hydroxide/piperidine. The yield was 60-80% within 5-9 h of reflux.

\[
\begin{align*}
\text{Ar} & = C_6H_5, 4-ClC_6H_5, 4-CH_3OC_6H_4, 3-O_2NC_6H_4 \\
\end{align*}
\]

Rare earth perfluoro octanoate [RE(PFO)$_3$] was used as a catalyst by Wang et al., 2006 for the condensation of dimeredone, aldehydes and malononitrile. The corresponding 5-oxo-5,6,7,8-tetrahydro-4H-benzo-[b]-pyran derivatives were obtained in 28 to 92% yield within 5 h.

\[
\begin{align*}
\text{Ar} & = C_6H_5, 4-BrC_6H_4, 4-OCH_3C_6H_4, 2,4-ClC_6H_4 \\
\end{align*}
\]
Khaksar et al., 2012 reported a one-pot three-component syntheses of 2-amino-3-cyano-4H-chromenes and tetrahydrobenzo[b]pyran derivatives by condensation of aldehydes, malononitrile and resorcinol or dimedone in the presence of 2,2,2-trifluoroethanol as a catalyst. The reaction took 5 h to afford a yield of 80-96%.

\[
\begin{align*}
\text{R} &= \text{C}_6\text{H}_5, 4-\text{ClC}_6\text{H}_5, 4-\text{FC}_6\text{H}_5, 4-\text{BrC}_6\text{H}_5, 4-\text{NO}_2\text{C}_6\text{H}_5, 2-\text{FC}_6\text{H}_5
\end{align*}
\]

Elnagdi et al., 2012 synthesized pyrans via the multi-component reaction of aromatic aldehydes, malononitrile and active methylenes in the presence of L-proline as a catalyst. The reaction was carried out in 4 h under reflux conditions to afford a yield of 60-90% in ethanol.

\[
\begin{align*}
\text{Ph} &= \text{C}_6\text{H}_5, 2-\text{CH}_3\text{C}_6\text{H}_5
\end{align*}
\]

Shaabani et al., 2009 developed the synthesis of functionalized chromene derivatives through the addition and subsequent cyclization of 2-hydroxynaphthalene-1,4-dione or

\[
\begin{align*}
\text{R} &= \text{H}, 2-\text{CH}_3, 4-\text{Br}, 4-\text{NO}_2, 4-\text{Cl}, 2-\text{OCH}_3
\end{align*}
\]
2,5-dihydroxy cyclohexa-2,5-dione to the condensation product of an aldehyde with malononitrile in the presence of catalytic amount of Et$_3$N in acetonitrile at ambient temperature. The products were obtained at 65-82 % yield within 24 h.

Moafi et al., 2010 described a three-component method for the synthesis of 2-amino-4-cyano-4H-chromenes by reaction of salicylaldehydes, malononitrile or cyanoacetamide and trimethylsilyl cyanide (TMSCN) in the presence of LiClO$_4$ at room temperature. The reaction was achieved within 24 h with a maximum yield of 83-93 %.

\[ \text{R = H, 4-CH}_3, 4-\text{Br} \]

7.3. Results and discussion

A new heterogeneous Ca/BN catalyst containing calcium and BN was prepared by simply stirring a mixture of Ca(OAc)$_2$ and BN in an inert atmosphere for 7 days.
The Ca/BN was characterized by several techniques such as XRD, SEM with EDX, BET, Raman spectroscopy, DSC-TGA and FTIR. The XRD spectrum of Ca/BN (Fig. 1) showed the crystalline nature: the characteristic Bragg’s XRD peaks of BN at 26.09, 41.05, 54.43 and 75.34 were indexed to (002), (100), (004) and (220), respectively whilst the Ca peaks at 43.19 and 49.56 were indexed to (110) and (112), respectively.

![Figure 1](image1.png)

Fig. 1. XRD spectrum of Ca/BN showing the characteristic Bragg’s XRD peaks of BN and Ca.

![Figure 2](image2.png)

Fig. 2. Scanning electron microscope images of Ca/BN at 20 µm (A), 10 µm (B), 2 µm (C) and 1 µm (D) and Energy dispersive X-ray spectrum (E).

The SEM images (Fig. 2A-D) illustrated the morphologies of Ca/BN at 20 µm, 10 µm, 2 µm and 1 µm. The plate-like particles were clearly visible. The spherical-shaped particles that were observed suggested the presence of B₂O₃ particles (Ertug et al., 2007) that explained the presence of moisture during sample analysis.
**Fig. 2E** shows the Energy Dispersive X-ray (EDX) spectrum of Ca/BN. The Ca peaks were observed at 0.3, 3.7 and 4.0 KeV. The appearance of gold (Au) peaks was due to the coating with Au during sample preparation.

**Fig. 3.** Brunauer-Emmett-Teller surface area and surface size for Ca/BN

**Fig. 4.** Raman spectra of Ca/BN
The Brunauer-Emmett-Teller (BET) spectrum showed the specific surface area of Ca/BN by nitrogen multi-layer adsorption (Fig. 3). The nitrogen adsorption and desorption isotherm and the pore size distribution of Ca/BN were determined: the observed BET surface area was 21.52 m²/g, the pore volume was 0.1028 cm³/g whilst the pore size was 191.15 Å.

The Raman Spectrum of Ca/BN (Fig. 4) clearly showed three peaks located at approximately 900 cm⁻¹, 1380 cm⁻¹ and 1795 cm⁻¹. The peak at 1380 cm⁻¹ was aligned to the BN phonon mode (Kalay et al., 2013) whilst the peak observed at 1795 cm⁻¹ was due to calcium (Antunes et al., 2014; Harris et al., 2015).

The thermal stability of Ca/BN was measured from room temperature to 800°C (Fig. 5). A broad exothermic peak on the DSC curve was observed at 150 °C.

![DSC and TGA curves of Ca/BN](image)

**Fig. 5.** Differential scanning calorimetry-Thermal gravimetric analysis of Ca/BN

The corresponding TGA curve revealed two mass losses viz., 0.2 wt % in the temperature range of 93 to 95 °C and a gradual loss of weight in the temperature range of 102 to 300 °C which was probably due to the partial decomposition of Ca/BN. A mass loss of water was observed at 100 °C. Above 400 °C, the weight was found to increase slightly by ~0.35 wt % which might be associated with the partial formation of Ca₃B₂.
The FTIR spectrum (Fig. 6) of Ca/BN showed BN as N-B-N at 1630 cm\(^{-1}\), B-N at 1530 cm\(^{-1}\) and B-N-B at 814 cm\(^{-1}\) whilst Ca-N at 940 cm\(^{-1}\) was assigned to an asymmetric stretch and 1532 cm\(^{-1}\) was assigned as the symmetric stretch. The peak observed at 1630 cm\(^{-1}\) for N-B-N was absent after loading calcium to form Ca/BN. Ca/BN was subsequently assessed for catalytic activity for the synthesis of 2-amino-4H-pyran-3-carbonitrile (APCs).

In a preliminary experiment, an equivalent quantity (1 mmol) of aromatic aldehyde (19), malononitrile (2) and dimedone (7), in toluene, was refluxed at 110\(^{\circ}\)C in the presence of pure BN. The reaction was monitored by TLC: after 24 h, trace amount of a new product was observed. However, when the reaction was conducted in the presence of 10 mol % Ca/BN, within 1 h, an intense new spot was observed on the TLC plate. The reflux was continued until all the reactants were used. The reaction took 6 h to afford a 95 % product yield (Scheme 1). After the usual work-up of the reaction, and purification by column chromatography, the product was identified as 20 by FTIR, \(^1\)H-NMR, \(^13\)C-NMR, DEPT 90º, DEPT 135º, HSQC, COSY, NOESY, HMBC and elemental analysis.
Scheme 1. The synthesis of 2-amino-4H-pyran-3-carbonitriles (20-33)

The IR spectrum (Fig. 7.1, Appendix VII) of 20 showed stretching frequencies (cm\(^{-1}\)) at 3394 for NH\(_2\), 1036 for C-N, 2201 for CN, 1456 for C=C, 1642 for C=O, 1036 for C=O and 2963 for CH. The \(^1\)H-NMR spectrum (Fig. 7.2) of the compound 20 showed four singlets, ie., CH (C4) at \(\delta\) 4.16, NH\(_2\) (C12) broad singlet at \(\delta\) 6.98 , methyl proton (CH\(_3\), C9) broad singlet at \(\delta\) 0.94 and CH\(_3\) (C10) broad singlet \(\delta\) 1.03 . The (CH\(_2\), C6) proton of dimedone, showed a doublet of triplet at \(\delta\) 2.50. A doublet was observed for one proton (C8 carbon, CH\(_2\)) at \(\delta\) 2.25 and a doublet at \(\delta\) 2.10 which showed the presence of another proton of C8 (CH\(_2\)). The C2'-H proton at \(\delta\) 7.13 of the phenyl group was a triplet for one proton with coupling constant, \(J=11.96\) Hz. The C3'-H proton of the phenyl group was a triplet for one proton with coupling constant, \(J=11.96\) Hz. The C4'-H proton of the phenyl group was a doublet at \(\delta\) 7.28 with coupling constant, \(J=0.96\) Hz. The C5'-H proton of the phenyl group at \(\delta\) 7.28 was a triplet for one proton with coupling constant,
$J=7.28$ Hz. The C6'-H of the phenyl group was a triplet for one proton at $\delta$, 7.13 with coupling constant, $J=11.96$ Hz. The selected $^1$H and $^{13}$C-NMR chemical shifts are shown in Fig. 7.

**Fig. 7.** Selected $^1$H and $^{13}$C-NMR chemical shifts of 20

The $^{13}$C-NMR spectrum (Fig. 7.3) showed the carbonyl group C5' at $\delta$ 195.61. The methyl carbons C9 and C10 were observed at $\delta$ 26.77 and 28.35. The C7 carbon of the dimedone skeleton showed a peak at $\delta$ 31.76, C8 showed a peak at $\delta$ 40.11 and C6 showed a peak at $\delta$ 49.95. The following $\delta$ values were observed for the pyran ring and dimedone carbons: C4 at 38.54, C3 at 58.30, C2 at 162.45, C11 at 119.66, C8a at 158.46 and C8 at 40.11. The absorption signals for the phenyl carbons were at the following $\delta$ values: C1' at 144.70, C2' at 127.10, C3' at 128.28, C4' at 126.52, C5' at 128.28 and C6' at 127.10. Furthermore, CH and CH$_2$ peaks were confirmed with DEPT 90º and DEPT 135º. The DEPT 90º spectra showed the CH (C4) peak at $\delta$ 35.54 (Fig. 7.4). The DEPT 135º showed the CH$_2$ peak at $\delta$ 49.95 for C6 carbon (Fig. 7.5).

The structure was further confirmed on the basis of 2D NMR spectral studies. The $^{13}$C, $^1$H-COSY correlation of carbon signals in ppm: $\delta$ 144.76, 128.28, 127.10, 126.52, 49.95, 35.54, 28.35 and 26.77 which were assigned to carbons C1’, C3’, C2’, C4’, C6, C4, C10 and C9, respectively (Fig. 7.6).
The $^1$H, $^1$H-COSY spectrum (Fig. 7.7) of the compound revealed the correlation between the singlet of CH (C4-H) proton at $\delta$ 4.16 and doublet of triplet of CH$_2$ (C6-H) proton at $\delta$ 2.50. The C4-H proton was correlated with C4’ at $\delta$ 126.52 and C3’ at $\delta$ 128.28. The $^1$H, $^1$H-NOESY spectrum (Fig. 7.8) of the compound revealed that the singlet of NH$_2$ at $\delta$ 6.98 was coupled with the doublet of triplet of CH$_2$ (C8-H) at $\delta$ 2.50.

The HMBC spectrum (Fig. 7.9) of 20 showed the long-range correlations as follows: The proton C4-H, CH group of 20 coupled with pyran ring carbon (C3) at $\delta$ 58.30, C2 at $\delta$ 162.45 and carbonitrile (C11) carbon at $\delta$ 119.66, phenyl ring carbons C1’ at $\delta$ 144.70 and C6’ at $\delta$ 127.10, dimedone carbons C4a at $\delta$ 112.73 and C8a at $\delta$ 158.46. This correlation of C4-H to the quaternary carbon (C1’), dimedone carbons C4a and C8a, pyran ring carbons C3 and C2, carbonitrile (C11) carbon and carbon (C6’) of phenyl ring indicated that the three groups were attached to C4 (CH). Thus it was evident the three different moieties were bonded to a common carbon and hence added valuable information to 20. The selected HMBC correlation chemical shifts are shown in Fig. 8 below:

![Fig. 8. The selected HMBC correlation for 20](image-url)
The phenyl group C3'-H proton coupled with C2' at δ 127.10 and C1’ at δ 144.70. The C2'-H phenyl proton was coupled with C4 at δ 35.54 and C3’ at δ 128.28. The C6-H proton was coupled with C9 at δ 26.77, C7 at δ 31.76 and C4a at δ 112.71. The C8-H proton was coupled with C7 at δ 31.76, C9 at δ 26.77, C5 at δ 195.61 and C4a at δ 112.71. The C9-H was coupled with C7 at δ 31.76 and C8 at δ 40.11. The C10-H was coupled with C9 at δ 26.77, C7 at δ 31.76 and C8 at δ 40.11. The C4’-H was coupled with C2’ at δ 127.10. The C12-H (NH2) was correlated with carbons C3 at δ 58.30 and C8a at δ 158.46. The selected ¹H, ¹³C-NMR and HMBC chemical shifts of the compound 20 is shown in Table 1.

Table 1. Selected ¹H, ¹³C-NMR and HMBC chemical shifts

<table>
<thead>
<tr>
<th>S.No</th>
<th>Protons</th>
<th>Correlated carbons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C4-H (s, 1H, CH) at δ 4.16 ppm</td>
<td>C3 (58.30), C4a (112.71), C11 (119.66), C6' (127.10), C1' (144.70), C8a (158.46) and C2 (162.45)</td>
</tr>
<tr>
<td>2</td>
<td>C3'-H (t, 1H, J=7.28 Hz) at δ 7.28 ppm</td>
<td>C2' (127.10) and C1' (144.70)</td>
</tr>
<tr>
<td>3</td>
<td>C2'-H (t, 1H, J= 7.28 Hz) at δ 7.28 ppm</td>
<td>C4 (35.54) and C3' (128.28)</td>
</tr>
<tr>
<td>4</td>
<td>C6-H (d, 2H) at δ 2.25 and 2.10 ppm</td>
<td>C9 (26.77), C7 (31.76) and C4a (112.71)</td>
</tr>
<tr>
<td>5</td>
<td>C8-H (dt, 2H) at δ 2.50</td>
<td>C7 (31.76), C9 (26.77), C5 (195.61) and C4a (112.71)</td>
</tr>
<tr>
<td>6</td>
<td>C9-H (s, 3H) at δ 0.96 ppm</td>
<td>C7 (31.76) and C8 (40.11)</td>
</tr>
<tr>
<td>7</td>
<td>C10-H (s, 3H) at δ 1.03 ppm</td>
<td>C9 (26.77), C7 (31.76) and C8 (40.11)</td>
</tr>
<tr>
<td>8</td>
<td>C4'-H (d, 1H, J= 0.96 Hz) at δ 7.19 ppm</td>
<td>C2' (127.10)</td>
</tr>
<tr>
<td>9</td>
<td>C12-H (brs, 2H) at δ 6.98 ppm</td>
<td>C3 (58.30) and C8a (158.46)</td>
</tr>
</tbody>
</table>

Based on the above spectral details and elemental analysis: Anal. Calc. for C₁₈H₁₈N₂O₂: C, 73.45; H, 6.16; N, 9.52; %. Found: C, 73.47; H, 6.14; N, 9.50; %, the structure was confirmed as 2-amino-7,7-dimethyl-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (20). The synthesis of 20 was used as a model reaction.

To determine the optimum mole % of Ca/BN, the model reaction was carried with different mole percentages: 5 mol %, 10 mol %, 15 mol % and 20 mol % (Table 2).
Table 2. Optimization of Ca/BN catalyst

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Mol %</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BN</td>
<td>25</td>
<td>up to 24</td>
<td>Trace</td>
</tr>
<tr>
<td>2</td>
<td>Ca/BN</td>
<td>5</td>
<td>6</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>Ca/BN</td>
<td>10</td>
<td>6</td>
<td>95</td>
</tr>
<tr>
<td>4</td>
<td>Ca/BN</td>
<td>15</td>
<td>6</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>Ca/BN</td>
<td>20</td>
<td>6</td>
<td>95</td>
</tr>
</tbody>
</table>

It was observed that on increasing the amount of Ca/BN to either 15 mol% or 20 mol % had no improvement in the yield (Table 2, entries 4 and 5). The best yield of 20 was obtained at 10 mol % of Ca/BN.

Table 2. Optimization of Ca/BN catalyst

Also, in the model reaction, the presence of 10 mol % Ca/BN was investigated in different solvents to determine the effect on the reaction yield (Table 3). Solvents such as dichloromethane (DCM), ethyl acetate, ethanol, acetonitrile, toluene, methanol and water were used. Ethanol was determined to be the ideal solvent for this reaction which afforded maximum yield (95%) of 20 (Table 3, entry 3) for 6 h under reflux at 110 °C. Increasing the reaction time or temperature did not improve the yield.

Table 3. The effect of different solvents on the reaction

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DCM</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl Acetate</td>
<td>8</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol</td>
<td>6</td>
<td>95</td>
</tr>
<tr>
<td>4</td>
<td>Acetonitrile</td>
<td>6</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>Toluene</td>
<td>8</td>
<td>78</td>
</tr>
<tr>
<td>6</td>
<td>Methanol</td>
<td>10</td>
<td>75</td>
</tr>
<tr>
<td>7</td>
<td>Water</td>
<td>10</td>
<td>72</td>
</tr>
</tbody>
</table>

To determine the catalytic efficiency of the recycled catalyst, five successive cycles for the synthesis of 20 were investigated under optimal reaction conditions. The procedure was simple: the final reaction mixture was filtered and the solid was washed with solvents, air-dried and then the catalyst was reused. It was observed that the activity of the catalyst did not produce any significant decrease in yield of 20 after five successive runs. The fifth run of the reaction produced 85% yield of 20 (Fig. 9).
A comparison of the results from our study with previously reported catalysts for the preparation of 2-amino-4H-pyran-3-carbonitrile (APC) derivatives (Table 4) indicated that some catalysts had longer reaction time which produced lower percentage yields.

Table 4. Comparison of reported catalysts with Ca/BN heterogeneous catalyst

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Condition</th>
<th>Solvent</th>
<th>Time</th>
<th>Yield (%)</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NaOH/Piperidine</td>
<td>MW</td>
<td>EtOH</td>
<td>5-9 h</td>
<td>71</td>
<td>Zhou, 2003</td>
</tr>
<tr>
<td>2</td>
<td>KF-Al₂O₃</td>
<td>Reflux</td>
<td>DMF</td>
<td>10-14 h</td>
<td>90</td>
<td>Wang et al., 2004</td>
</tr>
<tr>
<td>3</td>
<td>HTMAB</td>
<td>Reflux</td>
<td>Water</td>
<td>8 h</td>
<td>93</td>
<td>Jin et al., 2005</td>
</tr>
<tr>
<td>4</td>
<td>L-proline</td>
<td>Reflux</td>
<td>EtOH</td>
<td>4 h</td>
<td>90</td>
<td>Elnagdi et al., 2015</td>
</tr>
<tr>
<td>5</td>
<td>RE(PFO)₃</td>
<td>Reflux</td>
<td>EtOH</td>
<td>5 h</td>
<td>90</td>
<td>Wang et al., 2006</td>
</tr>
<tr>
<td>6</td>
<td>Trifluoroethanol</td>
<td>Reflux</td>
<td>EtOH</td>
<td>5 h</td>
<td>90</td>
<td>Khaksar et al., 2012</td>
</tr>
<tr>
<td>7</td>
<td>I₂</td>
<td>Reflux</td>
<td>DMSO</td>
<td>4 h</td>
<td>86</td>
<td>Tahmassebi et al., 2011</td>
</tr>
<tr>
<td>8</td>
<td>Ca/BN</td>
<td>Reflux</td>
<td>EtOH</td>
<td>6 h</td>
<td>95</td>
<td>This work</td>
</tr>
</tbody>
</table>

The catalysts such as KF-Al₂O₃, HTMAB, L-proline, RE(PFO)₃, and trifluoroethanol gave 90 % of yield in shorter reaction time. The major advantage of Ca/BN over other catalysts was the maximum yield of APCs and the recyclability potential.

After optimising the reaction condition for the synthesis of 20, the protocol was used with the appropriate starting compounds to synthesize 21-33 (Table 5). The 13 new derivatives were easily characterized from the spectroscopic data because there was only a minor change in their structure from 20. It was concluded that the one-pot MCR synthesis of APCs was successful. The FTIR, ¹H-NMR, ¹³C-NMR and elemental analysis is presented in Appendix-VII.
Table 5. Synthesis of 2-amino-4H-pyran-3-carbonitrile derivatives under reflux conditions in the presence of 10 mol % Ca/BN.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ar-CHO</th>
<th>1,3-diketone</th>
<th>Product</th>
<th>Yield (%)</th>
<th>Melting Point (℃)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Observed</td>
<td>Reported</td>
</tr>
<tr>
<td>1</td>
<td>H</td>
<td>C₈H₁₀O₂</td>
<td>20</td>
<td>95</td>
<td>233-235</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>234-235 (Gao et al., 2008)</td>
</tr>
<tr>
<td>2</td>
<td>4-NO₂</td>
<td>C₈H₁₀O₂</td>
<td>21</td>
<td>95</td>
<td>176-178</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>177-178 (Nemouchi et al., 2012)</td>
</tr>
<tr>
<td>3</td>
<td>4-F</td>
<td>C₈H₁₀O₂</td>
<td>22</td>
<td>85</td>
<td>199-201</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>200 (Osikoie et al., 2011)</td>
</tr>
<tr>
<td>4</td>
<td>4-MeO</td>
<td>C₈H₁₀O₂</td>
<td>23</td>
<td>80</td>
<td>199-201</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>199-200 (Osikoie et al., 2011)</td>
</tr>
<tr>
<td>5</td>
<td>2-NO₂</td>
<td>C₈H₁₀O₂</td>
<td>24</td>
<td>94</td>
<td>223-226</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>224-226 (Jin et al., 2004)</td>
</tr>
<tr>
<td>6</td>
<td>4-Cl</td>
<td>C₈H₁₀O₂</td>
<td>25</td>
<td>94</td>
<td>208-210</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>209-210 (Katkar et al., 2011)</td>
</tr>
<tr>
<td>7</td>
<td>4-Me</td>
<td>C₈H₁₀O₂</td>
<td>26</td>
<td>90</td>
<td>219-222</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>220-222 (Rong et al., 2006)</td>
</tr>
<tr>
<td>8</td>
<td>2-OH</td>
<td>C₈H₁₀O₂</td>
<td>27</td>
<td>75</td>
<td>175-177</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No literature</td>
</tr>
<tr>
<td>9</td>
<td>Furfural</td>
<td>C₈H₁₀O₂</td>
<td>28</td>
<td>94</td>
<td>215-217</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>216 (Lian et al., 2008)</td>
</tr>
<tr>
<td>10</td>
<td>H</td>
<td>C₆H₁₀O₂</td>
<td>29</td>
<td>95</td>
<td>194-196</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No literature</td>
</tr>
<tr>
<td>11</td>
<td>4-NO₂</td>
<td>C₆H₁₀O₂</td>
<td>30</td>
<td>94</td>
<td>177-179</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No literature</td>
</tr>
<tr>
<td>12</td>
<td>4-F</td>
<td>C₆H₁₀O₂</td>
<td>31</td>
<td>83</td>
<td>159-161</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No literature</td>
</tr>
<tr>
<td>13</td>
<td>2-NO₂</td>
<td>C₆H₁₀O₂</td>
<td>32</td>
<td>94</td>
<td>176-178</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No literature</td>
</tr>
<tr>
<td>14</td>
<td>4-Me</td>
<td>C₆H₁₀O₂</td>
<td>33</td>
<td>75</td>
<td>171-173</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No literature</td>
</tr>
</tbody>
</table>

A plausible mechanism for the formation of APCs is presented in Scheme 2. In this mechanism BN increased the electrophilicity of the carbonyl carbon of C-H activated compounds as well as aryl aldehydes and efficiently catalysed Michael addition (Molla and Hussain, 2013). In the first transformation, aryl aldehyde 1 and malononitrile 2 formed a Knoevenagel product 3. The methine carbon of 3 was activated by BN and reacted with the C-H activated compound of 1,3-diketone 4 in a Michael fashion, producing intermediate 5. Finally, the intermediate 5, underwent intramolecular cyclization followed by the formation of pyran derivatives 6.
Due to pathogenic bacteria developing resistance against antibiotics, there is a need to identify new compounds/drugs that could be used as alternate to these antibiotics. Therefore, research into medicinal raw material which can be used to identify new compounds that may have antibiotic action is imperative (Andrews, 2001). Various heterocyclic compounds have shown antimicrobial potential and pyran is one of the promising heterocyclic moieties. The incorporation of the pyran nucleus is an important synthetic strategy in drug discovery. As discussed in Chapter Two, several pyrans have been used as precursors for the synthesis of pharmacologically active compounds such as HIV protease inhibitors, antifungals, cardiotonics, anticonvulsants, antimicrobials, pheromones, natural pigments, antitumor agents and plant growth regulators. Pyran derivatives can be used for medicinal applications therefore these compounds were investigated for their antibacterial properties. In this investigation, we reported that some

**Scheme 2.** Plausible mechanism of the synthesis of 2-amino-4H-pyran-3-carbonitriles
pyran derivatives showed significant antibacterial activities against *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*).

The minimal inhibitory concentration (MIC) was determined by serially diluting the extracts using nutrient broth. A series of five APCs (29-33) were evaluated for antibacterial activity against two Gram-negative bacteria, *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) and one Gram-positive bacterium, *S. aureus* (ATCC 29213), Fig. 10, Fig. 11 and Fig. 12.

**Table 6. Antibacterial activities of APCs: Minimal Inhibitory Concentration**

<table>
<thead>
<tr>
<th>Entry</th>
<th>APCs</th>
<th><em>E. coli</em> (µg/mL)</th>
<th><em>P. aeruginosa</em> (µg/mL)</th>
<th><em>S. aureus</em> (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>128</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>256</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>256</td>
<td>256</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>256</td>
<td>256</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>256</td>
<td>-</td>
<td>256</td>
</tr>
</tbody>
</table>

Standard antibacterial drugs: Ciprofloxacin and Nalidixic acid.  
(-): has no bacterial effect.

**Fig. 10.** Antibacterial activity of 2-amino-4H-pyran-3-carbonitriles against *E. coli*
Fig. 11. Antibacterial activity of 2-amino-4H-pyran-3-carbonitriles against *P. aeruginosa*

Fig. 12. Antibacterial activity of 2-amino-4H-pyran-3-carbonitriles against *S. aureus*

1 = 29; 2 = 30; 3 = 31; 4 = 32; 5 = 33
Standard antibiotics, ciprofloxacin and nalidixic acid were used as positive controls and DMSO used as negative control. DMSO had no effect on the bacteria. The antibacterial activity results are presented in Table 6. The results indicate that the APCs showed moderate to good activity against the bacterial strains. It is interesting that compound 29 showed very good activity against all strains. Compounds 30 and 33 showed no inhibitory effect against P. aeruginosa. Furthermore, compounds 30, 31 and 32 showed no activity against S. aureus.

These results indicate that the APCs had good antibacterial activity against the strains used. The pyran nucleus is one of the active constituents present in many standard drugs and is known to increase the pharmacological activity of the molecule. The presence of substituents like fluorine, nitro and methyl groups in the APCs contributed to the net biological activity (Shah and Westwell, 2007; Chauvière et al., 2003; Deckers et al., 2000). Overall, these synthesized compounds showed moderate to promising activity as compared to standard drugs, although not against all tested bacterial strains.

7.4. Conclusion

In conclusion, a facile and convenient practical method for easy access to a wide range of pharmaceutically functionalized 2-amino-4H-pyran-3-carbonitriles in the presence of Ca/BN via a one-pot tandem Knoevenagel-cyclocondensation of aldehydes, malononitrile and 1,3-diketone in ethanol at room temperature was developed. Mild reaction conditions, excellent yields, operational simplicity, clean reaction profiles as well as the use of inexpensive solvent and catalysts are the key advantages of the present method. Moreover, reusability of the catalyst is an added advantage to this protocol. A total of five APCs were subjected to antibacterial studies against S. aureus, E. coli and P. aeruginosa. The MIC value for the synthesized 2-amino-4H-pyran-3-carbonitriles showed moderate to promising activity against the bacteria tested. Among them, compound 29 showed MIC values of 128, 16 and 4 µg/mL towards E.coli, P. aeruginosa and S. aureus. The synthesis of biologically relevant pyran-annulated heterocyclic scaffolds is important for medicinal research hence, the present methodology with mild reaction conditions and operational simplicity offers the possibility of its use in a cost-effective way for large-scale industrial syntheses.
7.5. Experimental

Preparation of calcium loaded boron nitride catalyst

To a solution of Ca(OAc)$_2$ (19.7 mg, including 7.6 % mg of Ca metal; 0.5 wt % of Ca metal vs. BN) in methanol (50 mL), boron nitride (2.66 g) was added and the mixture was stirred at room temperature for a week. The resulting suspension was filtered and the solid was washed with MeOH, dried under reduced pressure to yield 0.3 % Ca/BN catalyst as a white powder; yield: 2.679 g.

General Procedure for the preparation of 2-amino-4H-pyran-3-carbonitrile derivatives

A 100 mL round bottom flask containing the selected aryl aldehyde (1 mmol), malononitrile (1 mmol) and Ca/BN (0.10 g, 10 mol % of the substrate), in ethanol (15 mL), was set up for reflux, with stirring, on an oil bath. After one h of reflux, 5,5-dimethylcyclohexane-1,3-dione was slowly added with stirring. Reflux was continued whilst the progress of the reaction was intermittently monitored by TLC. Following completion of the reaction, the mixture was filtered, the filtrate was collected and the solvent was removed in vacuo. The product was purified by column chromatography with an eluent system of ethyl acetate: petroleum ether, 50 % (1:1). The products were characterized by IR, $^1$H-NMR and $^{13}$C-NMR and elemental analysis. The solid in the filtration process was collected, washed with CHCl$_3$: MeOH mixture followed by acetone and kept aside for further use.

Antibacterial studies

The preparation of Media

Fresh Nutrient Agar, Oxoid Ltd (Hampshire, England) was prepared according to the manufacturer’s guidelines as follow:

- 28 g was weighed out into each of 3 separate one litre glass bottles
- 1L of distilled water was added to each bottle
- The solution was mixed until the powder completely dissolved.
- Bottles were sterilized by autoclaving for 15 minutes at 121°C.
- The agar was poured into plates to solidify.
The preparation of the Nutrient Broth

Fresh Mueller Hinton Broth (Sigma-Aldrich) was made up according to manufacturer’s guidelines as follows:

- 23 g of Nutrient Broth powder was weighed into a one litre glass bottle
- 1L of distilled water was added. The solution was mixed until the powder completely dissolved
- This was dispensed into bijou bottles before autoclaving
- Bijou bottles were sterilized by autoclaving for 15 minutes at 121°C

Bacterial cultures

The cultures of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were maintained on nutrient agar slopes at 4°C and sub-cultured on to blood agar plates for 24 h before use.

Preparation of reagent: Microplate Alamar Blue Assay (MABA)

0.2 g of Resazurin powder was dissolved in 10 mL autoclaved distilled water. The dye solution was vortexed vigorously. The solution was immediately covered with aluminium foil as it was light sensitive. Each well was inoculated with a bacterial suspension containing 1x10⁶ CFU/mL and incubated at 37°C for 24 h. The MIC was regarded as lowest concentration of the extract which would not permit any visible growth when compared with extract free broths inoculated with each of the bacterial suspensions. They were incubated at 37°C for 24 h and then examined for growth. The antibacterial activities of the synthesized compounds were studied by MABA using 96-wells microplates including positive control (containing standard antibiotic) and growth control (containing culture broth without testing materials). 20 µL of each concentration of the synthesized compounds were added in duplicate except for positive and growth control wells. After adding Alamar Blue (20 µL) to all 96 wells the total volume in each well reached 200 µL. The final concentrations of the tested compounds were 256, 128, 64, 32, 16, 8, 4, 2, 1 µg/mL. After incubation, the results were recorded and MIC was determined.
References


for the efficient synthesis of poly substituted pyrans, thiopyrans, pyridines, and pyrazoles. Molecular Diversity, (19) 625-651.


7.1. 2-Amino-7,7-dimethyl-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (20)

White solid, m.p = 233-235°C; IR (ATR, cm\(^{-1}\)) : 3394 NH\(_2\), 1036 C-N, 2963 CH. \(^1\)H-NMR (400 MHz, DMSO-d\(_6\)) : \(\delta\) (ppm) 7.28 (t, 2H, J=7.28 Hz, Ar-H), 7.19 (d, 1H, J=0.96 Hz, Ar-H), 7.13 (t, 2H, J=11.96 Hz, Ar-H), 6.98 (brs, 2H, NH\(_2\)), 4.16 (s, 1H, CH), 2.50 (dt, 2H, J=2.04 Hz, CH\(_2\)), 2.25 (d, 1H, J=16.08 Hz, CH\(_2\)), 2.10 (d, 1H, J=16.08 Hz, CH\(_2\)), 0.94 (s, 3H, CH\(_3\)). \(^1\)C-NMR (100 MHz, DMSO-d\(_6\)) : \(\delta\) (ppm) 195.61, 162.45, 158.46, 144.70, 128.28, 127.10, 126.52, 119.66, 112.71, 58.30, 49.95, 40.11, 35.54, 31.76, 28.35, 26.77.

Elemental Analysis: Anal. Calc. for C\(_{18}\)H\(_{21}\)N\(_2\)O\(_2\): C, 73.45; H, 6.16; N, 9.52; %. Found: C, 73.47; H, 6.14; N, 9.50; %.

7.2. 2-Amino-7,7-dimethyl-4-(4-nitrophenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (21)

Yellow solid, m.p = 185-187°C; IR (ATR, cm\(^{-1}\)) : 3314 NH\(_2\), 1043 C-N, 2191 CN, 1514 C=C, 1498 C-O, 1485 C-H, 1427 C-H, 1417 C-H, 1394 C-H, 1376 C-H, 1330 C-H, 1307 C-H, 1297 C-H, 1281 C-H, 1112 C-H, 714 C-H. \(^1\)H-NMR (400 MHz, DMSO-d\(_6\)) : \(\delta\) (ppm) 8.15-8.17 (d, J =7.12 Hz, 2H, Ar-H), 7.43-7.45 (d, J = 5.36, 2H, Ar-H), 7.16 (brs, 2H, NH\(_2\)), 4.36 (s, 1H, CH), 2.49-2.53 (t, J = 3.52 Hz, 2H, CH\(_2\)), 2.23 (d, J = 16.08 Hz, 1H, CH\(_2\)), 2.01 (d, J = 16.04 Hz, 1H, CH\(_2\)), 1.03 (s, 3H, CH\(_3\)), 0.94 (s, 3H, -CH\(_3\)). \(^1\)C-NMR (100 MHz, CDCl\(_3\)) : \(\delta\) (ppm) 163.27, 149.80, 148.53, 142.27, 134.89, 130.75, 130.20, 130.08, 127.35, 125.94, 125.90, 125.86, 120.13, 104.46, 62.12, 52.66, 52.16, 38.49. Elemental Analysis: Anal. Calc. for C\(_{19}\)H\(_{17}\)N\(_3\)O\(_4\): C, 63.71; H, 5.05; N, 12.38; %. Found: C, 63.72; H, 5.08; N, 12.39; %.

7.3. 2-Amino-4-(4-fluorophenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (22)

White solid, m.p = 130-132°C; IR (ATR, cm\(^{-1}\)) : 3408 NH\(_2\), 1063 C-N, 2234 CN, 1508 C=C, 1594 C-O, 938 C-O, 3049 CH. \(^1\)H-NMR (400 MHz, CDCl\(_3\)) : \(\delta\) (ppm) 11.85 (brs, 2H, NH\(_2\)), 7.00-7.03 (m, 2H, Ar-H), 6.90-6.94 (m, 2H, Ar-H), 5.46 (s, 1H, CH), 2.31-
2.41 (m, 4H, CH₂), 1.19 (s, 3H, CH₃), 1.07 (s, 3H, -CH₃).

¹³C-NMR (100 MHz, DMSO-d₆): δ (ppm) 146.09, 143.92, 130.02, 129.96, 129.82, 128.24, 123.92, 123.40, 119.43, 116.34, 116.34, 116.10, 114.36, 114.30, 97.07, 62.05, 40.10, 38.85, 15.10.

Elemental Analysis: Anal. Calc. for C₁₈H₁₇FN₂O₂: C, 69.22; H, 5.49; N, 8.97; %. Found: C, 69.25; H, 5.47; N, 8.99; %.

7.4. 2-Amino-4-(4-methoxyphenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (23)

Pale yellow solid, m.p = 212-214°C; IR (ATR, cm⁻¹): 3334 NH₂, 1075 C-N, 2197 CN, 1456 C=C, 1614 C=O, 1036 C-O, 2962 CH. ¹H-NMR (400 MHz, CDCl₃): δ (ppm) 7.11-7.13 (dd, J = 4.56 Hz, 2H, Ar-H), 6.78-6.80 (dd, J = 4.60 Hz, 2H, Ar-H), 4.48 (brs, 2H, NH₂), 4.33 (s, 1H, CH), 3.75 (s, 3H, OCH₃), 2.41 (s, 2H, CH₂), 2.18-2.19 (d, J = 5.08 Hz, 2H, CH₂), 1.08 (s, 3H, CH₃), 1.01 (s, 3H, -CH₃). ¹³C-NMR (100 MHz, CDCl₃): δ (ppm) 195.94, 161.21, 158.61, 157.32, 135.45, 128.62, 118.70, 114.24, 113.98, 77.33, 76.70, 63.90, 5.21, 50.69, 40.67, 34.74, 32.19, 28.88, 27.68. Elemental Analysis: Anal. Calc. for C₁₉H₂₀N₂O₃: C, 70.35; H, 6.21; N, 8.64; %. Found: C, 70.37; H, 6.20; N, 8.66; %.

7.5. 2-Amino-7,7-dimethyl-4-(2-nitrophenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (24)

Dark yellow solid, m.p = 234-236°C; IR (ATR, cm⁻¹): 3324 NH₂, 1145 C-N, 1521 C=C, 1602 C=O, 1042 C-O, 2959 CH. ¹H-NMR (400 MHz, DMSO-d₆): δ (ppm) 7.80-7.82 (d, J = 8.04 Hz, 1H, Ar-H), 7.63-7.67 (t, J = 7.56 Hz, 1H, Ar-H), 7.34-7.36 (t, J = 7.76 Hz, 2H, Ar-H), 0.87 (s, 3H, -CH₃), 7.34-7.35 (d, J = 7.8 Hz, 2H, NH₂), 4.93 (s, 1H, CH), 1.00 (s, 3H, CH₃), 2.50 (m, 2H, CH₂), 2.17-2.21 (d, J = 16.12 Hz, 1H, CH₂), 1.99-2.03 (d, J = 16.12 Hz, 1H, CH₂). ¹³C-NMR (100 MHz, DMSO-d₆): δ (ppm) 196.27, 163.19, 159.65, 149.43, 139.42, 133.82, 130.74, 128.33, 124.18, 119.52, 112.78, 56.82,
7.6. 2-Amino-4-(4-chlorophenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (25)

White solid, m.p = 217-219°C; IR (ATR, cm⁻¹): 3330 NH₂, 1091 C-N, 2969 CH. ¹H-NMR (400 MHz, CDCl₃): δ (ppm) 7.33-7.35 (d, J = 8.16 Hz, 2H, Ar-H), 7.16-7.18 (d, J = 8.2 Hz, 2H, Ar-H), 7.05 (brs, 2H, NH₂), 4.19 (s, 1H, CH), 2.50 (m, 2H, CH₂), 2.22-2.26 (d, J = 16.08 Hz, 1H, CH₂), 2.08-2.12 (d, J = 16.04 Hz, 1H, CH₂), 1.02 (s, 3H, CH₃), 0.94 (s, 3H, CH₃). ¹³C-NMR (100 MHz, CDCl₃): δ (ppm) 196.12, 163.07, 158.90, 144.20, 131.58, 129.57, 128.74, 120.00, 112.81, 58.27, 55.34, 50.42, 40.61, 39.36, 35.57, 32.25, 28.77, 27.33. Elemental Analysis: Anal. Calc. for C₁₈H₁₇ClN₂O₂: C, 65.75; H, 5.21; N, 8.52; %. Found: C, 65.77; H, 5.23; N, 8.54; %.

7.7. 2-Amino-7,7-dimethyl-5-oxo-4-(p-tolyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (26)

White solid, m.p = 222-224°C; IR (ATR, cm⁻¹): 3333 NH₂, 1143 C-N, 2194 CN, 1512 C=C, 1603 C=O, 1037 C-O, 2961 CH. ¹H-NMR (400 MHz, DMSO-d₆): δ (ppm) 7.09-7.07 (d, J = 7.84, 2H, Ar-H), 7.00-7.02 (d, J = 7.8, 2H, Ar-H), 6.95 (brs, 2H, NH₂), 4.12 (s, 1H, CH), 2.45-2.55 (m, J = 4.4 Hz, 3H, Ar-CH₃), 2.22-2.26 (t, J = 6.92, 4H, CH₂), 1.03 (s, 3H, CH₃), 0.94 (s, 3H, -CH₃). ¹³C-NMR (100 MHz, DMSO-d₆): δ (ppm) 196.10, 162.75, 158.90, 142.27, 136.08, 129.33, 127.53, 120.21, 113.34, 58.94, 50.46, 40.59, 39.75, 39.34, 35.64, 32.24, 28.88, 27.22, 21.04. Elemental Analysis: Anal. Calc. for C₁₉H₂₀N₂O₂: C, 74.00; H, 6.54; N, 9.08; %. Found: C, 74.03; H, 6.56; N, 9.06; %.

7.8. 2-Amino-4-(2-hydroxyphenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (27)

Dark brown solid, m.p = 175-177°C; IR (FTIR, cm⁻¹): 2958 NH₂, 1147 C-N, 2201 CN, 1579 C=C, 1642 C=O, 1025 C-O, 2900 CH. ¹H-NMR (400 MHz, CDCl₃): δ (ppm) 10.50
(s, 1H, OH), 7.10-7.11 (m, 1H, Ar-H), 6.99-7.00 (m, 3H, Ar-H), 7.11 (brs, 2H, NH2), 4.64 (s, 1H, CH), 2.47 (s, 2H, CH2), 1.15 (s, 2H, CH2), 1.00 (s, 3H, CH3), 0.98 (s, 3H, -CH3). 13C-NMR (100 MHz, CDCl3): δ (ppm) 200.92, 169.17, 151.03, 127.98, 127.52, 124.58, 124.29, 118.32, 115.74, 111.03, 49.92, 41.55, 32.29, 30.95, 29.69, 29.16, 27.76, 27.18.
Elemental Analysis: Anal. Calc. for C18H18N2O3: C, 69.66; H, 5.85; N, 9.03; %. Found: C, 69.68; H, 5.87; N, 9.05; %.

7.9. 2-Amino-4-(furan-2-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (28)

Brown solid, m.p = 220-222°C; IR (FTIR, cm⁻¹): 2925 NH₂, 1174 C-N, 2224 CN, 1465 C=C, 1647 C=O, 1082 C-O, 2852 CH. 1H-NMR (400 MHz, CD3OD): δ (ppm) 7.32-7.33 (d, J = 4.0, 1H, Ar-H), 6.28-6.30 (m, 1H, Ar-H), 6.10 (d, J = 3.16 Hz, 1H, Ar-H), 7.32 (brs, 2H, NH2), 4.44 (s, 1H, CH), 2.47-2.55 (q, 2H, CH2), 2.22-2.36 (q, 2H, CH2), 1.08 (s, 3H, CH3), 1.05 (s, 3H, CH3). 13C-NMR (100 MHz, CDCl3): δ (ppm) 164.77, 163.00, 141.64, 137.84, 129.69, 128.60, 122.46, 120.47, 115.13, 111.49, 50.87, 40.99, 32.18, 31.18, 29.28, 27.04. Elemental Analysis: Anal. Calc. for C16H16N2O2: C, 71.62; H, 6.01; N, 10.44; %. Found: C, 71.64; H, 6.03; N, 10.46; %.

7.10. 2-Amino-6-methyl-4-phenyl-5-propionyl-4H-pyran-3-carbonitrile (29)

White solid, m.p = 194-196°C; IR (ATR, cm⁻¹): 3395 NH₂, 1061 C-N, 2190 CN, 1411 C=C, 1693 C=O, 1121 C-O, 2970 CH. 1H-NMR (400 MHz, DMSO-d6): δ (ppm) 7.22-7.32 (t, J = 7.28, 2H, Ar-H), 7.18-7.22 (t, J = 8.08 Hz, 1H, Ar-H), 7.13-7.15 (d, J = 7.04 Hz, 2H, Ar-H), 6.89 (brs, 2H, NH2), 4.28 (s, 1H, CH), 3.94-3.97 (m, 2H, CH2), 2.12 (s, 3H, CH3), 1.00-1.03 (t, J = 7.08, 3H, CH3). 13C-NMR (100 MHz, DMSO-d6): δ (ppm) 206.48, 165.40, 158.43, 156.55, 144.83, 128.39, 127.14, 126.77, 119.68, 107.20, 60.10, 57.21, 38.82, 30.62, 18.07 13.66. Elemental Analysis: Anal. Calc. for C16H16N2O2: C, 71.62; H, 6.01; N, 10.44; %. Found: C, 71.64; H, 6.03; N, 10.46; %.
7.11. 2-Amino-6-methyl-4-(4-nitrophenyl)-5-propionyl-4H-pyran-3-carbonitrile (30)

Yellow solid, m.p = 177-179°C; IR (ATR, cm⁻¹): 3406 NH₂, 1120 C-N, 2201 CN, 1519 C=C, 1682 C=O, 1055 C-O, 2985 CH. ¹H-NMR (400 MHz, DMSO-d₆): δ (ppm) 8.17-8.20 (d, J = 8.67 Hz, 2H, Ar-H), 7.42-7.44 (d, J = 8.63 Hz, 2H, Ar-H), 7.06 (d, J = 8.63 Hz, 2H, Ar-H), 4.46 (s, 1H, CH), 3.92-3.97 (m, 2H, CH₂), 2.34 (s, 3H, CH₃), 0.99-1.02 (t, J = 7.12, 3H, -CH₃), ¹³C-NMR (100 MHz, DMSO-d₆): δ (ppm) 165.05, 158.52, 157.90, 152.51, 146.35, 128.50, 123.77, 119.29, 105.95, 60.33, 56.10, 39.99, 39.78, 38.73, 18.27, 13.64. Elemental Analysis: Anal. Calc. for C₁₆H₁₅N₃O₄: C, 61.34; H, 4.83; N, 13.41%; Found: C, 61.36; H, 4.85; N, 13.43%; %.

7.12. 2-Amino-4-(4-fluorophenyl)-6-methyl-5-propionyl-4H-pyran-3-carbonitrile (31)

White solid, m.p = 159-161°C; IR (ATR, cm⁻¹): 3407 NH₂, 1094 C-N, 2194 CN, 1508 C=C, 1605 C=O, 1057 C-O, 2985 CH. ¹H-NMR (400 MHz, CDCl₃): δ (ppm) 7.32-7.35 (m, 2H, Ar-H), 7.13-7.18 (t, J = 8.52 Hz, 2H, Ar-H), 7.24 (d, J = 8.63 Hz, 2H, Ar-H), 3.91-3.96 (m, 1H, CH), 2.59 (s, 3H, CH₃), 2.14 (s, 2H, CH₂), 0.85-0.89 (t, J = 7.12, 3H, -CH₃), ¹³C-NMR (100 MHz, CDCl₃): δ (ppm) 164.96, 163.05, 160.31, 127.41, 126.80, 125.40, 124.09, 120.32, 117.08, 116.85, 103.83, 61.52, 53.63, 33.70, 13.86, 13.54. Elemental Analysis: Anal. Calc. for C₁₆H₁₅FN₂O₂: C, 67.12; H, 5.28; N, 9.78%; Found: C, 67.14; H, 5.30; N, 9.79%; %.

7.13. 2-Amino-6-methyl-4-(2-nitrophenyl)-5-propionyl-4H-pyran-3-carbonitrile (32)

Dark yellow solid, m.p = 176-178°C; IR (ATR, cm⁻¹): 3455 NH₂, 1127 C-N, 2209 CN, 1525 C=C, 1602 C=O, 1064 C-O, 2987 CH. ¹H-NMR (400 MHz, CDCl₃): δ (ppm) 7.67-7.69 (t, J = 6.72, 1H, Ar-H), 7.47-7.48 (m, 1H, Ar-H), 7.43-7.47 (m, 2H, Ar-H), 7.42 (t, J = 8.63 Hz, 2H, Ar-H).
NH$_2$), 5.01 (s, 1H, CH), 3.85-3.90 (m, $J=6.92$, 2H, CH$_2$), 2.84 (s, 3H, OCH$_3$), 0.89-0.92 (t, $J=7.08$, 3H, -CH$_3$), $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ (ppm) 164.79, 158.93, 158.25, 148.47, 139.55, 133.73, 130.41, 128.06, 123.70, 118.91, 106.30, 60.25, 55.8532, 18.27, 13.43. Elemental Analysis: Anal. Calc. for C$_{16}$H$_{15}$N$_3$O$_4$: C, 61.34; H, 4.83; N, 13.41; %. Found: C, 61.36; H, 4.85; N, 13.44; %.

7.14. 2-Amino-6-methyl-5-propionyl-4-(p-tolyl)-4H-pyran-3-carbonitrile (33)

White solid, m.p = 121-123°C; IR (FTIR, cm$^{-1}$): 3049 NH$_2$, 1133 C=N, 2229 CN, 1575 C=C, 1615 C=O, 1046 C-O, 2979 CH. $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ (ppm) 7.95-7.97 (t, $J=8.92$, 2H, Ar-H) 7.15-7.18 (t, $J=7.08$, 2H, Ar-H), 4.48 (brs, 2H, NH$_2$), 4.38 (s, 1H, CH), 4.01-4.03 (m, 2H, CH$_2$), 2.33 (s, 3H, Ar-CH$_3$), 2.27 (s, 3H, CH$_3$), 1.01-1.07 (t, $J=7.16$, 3H, -CH$_3$), $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ (ppm) 207.46, 165.99, 157.61, 156.58, 140.87, 136.71, 129.69, 129.24, 127.38, 119.14, 108.13, 62.21, 60.64, 38.29, 30.90, 21.04, 18.38. Elemental Analysis: Anal. Calc. for C$_{17}$H$_{18}$N$_2$O$_2$: C, 72.32; H, 6.43; N, 9.92; %. Found: C, 72.34; H, 6.46; N, 9.93; %. 
7.1. 2-Amino-7,7-dimethyl-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (20)

Fig. 7.1. IR spectrum of 20

Fig. 7.2. $^1$H-NMR spectrum for 20
Fig. 7.3. $^{13}$C-NMR spectrum for 20

Fig. 7.4. DEPT 90° spectrum for 20
Fig. 7.5. DEPT 135° spectrum for 20

Fig. 7.6. HSQC spectrum for 20
Fig. 7.7. COSY spectrum for 20

Fig. 7.8. NOESY spectrum for 20
7.2. 2-Amino-7,7-dimethyl-4-(4-nitrophenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (21)

Fig. 7.9. HMBC spectrum for 20

Fig. 7.10. IR spectrum of 21
Fig. 7.11. $^1$H-NMR spectrum for 21

Fig. 7.12. $^{13}$C-NMR spectrum for 21
7.3. 2-Amino-4-(4-fluorophenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-
chromene-3-carbonitrile (22)

Fig. 7.13. IR spectrum of 22

Fig. 7.14. $^1$H-NMR spectrum for 22
7.4. 2-Amino-4-(4-methoxyphenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (23)
Fig. 7.17. $^1$H-NMR spectrum for 23

Fig. 7.18. $^{13}$C-NMR spectrum for 23
7.5. 2-Amino-7,7-dimethyl-4-(2-nitrophenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (24)

Fig. 7.19. IR spectrum of 24

Fig. 7.20. $^1$H-NMR spectrum for 24
7.6. 2-Amino-4-(4-chlorophenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (25)

**Fig. 7.21.** $^{13}$C-NMR spectrum for 24

**Fig. 7.22.** IR spectrum of 25
Fig. 7.23. $^1$H-NMR spectrum for 25

Fig. 7.24. $^{13}$C-NMR spectrum for 25
7.7. 2-Amino-7,7-dimethyl-5-oxo-4-(p-tolyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (26)

Fig. 7.25. IR spectrum of 26

Fig. 7.26. $^1$H-NMR spectrum for 26
7.8. 2-Amino-4-(2-hydroxyphenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (27)
Fig. 7.29. $^1$H-NMR spectrum for 27

Fig. 7.30. $^{13}$C-NMR spectrum for 27
7.9. 2-Amino-4-(furan-2-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (28)

Fig. 7.31. IR spectrum of 28

Fig. 7.32. $^1$H-NMR spectrum for 28
Fig. 7.33. $^{13}$C-NMR spectrum for 28

7.10. 2-Amino-6-methyl-4-phenyl-5-propionyl-4H-pyran-3-carbonitrile (29)

Fig. 7.34. IR spectrum of 29
Fig. 7.35. $^1$H-NMR spectrum for 29

Fig. 7.36. $^{13}$C-NMR spectrum for 29
7.11. 2-Amino-6-methyl-4-(4-nitrophenyl)-5-propionyl-4H-pyran-3-carbonitrile (30)

Fig. 7.37. IR spectrum of 30

Fig. 7.38. $^1$H-NMR spectrum for 30
7.12. 2-Amino-4-(4-fluorophenyl)-6-methyl-5-propionyl-4H-pyran-3-carbonitrile (31)

Fig. 7.39. $^{13}$C-NMR spectrum for 30

Fig. 7.40. IR spectrum of 31
Fig. 7.41. $^1$H-NMR spectrum for 31

Fig. 7.42. $^{13}$C-NMR spectrum for 31
7.13. 2-Amino-6-methyl-4-(2-nitrophenyl)-5-propionyl-4H-pyran-3-carbonitrile (32)

Fig. 7.43. IR spectrum of 32

Fig. 7.44. $^1$H-NMR spectrum for 32
7.14. 2-Amino-6-methyl-5-propionyl-4-(p-tolyl)-4H-pyran-3-carbonitrile (33)

Fig. 7.45. $^{13}$C-NMR spectrum for 32

Fig. 7.46. IR spectrum of 33
Fig. 7.47. $^1$H-NMR spectrum for 33

Fig. 7.48. $^{13}$C-NMR spectrum for 33
Conclusion and recommendation for future studies

An efficient synthesis of a library of biologically active oxygen, nitrogen and sulfur based heterocyclic scaffolds were successfully reported. A total of 71 small molecules were prepared by using multi-component reactions by using new catalysts.

- In chapter III, a total of new 14 quinoline and quinolone based peptides were synthesised by Ugi four-component reaction under the action of microwave irradiation. A total of eight peptides were subjected to antimicrobial, antioxidant and toxicity evaluation; compounds 41, 43, 46, 47 showed positive results for antimicrobial activity and 41, 46, 47 showed positive results for antioxidant activity. Molecular docking studies of 41 and 43 showed a higher binding affinity (183.24 kcal/mol and 165.01 kcal/mol) towards DNA gyrase than ciprofloxacin based on Libdock score.

- In chapter IV, a total of 15 new quinoline, quinolone and indole based pyrans were synthesized by a three-component reaction under the action of microwave irradiation in the presence of a new catalyst: humic acid supported 1-butyl-3-methylimidazolium thiocyanate ionic liquid. All the compounds were subjected to antimicrobial, antioxidant and toxicity evaluation. Seven QPs (31, 34, 37, 38, 42, 43 and 44) and one QOP (40) showed good potential against B. cereus, S. aureus, E. coli and E. faecalis whilst nine (32, 34, 35, 36, 37, 38, 42, 43 and 44) QPs showed antioxidant activity. The brine shrimp test showed five QPs (34, 37, 42, 43 and 44) with mortality rate less than 50 % up to 48 h. Molecular docking showed higher binding affinity of 96.96 kcal/mol for 42 based on Libdock score with Mtb DNA gyrase.

- In chapter V, a total of 14 new BTQP derivatives were synthesized in the presence of new catalyst: iron loaded boron nitride by using water as medium under microwave irradiation. A total of 10 BTQPs evaluated for antibacterial and antioxidant activities: compounds 24, 25, 27, 28, 29, 31, 32 and 36 showed positive results for antibacterial activity and 23, 24, 26, 27, 29 and 36 showed positive results for antioxidant potential, respectively. Among these compounds, 31 exhibited potential binding affinity with Staphylococcus aureus gyrase based on in-silico molecular docking studies.
In chapter VI, a total of 14 novel BTQ-DHP derivatives were synthesized in the presence of new catalyst: iodine loaded boron nitride by using water as solvent under ultrasonic irradiation. A total of 10 BTQ-DHPs were subjected to antimicrobial, antioxidant, toxicity assessment and molecular docking studies. Among them, compounds 24, 26, 27, 28, 29, 30 and 31 showed positive results for antibacterial activity and 22, 24, 28 and 31 showed positive results for antioxidant activity respectively. Compound 24 showed stronger potency toward Staphylococcus aureus gyrase on molecular docking studies.

In chapter VII, a total of 14, 2-amino-4H-pyran-3-carbonitriles in the presence of calcium loaded boron nitride catalyst via a one-pot tandem Knoevenagel-cyclocondensation reaction were synthesized in ethanol. A total of five APCs were subjected to antibacterial studies against S. aureus, E. coli and P. aeruginosa. The MIC value for the synthesized 2-amino-4H-pyran-3-carbonitriles showed moderate to promising activity against the bacteria tested. Among them, compound 29 showed MIC values of 128, 16 and 4 µg/mL towards E.coli, P. aeruginosa and S. aureus.

All the synthesized molecules (QLPs, QOLP, QPs, QOPs, IP, BTQPs, BTQ-DHPs and APCs) were confirmed by FTIR, 1H-NMR, 13C-NMR and elemental analysis. Moreover, 19F-NMR, 31P-NMR and TOF-MS analysis were included for some selected compounds. The advantages of the synthetic methodology of this project are its green approach, easy work up, mild reaction conditions, the use of an inexpensive solvent, short reaction times with higher yields and recyclability of the catalyst.

Recommendation for future studies

Since all the synthesized new compounds showed good antibacterial and antioxidant activity and are also safe for biological studies, they could be further investigated for pharmaceutical applications. Peptides, pyrans, aminophosphonates and dihydropyridines are involved in a variety of physiological and pathological processes and play very important roles in modulating various cell functions. Also, O, N and S based heterocyclic drugs have been successfully applied in treating human diseases. By keeping this in mind, the synthesized new quinoline based O, N and S heterocycles with different functional groups will be studied with the following applications: NR8383 and ESAT-6 tuberculosis cell lines, secretory protein and potent T cell antigen for M.tuberculosis.
Also, the compounds will be investigated for breast cancer application and targeted with the following cell lines: MCF-7, MDA-MB-231, SKBr3 and T47D. A549 lung cancer cell lines will also be used to find the cytotoxicity of the synthesized compounds. Effective synthesized drugs are planned to do human lymphoblastic T cell lines, Sup T1, Jurkat and CEM and the human embryonic kidney (HEK) 293T. All the synthesized compounds aimed to screen twice with antibacterial, antifungal, antioxidant (DPPH), toxicity assessment (Artemia larvae) and molecular docking studies to continue their studies on cancer applications.
List of conferences attended

Presentation

International & National

“Efficient one-pot synthesis of 2-amino-4H-pyran-3-carbonitriles catalyzed by calcium-boron nitride frame works” at Second International conference on Composites, Biocomposites and Nanocomposites conducted by Composite Research Group, Department of mechanical Engineering, Durban University of Technology, Durban.

“Eco-friendly approach: graphene like boron nitride modified calcium material for the synthesis of 2-amino-4H-pyran-3-carbonitrile derivatives” at SACI national conference, Durban University of Technology, Durban.

“Calcium loaded boron nitride catalyst for the ultrasonicated synthesis of quinolinyl-lipoyl peptides”, at Institutional Research day-2017, ICC, Durban.

Participation in conferences

Participated an international conference on “Current Trends in Chemistry”, conducted by School of Chemical Sciences, Bharathiyar University sponsored by UGC XI plan, UGC, SAP, DRS –II, India.

Participated a national level workshop on “SPECTROSCOPY” held at School of Chemical Sciences, Bharathiyar University, India.
List of publications

   DOI: 10.5185/amlett.2016.6850

   https://doi.org/10.1016/j.jphotobiol.2017.11.019

   https://doi.org/10.1080/10426507.2015.1130046


Synthesis, molecular docking, antimicrobial, antioxidant and toxicity assessment of quinoline peptides

Muthu Thangaraju, Robert Moonsamy Gengan, Bibhuti Ranjan, Ramesh Muthusamy

A B S T R A C T

A series of quinoline based peptides were synthesized by a one-pot reaction through Ugi-four component condensation of lipoic acid, cyclohexyl isocyanide, aniline derivatives and 2-methoxy quinoline-3-carbaldehyde derivatives under microwave irradiation. The products were obtained in excellent yields and high purity. Solvent optimization and the effect of microwave irradiation with various powers were also observed. All the synthesized compounds were characterized by FTIR, NMR spectral data and elemental analysis. A total of eight peptides were subjected to antimicrobial, antioxidant and toxicity evaluation. Among them, four peptides showed potential towards antibacterial screening with Bacillus cereus, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis and Candida albicans, Candida utilis and three peptides showed antioxidant test positive (DPPH). Besides, toxicity of all the peptides were evaluated by using brine shrimp and it was observed that four peptides showed mortality rate less than 50% up to 48 h. Molecular docking studies revealed that the higher binding affinity of the two peptides toward DNA gyrase than ciprofloxacin based on Libdock score. The described chemistry presents a facile tool to synthesize complex heterocycles of pharmaceutical relevance in a highly efficient and one-pot fashion. The advantages of this method are its green approach, inexpensive solvent, shorter reaction times and excellent yields.

1. Introduction

Microbial infections are one of the leading diseases which are responsible for millions of deaths every year because of lack of effective antimicrobial therapy and this situation becomes more complicated because of microbial resistance towards conventional antibiotics [1]. Occurrence of the antibiotic resistance pathogen has become a severe health issue and thus, numerous studies have been stated to improve obtainable through variation of the starting materials.

Quinoline is an important class of heterocyclic compounds found in many synthetic and natural products with a wide range of pharmacological activities such as anti-inflammatory [8], antimalarial [9], antimicrobial [10], anticonvulsant [11], antineoplastic [12], vasorelaxing [13], antiproliferative [14] and platelet derived growth factor receptor tyrosine kinase inhibiting agents [15] which can be well illustrated by the large number of drugs in the market. Ugi four-component derivatives have also been found to have various applications (viz., aesthetics, antibiotics, natural product isolation, HIV protease inhibitor crixivan, [16–18] etc.). Therefore, it is of significance to develop novel
Eco-friendly approach: Graphene like boron nitride modified calcium material for the synthesis of 2-amino-4H-pyran-3-carbonitrile derivatives

T. Muthu, K. Anand, M. Sureshkumar, R. M. Gengan*

Department of Chemistry, Faculty of Applied Sciences, Durban University of Technology, Durban 4001, South Africa

*Corresponding author. Tel: (+27) 31 3732309; Fax: (+27) 31 2022671; E-mail: genganrm@dut.ac.za

Received: 09 April 2016, Revised: 06 June 2016 and Accepted: 17 June 2016

ABSTRACT

An efficient one-pot multi-component synthesis of medicinally important 2-amino-4H-pyran-3-carbonitrile derivatives using a new heterogeneous calcium loaded boron nitride (CaBNT) catalyst is described herein. This transformation transpires by Knoevenagel condensation, Michael addition and intramolecular cyclization. Alkaline earth metal-based green catalyst was successfully prepared and characterized by XRD, SEM with EDX, Raman spectroscopy, BET, DSC-TGA and FT-IR. The reaction works up is facile and CaBNT catalyst can easily be separated from the reaction mixture and re-used more than five times in subsequent reactions. This methodology offers several advantages such as excellent yields, use of inexpensive solvent and relatively shorter reaction time. Copyright © 2016 VBRI Press.

Keywords: Heterogeneous catalyst; calcium; boron nitride; multi-component synthesis.

Introduction

One-pot multi-component reactions (MCRs) are simple and efficient synthetic routes for sustaining diverse heterocycles. These reactions are a straightforward one-step transformation which offers significant advantages over conventional linear type synthesis due to its flexible, convergent and atom efficient nature. Thus, the development of multi-component reactions has attracted considerable attention from the view of ideal synthesis by virtue of their efficiency, facile implementation and generally high yield of the products. These reactions are designed to produce elaborate biologically active compounds hence are an important area of research in organic, combinatorial and medicinal chemistry.

The known multi-component procedures for the synthesis of 2-amino-4H-pyran-3-carbonitriles employ a three-component condensation of cyclic 1,3-diketones, aldehydes and malononitrile and is performed under a variety of reaction conditions. Catalysts such as piperidine/ammonium acetate [1], triethylamine [2] are reported and the yields are in the range of 70-85 %. Alkyl ammonium salts in water [3], (S)-proline in aqueous media [4] afford the corresponding carbonitriles in higher yields (75-95 %), however they suffer from long reaction times up to 10 hours. As a consequence, reagents such as benzyltriethylammonium chloride (TEBA) [5], NaBr [6], microwave irradiation [7] and amino functionalized ionic liquid [8] are reported to catalyze these reactions including ammonium chloride [9], ethylenediamine diacetate [10], surfactant metal carboxylates [11] and b-cyclodextrin [12]. However, some of the reporting procedures have drawbacks such as tedious work-up, use of expensive reagents, long reaction times and low yields of products.

Insulating oxides such as SiO₂, Al₂O₃, silica-alumina and various zeolites are the materials commonly used as catalyst supports [13]. These oxides possess low thermal conductivity, generating sintering of the assisted metal on hot spots, various acidic and basic sites and the coverage of the catalyst with water at low temperature due to its hydrophobic surface. Various two-dimensional (2D) nanomaterials have received considerable developments for heterogeneous catalysis. Among them, boron nitride has attracted more attention because of its high elastic modulus, high melting-point, excellent thermal conductivity and a large and direct band gap. Such properties can be of high value for ultraviolet-light emitters, advanced ceramic composites, electrical insulators, solid lubricants and ideal substrates [14] so we have fixed boron nitride (BNT) as catalyst support. The graphene like hexagonal boron nitride [15] is a most stable isomer. A giant planar network of hexagonal boron nitride has an acid-base resistance, good thermal and electrical conductivity and chemically very inert. Moreover, BNT is hydrophobic, hence preventing moisture condensation on its surface. Activated BNT exhibits an excellent adsorption performance for various metal ions such as Cr⁶⁺, Co⁶⁺, Ni⁰³⁺, Ce⁴⁺, Pb⁴⁺ and organic pollutants (tetracycline, methyl orange and congo red) in water, as well as volatile organic compounds (benzene) in air [16].

Herein we report the synthesis and characterization of a new calcium loaded boron nitride catalyst which is subsequently used for a one-pot three component synthesis of 2-amino-4H-pyran-3-carbonitrile derivatives.
Cobalt boron nitride: A novel heterogeneous catalyst for the synthesis of medicinally important α-amino quinoline phosphonates

M. Sureshkumar, K. Anand, T. Muthu, and R. M. Gengan

Department of Chemistry, Durban University of Technology, Durban, South Africa

ABSTRACT

A novel cobalt supported on boron nitride (CoBNT) heterogeneous catalyst for the synthesis of α-amino quinoline phosphonates (AQPs) is reported in the present work. The CoBNT was synthesised by simply mixing boron nitride in a solution of cobalt acetate, under an inert atmosphere for 7 d followed by filtration; the yield was 94%. It exhibited excellent catalytic properties for the synthesis of 16 novel AQPs in a one pot mixture containing 2-methoxy-3-formyl quinoline, aniline derivatives and diethyl phosphite. Reactions were rapid, products were easily worked-up and were obtained in more than 90% yield. The CoBNT also exhibited higher catalytic activity than conventional catalysts and was re-used five times without significant decrease in catalytic activity.

1. Introduction

Over the next few decades, the synthesis of more effective drugs will increase all over the world owing to the outbreak of new diseases and illnesses. Therefore, more efforts are expected by the scientific community to address this dilemma by designing, synthesizing, and assessing new drugs for their biological potency. In particular, the synthesis of α-amino phosphonates (APS) has attracted significant interest in recent years because they exhibit useful biological activities such as the mimicking of peptides,1,2 and acting as enzyme inhibitors,3 antibiotics,4 pharmacological,5 and anticancer agents.6,7

Although several methods are reported for the synthesis of α-amino phosphonates,8 the Kabachnik–Fields (KF) and Pudovik reactions are commonly used. In the KF reaction, a carbonyl substrate and an amine are reacted with dialkyl or trialkyl phosphites in the presence of a catalyst (Scheme 1). Various catalysts are reported such as Bronsted–Lewis acids and heterogeneous catalysts such as SnCl4,9 ZnCl2,10 scandium tris (dodecyl sulfate),11 samarium diiodide,12 bismuth nitrate pentahydrate,13 bmimBF4,14 lithium perchlorate,15 montmorillonite KSF,16 and ZrCl4.17 These protocols however suffer from drawbacks such as long reaction time, low yield of the products, and formation of large amount of waste, while some catalysts are expensive,18–20 require relatively high amounts and display limited reusability. Hence, our aim was to synthesize novel α-amino quinoline phosphonates (AQP) but with the aid of an alternative novel heterogeneous catalysts. Also, we decided to