ACID CATALYZED THREE-COMPONENT ONE-POT SYNTHESIS OF SUBSTITUTED SIX MEMBERED HETEROCYCLIC COMPOUNDS OF BIOLOGICAL INTEREST

A Dissertation Submitted in Partial Fulfillment of the Requirement for the Degree of MASTER OF SCIENCE (M.Sc.) IN CHEMISTRY

SUBMITTED
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Session: April, 2016

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July, 2019
Dedicated to
My Beloved Family,
Beloved Grandfather
&
Honorable Supervisor
BANGLADESH UNIVERSITY OF ENGINEERING & TECHNOLOGY (BUET), DHAKA
DEPARTMENT OF CHEMISTRY

THESIS ACCEPTANCE LETTER

The thesis entitled “ACID CATALYZED THREE-COMPONENT ONE-POT SYNTHESIS OF SUBSTITUTED SIX MEMBERED HETEROCYCLIC COMPOUNDS OF BIOLOGICAL INTEREST” submitted by Zannatul Ferdous Sonia, Roll No: 0416032605F, Session- April/2016 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Master of Science (M.Sc) in chemistry on July 03, 2019.

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Fig. 11: $^1$H NMR spectrum of compound 10.

Fig. 12: $^{13}$C NMR spectrum of compound 10.

Fig. 13: IR spectrum of compound 13.

Fig. 14: $^1$H NMR spectrum of compound 13.

Fig. 15: $^{13}$C NMR spectrum of compound 13.

Fig. 16: IR spectrum of compound 15.

Fig. 17: $^1$H NMR spectrum of compound 15.

Fig. 18: $^{13}$C NMR spectrum of compound 15.

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<table>
<thead>
<tr>
<th>Elaborations</th>
<th>Abbreviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broad singlet</td>
<td>bd, s</td>
</tr>
<tr>
<td>Singlet</td>
<td>s</td>
</tr>
<tr>
<td>Doublet</td>
<td>d</td>
</tr>
<tr>
<td>Double doublet</td>
<td>dd</td>
</tr>
<tr>
<td>Triplet</td>
<td>t</td>
</tr>
<tr>
<td>Multiplet</td>
<td>m</td>
</tr>
<tr>
<td>Hertz</td>
<td>Hz</td>
</tr>
<tr>
<td>Mega Hertz</td>
<td>MHz</td>
</tr>
<tr>
<td>Infrared Spectroscopy</td>
<td>IR</td>
</tr>
<tr>
<td>Nuclear Magnetic Resonance</td>
<td>NMR</td>
</tr>
<tr>
<td>Proton NMR</td>
<td>$^{1}$H-NMR</td>
</tr>
<tr>
<td>Carbon-13 NMR</td>
<td>$^{13}$C-NMR</td>
</tr>
<tr>
<td>Coupling constant</td>
<td>$J$</td>
</tr>
<tr>
<td>Deuterated chloroform</td>
<td>CDCl$_3$</td>
</tr>
</tbody>
</table>


*p*-Toluenesulphonic acid is a highly effective and efficient catalyst for the one-pot multicomponent coupling of phenylaminoethylacrylate derivatives, substituted amines and substituted aldehydes in ethanol at ambient temperature to give highly functionalized piperidines in high yields. This one-pot reaction has some important advantages such as the easy workup procedure, simple and relatively available precursors and inexpensive catalyst and high yields. The multicomponent one-pot products showed moderate to good antibacterial and antifungal activity against *Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Citrobacter freundii, Aspergillus niger* and *Tricoderma harzianum*.

\[
\begin{align*}
\text{R}_1 = & -\text{OCH}_3, -\text{CH}_3, -\text{Br}, \text{H} \\
\text{R}_2 = & \text{H, Cl, NO}_2
\end{align*}
\]

**Scheme 1:** Synthesis of substituted six membered heterocyclic compounds

**Fig. 1:** Synthesis of piperidine derivative
<table>
<thead>
<tr>
<th>SI. No</th>
<th>Starting Material</th>
<th>Product</th>
<th>M.P.(°C)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>![1]</td>
<td>![4]</td>
<td>178-182</td>
<td>92</td>
</tr>
<tr>
<td>2</td>
<td>![2]</td>
<td>![7]</td>
<td>185-187</td>
<td>91</td>
</tr>
<tr>
<td>3</td>
<td>![3]</td>
<td>![10]</td>
<td>161-163</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>![4]</td>
<td>![13]</td>
<td>210-212</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>![5]</td>
<td>![15]</td>
<td>190-192</td>
<td>92</td>
</tr>
<tr>
<td>6</td>
<td>![6]</td>
<td>![17]</td>
<td>175-177</td>
<td>90</td>
</tr>
</tbody>
</table>

Table-1: Synthesis of Piperidine derivatives
Antibacterial and Antifungal Tests

Piperidine derivatives showed moderate to good antibacterial and antifungal activity against *Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Citrobacter freundii, Aspergillus niger* and *Tricoderma harzianum*. All the compounds were tested at 300 µg/disc concentration.

**Table 2: List of antimicrobial tested compounds’ names**

<table>
<thead>
<tr>
<th>Sample Code on Petri Plates</th>
<th>Compound no</th>
<th>Sample / compounds’ names</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>13</td>
<td><em>N</em>(4-bromophenyl)-2,6-diphenyl-3-ethylacetato-4(4-bromophenylamino)-piperidine-3-ene</td>
</tr>
<tr>
<td>16</td>
<td>15</td>
<td><em>N</em>-phenyl-2,6-di(4-chlorophenyl)-3-ethylacetato-4-phenylamino-piperidine-3-ene</td>
</tr>
<tr>
<td>17</td>
<td>10</td>
<td><em>N</em>-phenyl-2,6-diphenyl-3-ethylacetato-4-phenylamino-piperidine-3-ene</td>
</tr>
</tbody>
</table>
Table 3: List of antimicrobial tested compounds’ structures

<table>
<thead>
<tr>
<th>Sample Code on Petri Plates</th>
<th>Compound no</th>
<th>Sample / compounds’ structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>13</td>
<td><img src="image1" alt="Structure 13" /></td>
</tr>
<tr>
<td>16</td>
<td>15</td>
<td><img src="image2" alt="Structure 15" /></td>
</tr>
<tr>
<td>17</td>
<td>10</td>
<td><img src="image3" alt="Structure 10" /></td>
</tr>
</tbody>
</table>

The zones of inhibition produced by synthesized compounds (13, 15 and 10) were compared against the standard Ciprofloxacin for bacteria and Miconazole for fungi.

Microbial results revealed that the synthesized compounds 13 and 15 exhibited moderate to good antibacterial activity against *Bacillus subtilis*, *Escherichia coli* and *Citrobacter freundii*. Compound 10 showed moderate activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Citrobacter freundii*. Moderate fungicidal activity was observed against *Aspergillus niger* and *Tricoderma harzianum* (Table 8).
CHAPTER-1

INTRODUCTION
1.1 Introduction of Heterocyclic Compounds

Heterocyclic compounds play an important role in the field of synthetic organic chemistry. These compounds can have a variety of structures. The structures can be acyclic or cyclic. The cyclic system containing only carbon atoms are called carbocyclic and the cyclic systems containing carbons and at least one other element are called heterocyclic compounds. Though a number of heteroatom are known to be part of the heterocyclic rings, the most common heteroatom are nitrogen, oxygen and sulphur. A heterocyclic ring may contain one or more heteroatoms which may or may not be same. Also the rings may be saturated or unsaturated. Many heterocyclic compounds occur naturally and are actively involved in biological process e.g. nucleic acids (purine and pyrimidine bases), vitamins (Thiamine B$_1$, Riboflavin B$_2$, Nicotinamide B$_3$, Pyridoxol B$_6$ and Ascorbic acid C), heme and chlorophyl, penicillins, cephalosporins, macrolides etc. The study of heterocyclic chemistry is a vast and expanding area of chemistry because of their applications in medicine, agriculture, photodiodes and other fields. Heterocyclic compounds may be classified as aliphatic and aromatic heterocycles. The aliphatic heterocycles are the cyclic analogues of amines, ethers and thioethers and their properties are influenced by the ring strain. The three and four membered aliphatic heterocyclic rings are more strained and reactive compared to five and six membered rings. The common aliphatic heterocyclic compounds are aziridine, oxirane, thirane, azetidine, oxetane, thietane, pyrrolidine, tetrahydrofuran, tetrahydrothiophene and piperidine.
The heterocycles which show aromatic behavior as in benzene are called the aromatic heterocyclic compounds. These compounds follow the Hückel's rule which states that cyclic conjugated and planar systems having \((4n+2)\) \(\pi\) electrons are aromatic. Some simple aromatic heterocyclic compounds are pyrrole, furan, thiophene, imidazole, pyrazole, oxazole, thiazole and pyridine.
Nitrogen-containing heterocyclic compounds are widespread in nature, and their applications to biologically active pharmaceuticals, agrochemicals, and functional materials are becoming more and more important [1,2]. Therefore, the development of new efficient methods for synthesis of N-heterocycles is one major interest of modern synthetic organic chemistry [3–5]. Polyfunctionalized heterocyclic compounds play important roles in the drug discovery process and analysis of drugs in the latest development.

Piperidine is an organic compound with the molecular formula \((\text{CH}_2)_3\text{NH}\). This heterocyclic amine consists of a six-membered ring containing five methylene bridges (–CH\(_2\)–) and one amine bridge (–NH–). The name comes from the genus name Piper, which is the Latin word for pepper [6]. Although piperidine is a common organic compound, it is best known as a representative structure element within many pharmaceuticals and alkaloids.

1.2 Natural Occurrence

Piperidine was first reported in 1850 by the Scottish chemist Thomas Anderson and again independently in 1852 by the French chemist Auguste Cahours, who named it [7-9]. Both chemist obtained piperidine by reacting piperine with nitric acid.
Piperine 1 was discovered in 1819 by Hans Christian Ørsted, who isolated it from the fruits of Piper nigrum, the source plant of both black and white pepper [10]. Piperine was also found in Piper longum and Piper officinarum (Miq.) C.D.C.(Piper retrofractum Vahl), two species called “long pepper” [11].

Piperine is extracted from black pepper using dichloromethane [12]. Aqueous hydrotropes can be used in the extraction to result in high yield and selectivity [13]. The amount of piperine varies from 1–2% in long pepper, 5–10% in commercial white and black peppers [14]. Further, it may be prepared by treating the solvent-free residue from an alcoholic extract of black pepper, with a solution of potassium hydroxide to remove resin(said to contain chavicine, an isomer of piperine) and solution of the washed, insoluble residue in warm alcohol, from which the alkaloid crystallizes on cooling [15].

A component of pungency by piperine results from activation of the heat and acid sensing TRPV ion channels, TRPV1 and TRPA1 on nociceptors, the pain-sensing nerve cells [16]. Piperine is under preliminary research for its potential to affect bioavailability of other compounds in food and dietary supplements, such as a possible effect on the bioavailability of curcumin [17].

Piperidine itself has been obtained from black pepper [18,19], from Psilocaulon absimile (Aizoaceae) [20], and in Petrosimonia monandra [21]. The piperidine structural motif is present in numerous natural alkaloids. These include piperine, which gives black pepper its spicy
taste. Other examples are the fire antitoxin solenopsin [22], the nicotine analog anabasine of tree tobacco (Nicotiana glauca), lobeline of Indian tobacco, and the toxic alkaloid coniine from poison hemlock, which was used to put Socrates to death [23].

Piperidine alkaloids constitute one of the major classes of alkaloids and have been the subject of numerous reviews. Piperidine itself is a naturally occurring compound found in plants such as Piper nigrum L., Piperaceae and piperidine alkaloids are classified according to their natural source. Piperidine alkaloids can also be categorized on the basis of their structure, for example, 2,6-disubstituted piperidines, fused-ring piperidines, N-acylpiperidines, steroidal piperidines, piperidine alcohols, etc.

1.3 Synthetic Aspects

Industrially, piperidine is produced by the hydrogenation of pyridine, usually over a molybdenum disulfide catalyst [24].

\[
\begin{array}{cc}
\text{Pyridine} & \text{Piperidine} \\
\text{H}_2 & \text{H}_2 \\
\end{array}
\]

\text{Scheme 2: Synthesis of piperidine from pyridine}

Pyridine can also be reduced to piperidine 2 via a modified Birch reduction using sodium in ethanol [25].

A new route for the industrial preparation of piperidine has been developed recently at Mitsubishi’s fine chemical laboratory [26]. The key to this new process is the catalytic hydrogenolysis of tetrahydrofurfurylamine with cobalt in a suspended catalytic bed. Furfural is made to react with furfurylamine or tetrahydrofurfurylamine and the product undergoes hydrogenolysis in the presence of a slight excess of ammonia. The main product is piperidine. Because the catalyst is not 100% selective, some by-products are formed, which are minimized by adroit operation of the reactor.
On the laboratory scale, reduction of pyridine derivatives is also the most useful route for the synthesis of piperidine compounds [27-29]. Although the most common reagent was sodium and alcohol [30, 31] catalytic hydrogenation is the present method of choice. A variety of catalysts (nickel, palladium, ruthenium etc.) have been reported but platinum oxide in acetic acid normally gives very good results. An example of selectivity in the reduction of a pyridine derivative is given in scheme 3 [32]. Reduction of pyridine to piperidine can also be carried out electrochemically using a lead electrode and an aqueous sulphuric acid medium [33].

Scheme 3: Synthesis of piperidine derivative from the reduction of pyridine derivative

A variety of methods for building up the piperidine ring from aliphatic compounds have been described and widely applied in the piperidine alkaloid synthesis [34]. Ring closure takes place on 1,5-dihalides, 1,5-aminohalides, 1,5-diamines, 1,5-aminoalcohols etc. The classical reactions have been reviewed Elderfield [35]. Their usefulness relies upon the synthetic available of the aliphatic linear precursors. An example of piperidine ring elaboration by intramolecular cyclization is the synthesis of (±)-pseudoconhydrine 4 via reductive cyclization of a δ-nitroketone [36].
Scheme 4: Synthesis of piperidine derivative by intramolecular cyclization

Nevertheless, the great interest in piperidine alkaloids over the last few years due to their biological activity [34] has motivated the development of many reactions directed towards the chiral synthesis of such compounds or towards new synthetic methods.

A good example of piperidine ring elaboration is the different synthetic approaches described for the preparation of the alkaloid nitramine 5, a spiropiperidine alkaloid isolated from plants of the genus Nitraria which is of interest due to its spirocyclic structure related to the neurotoxic alkaloid histrionicotoxin 6 [37 a,b].
An enantioselective synthesis of (+)-nitramine was reported involving piperidine ring formation via intramolecular ring opening of the chiral epoxysulfone 7 [37c,d].

![Scheme 5: piperidine ring formation via intramolecular ring opening of compound 7](image)

Racemic nitramine was synthesized using an intramolecular Mannich reaction to set up the spirocyclic ring system. In this reaction the C\textsubscript{2}-C\textsubscript{3} piperidine bond was generated by attack of an enol ether, formed by acid catalyzed dioxolane ring opening upon the iminium salt 8 [38].

![Scheme 6: Synthesis of racemic nitramine using an intramolecular Mannich reaction](image)
A new approach to cis-2,6-dialkylpiperidine alkaloids such as (±)-dihydropinidine 9, was recently reported based on a highly region and stereoselective intramolecular acyl nitroso Diels-Alder cycloaddition leading to bicyclic oxazinolactam 10 which, by a stereocontrolled process involving a Grignard reaction followed by reduction, afforded bicyclic oxazines 11. Reductive N-O bond cleavage gave the corresponding piperidine derivative [39].

\[ \text{NHOOH} \rightarrow \text{O} \]

\[ \text{O} \]

\[ \text{H}_3\text{C}^- \]

\[ \text{H}_3\text{C}^- \]

\[ \text{OH} \]

\[ (\pm) \text{ Dihydropinidine} \]

\[ \text{Scheme 7: Synthesis of cis-2,6-dialkylpiperidine alkaloids} \]
Recently, a new method for obtaining trans-2,6-dialkylpiperidine alkaloids in high optical purity, such as (+)-deoxoprosopinine 12, based on the chemoenzymatic aza-Achmatowicz method has been published. This procedure permits the stereospecific generation of protected piperidines [40].

\[\text{Scheme 8: Synthesis of trans-2,6-dialkylpiperidine alkaloids}\]
Intramolecular amino- and amidomercuration of aminoolefins $\delta$-alkenylcarbamates, respectively have been used for piperidine ring elaboration in the synthesis of solenopsin A [41], an interesting antibacterial alkaloid, prosopis alkaloids [42] and (±)-pseudoconhydrine 13 [43].

An original strategy for obtaining piperidine alkaloids via an asymmetric synthesis consists of the formation of the piperidine ring functionalized on position 2 and 6 by means of a condensation of (-)-phenylglycinol and glutaraldehyde with addition of KCN to provide the 2-cyanopiperidine 14 in one step. This methodology, known as the CN(R,S) method has been widely applied as for example in the enantiospecific synthesis of (+)-coniine [44], (+)-solenopsine 15 [45] and the more complex piperidine derivative (+)-tetraponerine-8 [46].
Intramolecular cyclization of vinylsulfoxides has been very recently used in the enantioselective synthesis of (-)- and (+)-sedamine 16. Thus intramolecular addition of an amine anion generated by the action of benzyltriethylammonium hydroxide at -40 °C to (E)- and (Z)-vinylsulfoxides 17 occurs in the same diastereofacial manner giving chiral piperidines that differ in the relative stereochemistry at C-2 [47].

**Scheme 10:** Synthesis of (+)-solenopsine by CN(R,S) method
Scheme 11: Synthesis of (±)-sedamine by intramolecular cyclization

Acid-catalyzed reaction of cyclopentane tertiary azides affords α-substituted piperidines in good yields and this rearrangement has been exploited for the synthesis of (±)-dihydropinidine, (±)-coniceine and (±)-coniine [48]. Similarly, the formation of an epoxy azide via the corresponding olefin by epoxidation followed by its transformation into the amine has been used to prepare (±)-β-conhydrine 18 [49] following the sequence represented below:
Scheme 12: Synthesis of β-conhydrine

Very recently the importance of several polyhydroxylated piperidines as glucosidase inhibitors has been evidenced and the elaboration of piperidine ring in these cases presents a particular difficulty due to its high degree of functionalization [50,51]. For instance, the synthesis of nojirimycin δ-lactam 19 has been accomplished by reduction of an azidolactone by tin (II) chloride in methanol followed by treatment with potassium carbonate and final hydrogenolysis of the benzyl group. Formation of the piperidine ring in the analogue deoxynojirimycin 20 was carried out by generation of the bond between N and C-6 by intramolecular cyclization of an aminotriflate generated by reduction of a mixture of the azidotriflates with tin (II) chloride in methanol [52,53].
Scheme 13: Synthesis of nojirimycin δ-lactam & deoxynojirimycin

Recently, the synthesis of functionalized piperidines from a Mannich type cyclization involving iminium ion-vinylsilane cyclization [54], δ-chloroimines [55], 1,1-disubstituted hydrazines via the products of double condensation with benzotriazole and glutaraldehyde [56] and intermolecular conjugate addition of a nitrogen nucleophile to an electrophilic olefin followed by intramolecular tropping of the generated enolate by a built-in α,β-unsaturated acceptor [57] have also been reported.

1.4 Applications

1.4.1 In Pharmacology

The medicinal use of piperidine is very ancient. Because of being an alkaloid and a fragment of morphine, piperidine itself and its derivatives are pervasive component for the synthesis of many significant pharmaceuticals. Furthermore, piperidine fragment was substituted via variety of
synthetic reactions to develop more improved moieties with enhanced activities and to suppress the side effects when taken as medicine for different ailments [58, 59].

Many naturally occurring piperidine compounds have been experimentally proved excellent antibacterial agents [60] as like black pepper (Piper nigrum Longum), the main source of piperidine, showed 75% bactericidal inhibition against different genera of bacteria obtained from oral cavity [61, 62]. Various derivatives of piperidine molecule are used as antimicrobial agents and were found to show activity at a minimum inhibitory concentration [63, 64].

![Nalidixic acid](image)

Nalidixic acid 21 was the first nitrogen containing antibacterial agent [65]. Nalidixic acid is effective primarily against gram-negative bacteria, with minor anti-gram-positive activity. In lower concentrations, it acts in a bacteriostatic manner; that is, it inhibits growth and reproduction. In higher concentrations, it is bactericidal, meaning that it kills bacteria instead of merely inhibiting their growth. It has historically been used for treating urinary tract infections, caused, for example, by *Escherichia coli*, *Proteus*, *Shigella*, *Enterobacter*, and *Klebsiella*. It is no longer clinically used for this indication in the USA as less toxic and more effective agents are available. It is also a tool in studies as a regulation of bacterial division. It selectively and reversibly blocks DNA replication in susceptible bacteria. Nalidixic acid and related antibiotics inhibit a subunit of DNA gyrase and topoisomerase IV and induce formation of cleavage complexes [66]. It also inhibits the nicking-closing activity on the subunit of DNA gyrase that releases the positive binding stress on the supercoiled DNA.

Another were fluoroquinolones 22, broad-spectrum antibiotics that were well known drug for bacterial infections.
Fluoroquinolones are antibiotics that are commonly used to treat a variety of illnesses such as respiratory and urinary tract infections. Fluoroquinolones are often used for genitourinary infections and are widely used in the treatment of hospital-acquired infections associated with urinary catheters. In community-acquired infections, they are recommended only when risk factors for multidrug resistance are present or after other antibiotic regimens have failed. However, for serious acute cases of pyelonephritis or bacterial prostatitis where the person may need to be hospitalised, fluoroquinolones are recommended as first-line therapy [67]. Due to people with sickle-cell disease being at increased risk for developing osteomyelitis from the Salmonella, fluoroquinolones are the "drugs of choice" due to their ability to enter bone tissue without chelating it, as tetracyclines are known to do. Fluoroquinolones are featured prominently in guidelines for the treatment of hospital-acquired pneumonia [68].

The strong growth inhibition against Mycobacterium tuberculosis was examined by two substituted derivatives of the antihistaminic agents, Bamipine 23 and Diphenylpyraline 24,
both containing piperidine ring and also have strong H1-receptor antagonistic activity [69]. Bamipine is used as an antipruritic ointment [70]. Diphenylpyraline is a first-generation antihistamine with anticholinergic effects of the diphenylpiperidine class [71,72,73]. It is marketed in Europe for the treatment of allergies [71,72,74]. DPP has also been found to act as a dopamine reuptake inhibitor and produces hyperactivity in rodents [75]. It has been shown to be useful in the treatment of Parkinsonism [76].

Activity of urease (E. C 3.5.1.5) has been shown to be an important virulence determinant in the pathogenesis of many clinical conditions, which are detrimental for human and animal health as well as for agriculture. Urease is directly involved in the formation of infectious stones and contributes to the pathogenesis of urolithiasis, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma, urinary catheter encrustation [77-79]. It is also known to be a major cause of pathologies induced by *Helicobacter pylori* (HP), which allows bacteria to survive at low pH of the stomach during colonization and therefore plays an important role in the pathogenesis of gastric and peptic ulcer [78]. In agriculture, high urease activity causes significant environmental and economic problems by releasing abnormally large amounts of ammonia into the atmosphere during urea fertilization. This further induces plant damage primarily by depriving them from their essential nutrient, and secondly ammonia toxicity and increase the pH of the soil [77, 78]. Therefore, strategies based on urease inhibition were considered as the first line of treatment for infections caused by urease producing bacteria, hence depending on these facts different piperidine derivatives were discovered having urease inhibition activity 25 [80].
Different antibacterial targets are selected to achieve bacterial inhibition successfully that are aimed while developing structure of molecule via synthesis as like the inhibition of bacterial immunoglobulin G (IgG) of pyrogenes, by IdeS cleaving enzyme. Peptides demonstrated negative inhibition against IdeS enzyme but prominently proved as a potent inhibitor when glycine residue of peptides was replaced by piperidine moiety and on the basis of this criteria a variety of antibacterial compounds were developed [81,82].

A highly bioactive compound

Piperidine nucleus is an important core of many drug molecules. Piperidine and its analogues are reported in literature for various pharmacological activities like antihistamines and antibacterial [83], AChE inhibitors [84], and antitubercular agents [85]. It is known that clinically useful drugs such as miconazole, bifonazole, clotrimazole, and oxiconazole exhibit strong antimicrobial activity.

Piperidines and their derivatives comprise a major class of N-heterocycles of biological interest. Compounds bearing the piperidine moiety exhibit a broad range of biological properties, including antihypertensive [86], antimalarial [87], antiinflammatory [88], analgesic [89], [90], antioxidant [91], and antiproliferative activities [92]. Currently, several compounds containing the piperidine nucleus are employed in the current clinical as drug for treating diseases.
Donepezil 27 a potent, specific, non-competitive and reversible inhibitor of acetylcholinesterase is prescribed to treat patients with Alzheimer’s disease [93]. Pipamperone 28, other 1,4-substituted piperidine derivative, is indicated for patients with schizophrenia [94]. Vinblastine 29, a naturally occurring alkaloid derived from *Catharanthus roseus*, is used as anticancer agent for a wide variety of cancers including non-small cell lung cancer, breast cancer, bladder cancer, lymphomas, and leukemia [95].

1-(3-(2,4-Dichlorophenoxy)propyl)-1,2,3,6-tetrahydropyridine 30 works as a potent MAO-A inhibitor).
Prosopinine

6-(11-Hydroxydodecyl)-2-(hydroxymethyl)piperidine-3-ol

Prosopinine 31 was isolated from the leaves, stems, and roots of Prosopis africana Taub [96], and has a wide variety of biological activities such as acting as a sedative, hypotensive agent, spasmolytic, local anesthetic, antiseptic agent, etc.

2-Methyl-6-nonylpiperidin-4-ol

Piperidine 32 was isolated from the skin of poison-dart dendrobate frogs Dendrobates speciosus [97] and represents the only 2,6-disubstituted piperidine detected in frog skin extracts at more than trace levels. It blocks the action of acetylcholine by a non competitive blockade of the nicotinic receptor-channel complex.
Sedamine 33 was isolated from *Sedum acre* [98] and has been shown to competitively inhibit pea diamine oxidase [99]. These are belladonna alkaloids (made up of the drugs hyoscyamine, atropine, and scopolamine) and phenobarbital. Belladonna alkaloids help to reduce the symptoms of stomach and intestinal cramping. They work by slowing the natural movements of the gut and by relaxing the muscles in the stomach and intestines. Belladonna alkaloids belong to a class of drugs known as anticholinergics/antispasmodics. Phenobarbital helps to reduce anxiety. It acts on the brain to produce a calming effect. Phenobarbital belongs to a class of drugs known as barbiturate sedatives.

Adaline 34 is a defensive alkaloid isolated from the European two-spot ladybird beetle *Adalia bipunctata* [100]. Its structure has been determined by spectroscopic methods. This alkaloid was also shown to be present in all the life cycle stages of *Adalia bipunctata* as well as in the adults of a related species, *A. decempunctata.*
Histrionicotoxin 35 is one of the components of the defensive skin secretions of *Dendrobates* frogs, which acts as a venom as well as a mucosal tissue irritant toward mammals and reptiles. This alkaloid is believed to block the nicotinic acetylcholine receptor-channel complex as well as inhibit the binding sites associated with sodium, potassium, and calcium channels in brain membranes.

**Some Piperidine Medications**

Piperidine and its derivatives are ubiquitous building blocks in pharmaceuticals [101] and fine chemicals.

**a) Insect repellent**

Icaridin

1-(1-Methylpropoxycarbonyl)-2-(2-hydroxyethyl)piperidine 36
Icaridin 36, also known as picaridin, is an insect repellent. It has broad efficacy against various insects and ticks.

b) Stimulants and nootropics

![Methylphenidate Structure](image)

**Methylphenidate**

Methyl phenyl(piperidin-2-yl)acetate

37

Methylphenidate 37 is a stimulant medication used to treat attention deficit hyperactivity disorder (ADHD) and narcolepsy [102]. It is a first line medication for ADHD [102].

![Ethylphenidate Structure](image)

**Ethylphenidate**

(RS)-ethyl-2-phenyl-2-piperidine-2-ylacetate

38

Ethylphenidate (EPH) 38 is a psychostimulant and a close analog of methylphenidate. Ethylphenidate acts as both a dopamine reuptake inhibitor and norepinephrine reuptake inhibitor, meaning it effectively boosts the levels of the norepinephrine and dopamine neurotransmitters in
the brain, by binding to, and partially blocking the transporter proteins that normally remove those monoamines from the synaptic cleft.

\[
\text{Pipradrol}
\]
\[
\text{Diphenyl(piperidin-2-yl)methanol}
\]

Pipradrol (Meratran) \textbf{39} is a mild central nervous system stimulant (norepinephrine-dopamine reuptake inhibitor) that is no longer widely used in most countries due to concerns about its abuse potential. Pipradrol is still used in some European countries, and even rarely in the United States.

\[
\text{Desoxypipradrol}
\]
\[
\text{(RS)-2-benzhydrylpiperidine}
\]

Desoxypipradrol \textbf{40}, also known as 2-diphenylmethylpiperidine (2-DPMP), acts as a norepinephrine-dopamine reuptake inhibitor (NDRI) [103] developed by Ciba in the 1950s [104].
c) **SERM** (selective estrogen receptor modulators)

![Chemical structure of Raloxifene](image)

**Raloxifene**

[6-Hydroxy-2-(4-hydroxyphenyl)-benzo thiophen-3-yl]-
[4-[2-(1-piperidyl)ethoxy]phenyl]-methanone

Raloxifene 41 is a medication used to prevent and treat osteoporosis in postmenopausal women and those on glucocorticoids [105]. For osteoporosis it is less preferred than bisphosphonates [105]. It is also used to reduce the risk of breast cancer in those at high risk [105].

**d) Vasodilators**

![Chemical structure of Minoxidil](image)

**Minoxidil** 42

6-(1-Piperidinyl)-pyrimidine-2,4-diamine-3-oxide
Minoxidil is an antihypertensive vasodilator medication, available as a generic medication and over the counter for the treatment of androgenic alopecia, a form of hair loss [106].

e) Antipsychotic medications

Droperidol

\[
\text{Droperidol} \\
3-[1-[4-(4-fluorophenyl)-4-oxobutyl]-3,6-dihydro-2H-pyridin-4-yl]-
1H-benzimidazol-2-one
\]

Droperidol (Inapsine, Droleptan, Dridol, Xomolix, Innovar [combination with fentanyl]) is an antidopaminergic drug used as an antiemetic (that is, to prevent or treat nausea) and as an antipsychotic. Droperidol is also often used as a sedative in intensive-care treatment.

Haloperidol

\[
\text{Haloperidol} \\
4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)-butan-1-one
\]

Haloperidol is a typical antipsychotic medication [107]. Haloperidol is used in the treatment of schizophrenia, tics in Tourette syndrome, mania in bipolar disorder, nausea and vomiting, delirium, agitation, acute psychosis, and hallucinations in alcohol withdrawal [107,108,109]. Haloperidol may result in a movement disorder known as tardive dyskinesia which may be permanent [107]. Neuroleptic malignant syndrome and QT interval prolongation may occur [107]. In older people with psychosis due
to dementia it results in an increased risk of death [107]. When taken during pregnancy it may result in problems in the infant [107,110]. It should not be used in people with Parkinson's disease [107].

\[
\text{Melperone}
\]

1-(4-fluorophenyl)-4-(4-methylpiperidin-1-yl)-butan-1-one

Melperone 45 is an atypical antipsychotic of the butyrophenone chemical class, making it structurally related to the typical antipsychotic haloperidol. It first entered clinical use in 1960s [111]. Melperone is reported to be Cytochrome P450 2D6 (CYP2D6) inhibitor [112,113,114].

\[
\text{Mesoridazine}
\]

10-[2-(1-methylpiperidine-2-yl)-ethyl]-2-methylsulfinylphenothiazine

Mesoridazine (Serentil) 46 is a piperidine neuroleptic drug belonging to the class of drugs called phenothiazines, used in the treatment of schizophrenia. It is a metabolite of thioridazine. It
Risperidone has central antiadrenergic, antidopaminergic, antiserotonergic and weak muscarinic anticholinergic effects. Serious side effects include akathisia, tardive dyskinesia and the potentially fatal neuroleptic malignant syndrome. Mesoridazine was withdrawn from the United States market in 2004 due to dangerous side effects, namely irregular heart beat and QT-prolongation of the electrocardiogram.

\[
\text{Risperidone}
\]

3-[2-{4-(6-fluoro-1,2-benzoxazo-3-yl)piperidine-1-yl}ethyl]-2-methyl-6,7,8,9-tetrahydropyrido[1,2-a]pyrimidin-4-one

Risperidone 47 is an antipsychotic [115]. It is used to treat schizophrenia, bipolar disorder, and irritability associated with autism [115].

\[
\text{Thioridazine}
\]

10-\{2-[(RS)-1-Methylpiperidine-2-yl]-ethyl\}-2-methylsulfanylphenothiazine
Thioridazine (Mellaril or Melleril) \( \text{48} \) is a piperidine typical antipsychotic drug belonging to the phenothiazine drug group and was previously widely used in the treatment of schizophrenia and psychosis. The branded product was withdrawn worldwide in 2005 because it caused severe cardiac arrhythmias. However, generic versions are still available in the US [116].

f) Opioids

\[
\text{Dipipanone}
\]

\[
\text{4,4-diphenyl-6-(1-piperidinyl)-heptan-3-one}
\]

Dipipanone (Pipadone) \( \text{49} \) [117] is a strong opioid analgesic drug, used for very severe pain in cases where other analgesics are unsuitable, for instance where morphine is indicated but cannot be used due to the patient being allergic to morphine.

\[
\text{Fentanyl}
\]

\[
\text{N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]propanamide}
\]

\( \text{50} \)
Fentanyl **50**, also spelled fentanil, is an opioid used as a pain medication and together with other medications for anesthesia [118]. Fentanyl is also used as a recreational drug, often mixed with heroin or cocaine [119]. It has a rapid onset and effects generally last less than two hours [118].

![Fentanyl structure]

**Loperamide**

4-[4-(4-Chlorophenyl)-4-hydroxypiperidine-1-yl]-N,N-dimethyl-2,2-diphenylbutanamide

**51**

Loperamide **51** is a medication used to decrease the frequency of diarrhea [120]. It is often used for this purpose in gastroenteritis, inflammatory bowel disease, and short bowel syndrome [120]. It is not recommended for those with blood in the stool [120].

![Loperamide structure]

**Pethidine**

Ethyl-1-methyl-4-phenylpiperidine-4-carboxylate

**52**
Pethidine 52, also known as meperidine, is a synthetic opioid pain medication of the phenylpiperidine class [121,122,123,124,125,126]. Pethidine is the prototype of a large family of analgesics including the pethidine 4-phenylpiperidines (piminodine, anileridine and others), the prodines (alphaprodine, MPPP, etc.), bemidones (ketobemidone, etc.) and others more distant, including diphenoxylate and analogues [127]. Pethidine is indicated for the treatment of moderate to severe pain. For much of the 20th century, pethidine was the opioid of choice for many physicians; in 1975, 60% of doctors prescribed it for acute pain and 22% for chronic severe pain [128].

![Prodine molecule]

**Prodine**

(1,3-Dimethyl-4-phenylpiperidine-4-yl)propanoate

**53**

Prodine (trade names Prisilidine and Nisentil) 53 is an opioid analgesic that is an analog of pethidine (meperidine). It was developed in Germany in the late 1940s.
Ditran is an anticholinergic drug mixture, related to the chemical warfare agent 3-Quinuclidinyl benzilate (QNB). Ditran is composed of a mixture of 70% 1-ethyl-2-pyrrolidinylmethyl-alpha-phenylcyclopentylglycolate 54 and 30% 1-ethyl-3-piperidyl-alpha-phenylcyclopentylglycolate 55. These compounds are structural isomers and have very similar pharmacological properties. The ditran mixture is more potent as an anticholinergic than the piperidyl benzilate drugs such as N-methyl-3-piperidyl benzilate, but is less potent than QNB [129,130]. There has been a modest amount of scientific research using this mixture [131,132].
N-Methyl-3-piperidyl benzilate (JB-336) is an anticholinergic drug related to the chemical warfare agent 3-quinuclidinyl benzilate. N-methyl-3-piperidyl benzilate is less potent and shorter acting than 3-quinuclidyl benzilate, but like 3-QNB its effects on the central nervous system predominate over peripheral effects. It produces deliriant and hallucinogenic effects similar to those of plants such as datura and may be used recreationally at low doses. Radiolabelled versions of this drug are used in scientific research to map the distribution of muscarinic acetylcholine receptors in the brain [133].

1.4.2 In Molecular Biology

Piperidine is also commonly used in chemical degradation reactions, such as the sequencing of DNA in the cleavage of particular modified nucleotides. Piperidine is also commonly used as a base for the deprotection of moc-amino acids used in solid-phase peptide synthesis.

Piperidine is listed as a Table II precursor under the United Nations Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances due to its use (peaking in the 1970s) in the clandestine manufacture of PCP (1-(1-phenylcyclohexyl)piperidine, also known as angel dust, sherms, wet, etc.) [134].

1.4.3 In Agrochemical

Prior to the development of a wide range of relatively low-toxic synthetic pesticides, some piperidines, like anabasine, were used as insecticides. Their use was limited by their high toxicity to humans [135].

\[
\text{Anabasine} \\
3-(2\text{-piperidyl})\text{-pyridine}
\]
Piperidines and their analogues have received attention owing to their biological activities [136,137], e.g., they act as antimalarial [138], antihypertensive [139], antibacterial [140], anticonvulsant, and anti-inflammatory agents [141]. As a result, various synthetic approaches have been developed for the synthesis of piperidines based on intramolecular Mannich reaction onto iminium ions [142], imino Diels–Alder reaction [143,144], aza-Prins cyclizations [145, 146], intramolecular Michael reaction [147], and cyclopropane ring-opening/Conia-ene cyclization [148]. Recently, the syntheses of functionalized piperidines have been reported using Multi-component reactions (MCRs). However, some of these methods have drawbacks, such as long reaction times, unsatisfactory yields, or use of expensive catalysts. Therefore, it is necessary to further develop a simple and greener synthesis of piperidines without these problems.

1.5 Multi-Component Reactions (MCRs)
Multi-component reactions (MCRs) are one-pot processes in which three or more reactants come together in a single reaction vessel to give a final product. Currently, multi-component reactions are an important part of numerous research works involved in the drug discoveries to achieve synthetic targets in effective way, because they are easy to carry out and provide rapid access to libraries of organic compounds with diverse substitution patterns [149-151].

One-pot reactions where several reactions sequences are conducted in the same reaction flask are one of the methods that can be used in order to conduct synthesis in a greener fashion. The chemistry is greener due to the reduction of work-up procedures and purification steps required compared to a more stepwise approach. In catalytic reaction it is possible to combine several catalytic processes in the same reaction vessel.

The most significant advantage, in the synthetic point of view is that it is less likely to lose material that would otherwise be lost during work up and purification.

The second advantage is the economy of utilizing chemicals and solvents. To work up the reaction at each step, it always needs to use some sort of solvents that would come to waste eventually. One-flask reaction multicenter involving multiple steps would only require final workup at the final step.

The third advantage is the minimal amount of work required. Instead of working up each step of the synthesis involving, say, three steps, it only need to do one work up at the end.
In chemistry a one-pot synthesis is a strategy to improve the efficiency of a chemical reaction whereby a reactant is subjected to successive chemical reactions in just one reactor. This is much desired by chemists because avoiding a lengthy separation process and purification of the intermediate chemical compounds would save time and resources while increasing chemical yield.

An example of a one-pot synthesis is the total synthesis of tropinone or the Gassman indole synthesis. Sequential one-pot syntheses can be used to generate even complex targets with multiple stereocentres, such as oseltamivir [152], which may significantly shorten the number of steps required overall and have important commercial implications. A sequential one-pot synthesis with reagents added to a reactor one at a time and without work-up is also called a telescoping synthesis. In one such procedure [153] the reaction of 3-N-tosylaminophenol I with acrolein II affords a hydroxyl substituted quinoline III through 4 sequential steps without workup of the intermediate products [154].

Scheme 14: Synthesis of hydroxyl substituted quinoline (III)
Multicomponent reactions are convergent reactions, in which three or more starting materials react to form a product, where basically all or most of the atoms contribute to the newly formed product. In an MCR, a product is assembled according to a cascade of elementary chemical reactions. Thus, there is a network of reaction equilibria, which all finally flow into an irreversible step yielding the product. The challenge is to conduct an MCR in such a way that the network of pre-equilibrated reactions channel into the main product and do not yield side products. The result is clearly dependent on the reaction conditions: solvent, temperature, catalyst, concentration, the kind of starting materials and functional groups.

In chemistry, a multi-component reaction (or MCR), sometimes referred to as a “Multi-component Assembly Process” (or MCAP), is a chemical reaction where three or more compounds react to form a single product. By definition, multicomponent reactions are those reactions whereby more than two reactants combine in a sequential manner to give highly selective products that retain majority of the atoms of the starting material. Multicomponent reactions have been known for over 150 years. First documented multicomponent reaction was the Strecker synthesis of α-amino cyanides in 1850 from which α-amino acids could be derived. A multitude of MCRs exist today, of which the isocyanide based MCRs are the most documented. Other MCRs include free-radical mediated MCRs, MCRs based on organoboron compounds and metal-catalyzed MCRs.

Multicomponent reaction (MCR) is a synthetic methodology in which three or more reactants come together in a single reaction vessel to form a new product. The characteristic aspect of MCRs is that the final products contain almost all portions of substrates, generating almost no by-products. That makes MCRs an extremely ideal and eco-friendly reaction system. Target compounds can be obtained in one pot with much fewer steps. Therefore, MCRs have been paid much attention in various research fields, such as discovery of lead compounds in medicinal chemistry, or combinatorial chemistry.
1.5.1 Some Examples of Multicomponent Reaction (MCR)

There have been a number of reports on MCRs so far, and typical examples are described as below:

**Strecker reaction**
(Three-component reaction: 3CR)

Strecker reaction (Amino acid synthesis)

\[
\begin{align*}
\text{RCHO} & \xrightarrow{\text{NaCN, NH}_3} \text{RCHNH}_2\text{CN} & \xrightarrow{\text{H}_3\text{O}^+} \text{RCHNH}_2\text{COOH}
\end{align*}
\]

**Scheme 15:** Synthesis of amino acid by Strecker reaction

This reaction was reported by A. Strecker in 1850, and is extremely famous as the synthesis of α-amino acids. This reaction is an MCR which comprises three components, aldehydes, hydrogencyanide, and ammonia as substrates, and is recognized as the world’s first MCR, [155].

**Hantzsch dihydropyridine synthesis (3CR)**

\[
\begin{align*}
\text{R}_2\text{O} & + \text{R}_3\text{CHO} + \text{NH}_3 \rightarrow \text{R}_2\text{O} & \text{R}_3\text{O} & \text{R}_1\text{N} & \text{R}_1\text{O} & \text{OR}_2
\end{align*}
\]

**Scheme 16:** Synthesis of 1,4-dihydropyridine derivatives

This reaction was reported by A. R. Hantzsch in 1881, and is the best-known three-component reaction, which affords 1,4-dihydropyridine derivatives using β-keto esters, aldehydes, and ammonia [156]. For an example, a calcium channel blocker “Nifedipine” is also synthesized by this reaction [157].
Scheme 17: Synthesis of nifedipine (a calcium channel blocker)

Biginelli reaction (3CR)

Scheme 18: Synthesis of dihydropyrimidinone derivatives

In 1891, an Italian chemist, P. Biginelli has reported the three component reaction using β-keto esters such as ethyl acetoacetate, aromatic aldehydes such as benzaldehyde, and ureas (or thioureas) in the presence of acid catalyst (Brönsted or Lewis acids), affording dihydropyrimidinone derivatives. [158] Dihydropyrimidinones have been paid much attention because of their various bioactivities such as anti-inflammatory or anti-bacterial activities. For an example of pharmaceuticals developed by using the reaction, several anti-tubercular agents have been reported.

Passerini reaction (3CR)

Scheme 19: Synthesis of acyloxy amide derivatives
In 1921, an Italian chemist, M. Passerini et al. have reported the three-component reaction using carboxylic acids, aldehydes, and isonitriles, affording acyloxy amides [159]. The Passerini reaction also has been applied into pharmaceutical research, for example, Hulme et al. have reported the library synthesis of novel norstatine derivatives bearing benzimidazole moieties [160].

**Gröebke-Blackburn-Bienaymé reaction [161]**

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{R}_1 \\
\text{H} & \quad \text{R}_2\text{-CHO} \\
\text{R}_3\text{-NC} & \quad \text{R}_3 \\
\text{H} & \quad \text{R}_2 \\
\text{N} & \quad \text{R}_1
\end{align*}
\]

Scheme 20: Synthesis of fused nitrogen-containing aromatic compounds

This reaction is a three-component reaction using aldehydes, isonitriles and aminoazines such as 2-aminimidazole or 2-aminopyridine in the presence of acid catalyst. The reaction is applicable for the synthesis of fused nitrogen-containing aromatic compounds.

**Kabachnik-Fields reaction (3CR)**

\[
\begin{align*}
\text{R}_1\text{H} & \quad \text{R}_2\text{-NH}_2 \\
\text{R}_2\text{H} & \quad \text{OR}_3 \\
\text{OR}_3 & \quad \text{OR}_3
\end{align*}
\]

Scheme 21: Synthesis of α-aminophosphonates
In 1952, M. I. Kabachnik et al. have reported the three-component reaction using aldehydes, amines, and dialkyl phosphites in the presence of acid catalyst (Brønsted or Lewis acids), afforded α-aminophosphonates [162].

In recent years, much attention has been paid to α-aminophosphonates since they can be considered as structural analogues of the corresponding α-amino acids and transition state mimics of peptide hydrolysis. Thus, α-aminophosphonates have been applied into several research areas, such as development of renin inhibitors or HIV protease inhibitors [163].

**Ugi reaction (4CR)**

\[
\begin{array}{c}
R_1^+H + R_2^-NH_2 + R_3^-NC + R_4^-COOH \\
\rightarrow
\end{array}
\]

**Scheme 22: Synthesis of α-aminoacyl amide derivatives**

This reaction is the four-component reaction reported by I. K. Ugi et al. in 1962 for the first time. It enables one-pot condensation of four components (aldehydes, amines, isonitriles, and carboxylic acids), thus, it can be said that the Ugi reaction is the most versatile among MCRs.

**Other Examples of MCR**

MCR using \( p \)-toluenesulfonylmethyl isocyanide (TosMIC) (3CR)

\( p \)-Toluenesulfonylmethyl isocyanide (TosMIC) (T1046) is a synthetic reagent, developed by Leusen et al., and has both an isonitrile group and a tosyl group (leaving group) in one molecule, [164]. Different from other isonitrile compounds with odor character, TosMIC is an odorless and solid compound. Because of its easy-handling property, TosMIC has been widely used for the synthesis of nitrogen-containing aromatic heterocyclic compounds, such as oxazoles [165]. TosMIC also has been used for MCRs, for example, Tsoleridis et al. reported the synthesis of quinoxaline derivatives via the three-component condensation of \( o \)-phenylenediamines, aromatic aldehydes and TosMIC [166].
Scheme 23: Synthesis of quinoxaline derivatives

Table 4: Synthesis of piperidine derivatives using TosMIC

<table>
<thead>
<tr>
<th>Entry</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Ar</th>
<th>Base</th>
<th>Quinoxaline (Y.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>H</td>
<td>H</td>
<td>Phenyl</td>
<td>DABCO</td>
<td>91%</td>
</tr>
<tr>
<td>2.</td>
<td>H</td>
<td>H</td>
<td>2,4-dimethylphenyl</td>
<td>DABCO</td>
<td>81%</td>
</tr>
<tr>
<td>3.</td>
<td>H</td>
<td>H</td>
<td>4-chlorophenyl</td>
<td>DABCO</td>
<td>84%</td>
</tr>
<tr>
<td>4.</td>
<td>Me</td>
<td>Me</td>
<td>Phenyl</td>
<td>DBU</td>
<td>86%</td>
</tr>
<tr>
<td>5.</td>
<td>Me</td>
<td>Me</td>
<td>2-methylphenyl</td>
<td>DBU</td>
<td>85%</td>
</tr>
</tbody>
</table>

MCR Using Benzynes (3CR)

Recently, there also have been several reports on MCRs using benzynes. For example, Yoshida et al. have reported the three-component MCR using in situ generated benzynes, imines, and carbon dioxide, affording benzoxadinones [167].

Recently, much attention has been paid on organic synthesis using carbon dioxide as a carbon source from the ecological point of view, thus, the reaction is an extremely useful and eco-friendly MCR.

Scheme 24: Synthesis of benzoxadinones
Thus, MCR is a strong synthetic methodology to enable condensation of various substrates in one pot, however, in some cases, reactions require long times for completion or result in undesired side reactions even after optimization of reaction conditions such as solvents or Lewis acid catalysts. For resolving these problems, there have been some successful reports on accelerating MCRs. For example, Shaabani et al. have reported the ionic liquid promoted Grobcke-Blackburn-Bienayme reaction. As indicated in the table below (Table 5), in the case of using ionic liquids as solvents, reactions proceed smoothly to afford the desired products in excellent yields. On the other hand, the yield of product is poor even in the prolonged reaction time. Moreover, as indicated in Entry 1, the ionic liquid can be reused for the same reactions but maintain the high yields.

![Scheme 25: Synthesis of fused nitrogen-containing aromatic compounds](image)

**Table 5:** Synthesis of fused nitrogen-containing compounds using ionic liquids as solvents

<table>
<thead>
<tr>
<th>Entry</th>
<th>R₁</th>
<th>R₂</th>
<th>Yield(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Br</td>
<td>Ph</td>
<td>98</td>
</tr>
<tr>
<td>2.</td>
<td>Me</td>
<td>Ph</td>
<td>98</td>
</tr>
<tr>
<td>3.</td>
<td>Me</td>
<td>Ph</td>
<td>25</td>
</tr>
<tr>
<td>4.</td>
<td>Me</td>
<td>4-CH₃C₆H₄</td>
<td>99</td>
</tr>
<tr>
<td>5.</td>
<td>Me</td>
<td>4-O₂NC₆H₄</td>
<td>92</td>
</tr>
<tr>
<td>6.</td>
<td>Me</td>
<td>4-Pyridly</td>
<td>97</td>
</tr>
</tbody>
</table>
All of the newly synthesized compounds were evaluated for their in vitro growth inhibitory activities against a panel of standard strains of pathogenic microorganisms including three Gram-positive bacteria, three Gram-negative bacteria, and three strains of fungi. The antimicrobial studies were assessed by minimum inhibitory concentration (MIC) using the broth dilution method [168,169]. MIC is the highest dilution of a compound which shows clear fluid with no development of turbidity.

Multi-component reactions (MCRs) are convergent reactions, in which three or more starting materials react to form a product, where basically all or most of the atoms contribute to the newly formed product [170]. In an MCR, a product is assembled according to a cascade of elementary chemical reactions. Thus, there is a network of reaction equilibria, which finally flow into an irreversible step yielding the product. The challenge is to conduct an MCR in such a way that the network of pre-equilibrated reactions channel into the main product and do not yield side products. The result is clearly dependent on the reaction conditions: solvent, temperature, catalyst, concentration, the kind of starting materials and functional groups. Such considerations are of particular importance in connection with the design and discovery of novel MCRs [171].

In the drug discovery process, MCR offers many advantages over traditional approaches. With only a limited number of chemists and technicians, more scaffold synthesis programs can be achieved within a shorter time. With one pot reactions, each synthetic procedure (weighing of reagents, addition of reagents, reaction time, control) and work-up procedure (quenching, extraction, distillation, chromatography, weighing, and analysis) needs to be performed only once, in contrast to multistep synthesis. MCRs are compatible with a solution phase approach, thus enabling a simple monitoring and they are easily amenable to automation. Moreover, each scaffold is expandable from a low number of compounds (scouting library) to a larger library. Thus, “hit-to-lead” transitions are normally accomplished easily and promptly. Certain physicochemical properties can be built into a library, e.g. lipophilicity and aqueous solubility, molecular weight, numbers of hydrogen donors and acceptors and the number of rotatable bonds, as well as the polar surface area. Finally, scale-up is often possible from a preclinical lab-scale (mg, gram) to clinical exploratory amounts (kg) using the same type of chemistry [172]. Drug molecules derived from MCR are very cost effective which is the need of the hour. MCRs have received considerable attention because of the complexity of the molecules that can be easily
achieved from readily available starting materials in one reaction sequence [173]. MCRs generally occur in one pot and exhibit high atom economy and product selectivity. In most of the cases, they yield a single product and thus MCRs are advantageous over linear stepwise synthesis because of operational simplicity, reduction in reaction time, ecological friendliness, saving of money and raw materials, inexpensive purification, and avoidance of protection and deprotection processes [174].

A simple, economical and one-pot protocol is developed for construction of multiple carbon–carbon bonds having C(sp2)-C(sp2) and C(sp2)-C(sp) hybridization, as these intriguing molecular architectures exhibit significant optoelectronic and medicinal properties. To this end, a sequential reaction is devised that allows one-pot three-component reactions of hydroxybenzaldehydes (C₆–C₁ unit), halo-phenylacetic acids (C₆–C₂ unit) and arylacetylenes (C₆–C₃ unit) resulting in the formation of highly π-conjugated complex arylethenyl-arylethynyl-arenes (C₆–C₂–C₆–C₂–C₆ unit) bearing a hydroxyl group via sequential perkin condensation–decarboxylation–Sonogashira coupling in polyethylene glycol with microwave irradiation. The process avoids protection–deprotection manipulations, generates CO₂ and H₂O as by-products and is successfully extended for the efficient construction of a wide series of novel aliphatic/ Alicyclic/ hetero/ aromatic arylethenyl-arylethynyl-arenes with pendant hydroxy functionality, which could undergo further chemical modification to achieve target biological and physical properties.

Synthetic methods that rapidly generate molecular complexity from simple starting materials in a tandem (consecutive or sequential) manner are highly attractive for the pot-economic synthesis of bioactive organic molecules, natural products and functional materials. Such methods preclude the isolation or purification of intermediates and are more efficient and sustainable. Thus, one-pot tandem [175] reactions have become innovative protocols for the construction of several bonds, including carbon–carbon bonds. In this way, construction of multiple carbon–carbon single bonds with double and triple bonds in one pot for the synthesis of privileged arylethenyl-arylethynylarenes with C(sp²)-C(sp²) and C(sp²)-C(sp) hybrid orbitals would be highly advantageous because such extended p-conjugated[2,3] scaffolds (with enyne bonds) are of considerable interest for their wide range of optoelectronic and medicinal properties.
Conventionally, construction [176] of these highly significant C(sp²)-C(sp²) and C(sp³)-C(sp) hybridized molecules has been mainly achieved either by Sonogashira coupling between halostilbenes and phenylacetylenes or by Cadiot–Chodkiewicz [177] coupling. Furthermore, Heck–Sonogashira sequences or the Suzuki–Miyaura [178] reaction (between potassium alkynyl-aryltrifluoroborates and aryl halides) are also useful for synthesizing such p-conjugated molecules. The synthesis of similar polyaromatic molecules can also be achieved by Sonogashira coupling followed by Wadsworth–Emmons reaction using diethyl (4-cyanobenzyl) phosphonate [179]. The Heck–Sonogashira reaction sequence has also been reported but this yielded only non-hydroxylated arylethenyl-arylethynyl-arene products [180]. Furthermore, use of expensive and unstable substrates, multistep synthesis and generation of waste, as well as protecting group manipulations [181] in the case of hydroxy-substituted arylethenyl-arylethynyl-arenes are of concern from green chemistry and economical perspectives. Highly p-conjugated hydroxylated aromatic molecules [182] have gained importance for their biological activities and applications as advanced materials, as the presence of hydroxy groups allows further chemical modification to achieve desired properties.

1.6 Use of Organic Catalyst
The use of organic catalysts has received considerable attention in organic synthesis owing to their important advantages such as the possibility of performing reactions with acid-sensitive substrates, milder reaction conditions, and selectivity [183–185]. *p*-Toluenesulfonic acid monohydrate (*p*-TsOH·H₂O) has received considerable attention in organic synthesis owing to its advantages such as low cost and ease of handling and isolation [186–188]. In continuation of our work on MCRs [189–193], in this work we report a simple and efficient procedure for the **one-pot multi-component** synthesis of highly substituted piperidine derivatives.
CHAPTER 2

EXPERIMENTAL
2.1 Synthesis of N(4-methoxyphenyl)-2,6-diphenyl-3-ethylacetato-4(4-methoxyphenylamino)-piperidine-3-ene (4)

A solution of 3(4-methoxyphenylamino)-3-methylethylacrylate 1 (1 mmol), 4-methoxyaniline 2 (1 mmol) and benzaldehyde 3 (2 mmol) in 5 ml EtOH was stirred for about 6 hours in the presence of 0.11 g p-TsOH.H2O at ambient temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC). After completion of the reaction, the thick white precipitate was filtered off and washed with ethanol (6 ml) to give the pure product 4 with 92% yield and the melting point was recorded as 179 °C-181 °C.

IR (KBr, cm⁻¹, Fig. 4): 3242 (N-H), 3060 (C-H, Ar), 2931, 2835 (C-H, aliphatic), 1650 (C=O), 1611, 1596, 1509, 1470 (C=C, aromatic and aliphatic), 1372 (C-O, bending).

¹H NMR (400 MHz, CDCl₃, δ ppm, Fig. 5): 1.46 (t, 3H, J=8.0 Hz, CH₃), 2.6 (dd, 1H, J=15.2 Hz, 2.4 Hz, H-5), 2.81 (dd, 1H, J=5.6 Hz, J=5.6 Hz, H'-5), 3.66 (s, 3H, OCH₃), 3.74 (s, 3H, OCH₃), 4.31 (m, 1H, CH₂-CH₃), 4.44 (m, 1H, CH₂-CH₃), 5.06 (d, 1H, J=3.2 Hz, H-6), 6.2 (d, 2H, J=8.8 Hz, Ar-H), 6.37 (bd, s, 1H, H-2), 6.46 (d, 2H, J=8.0 Hz, Ar-H), 6.61 (d, 2H, J=8.8 Hz, Ar-H), 6.67 (d, 2H, J=9.2 Hz, Ar-H), 7.20 (t, 3H, J=8.0 Hz, Ar-H), 7.26 (t, 5H, J=8.0 Hz, Ar-H), 7.30 (t, 2H, J=8.0 Hz, Ar-H), 10.16 (s, 1H, N-H).

¹³C NMR (100 MHz, CDCl₃, δ ppm, Fig. 6): 168.2 (1C, C=O), 157.7 (1C, Ar), 156.8 (1C, Ar), 150.8 (1C, Ar), 144.3 (1C, Ar), 143.2 (1C, Ar), 141.5 (1C, Ar), 130.6 (1C, Ar), 128.5 (1C, C=C), 128.1 (1C, C=C), 127.8 (3C, Ar), 127.0 (3C, Ar), 126.7 (3C, Ar), 126.1 (3C, Ar), 114.4 (2C, Ar), 114.0 (2C, Ar), 97.1 (1C, Ar), 59.4 (1C, OCH₃), 58.2 (1C, OCH₃), 55.6 (1C, C-6), 55.5 (1C, C-2), 55.3 (1C, C-5), 33.5 (1C, CH₂), 14.7 (1C, CH₃).
2.2 Synthesis of \(N(4\text{-methylphenyl})-2,6\text{-diphenyl}-3\text{-ethylaceto-}4(4\text{-methylphenylamino})\text{-piperidine-3-ene}\ (7)

\[
\begin{align*}
3(4\text{-methylphenylamino})-3\text{-methylethylacrylate} \ 5 & \quad (1 \text{ mmol}), \\
4\text{-methylaniline} \ 6 & \quad (1 \text{ mmol}) \quad \text{and} \\
\text{benzaldehyde} \ 3 & \quad (2 \text{ mmol}) \quad \text{in} \ 5 \text{ ml EtOH was stirred for about 1.30 hours in the} \\
& \quad \text{presence of} \ 0.11 \text{ g} \ p\text{-TsOH.H}_2\text{O} \text{ at ambient temperature. The progress of the reaction was} \\
& \quad \text{monitored by thin-layer chromatography (TLC). After completion of the reaction, the thick white} \\
& \quad \text{precipitate was filtered off and washed with ethanol (6 ml) to give the pure product} \ 7 \quad \text{with 91\% yield and the melting point was recorded as} 185 \degree C-187 \degree C.
\end{align*}
\]

\textbf{IR (KBr, cm}^{-1}, \text{ Fig. 7):} 3240 (N-H), 3026 (C-H, Ar), 2926, 2861 (C-H, aliphatic), 1650 (C=O), 1618, 1595, 1516, 1500 (C=C, aromatic and aliphatic), 1372 (C-O, bending).

\textbf{\textsuperscript{1}H NMR (400 MHz, CDCl} _3, \text{ δ ppm, Fig. 8):} 1.45 (t, 3H, \text{J}=8.0 \text{ Hz, CH}_3), 2.15 (s, 3H, CH}_3), 2.25 (s, 3H, CH}_3), 2.71 (dd, 1H, \text{J}=1.6 \text{ Hz, 1.6 Hz, H-5}), 2.83 (dd, 1H, \text{J}=5.6 \text{ Hz, 5.6 Hz, H'-5}), 4.33 (m, 1H, OCH}_2), 4.50 (m, 1H, OCH}_2), 5.11 (d, 1H, \text{J}=3.6 \text{ Hz, H-6}), 6.15 (d, 2H, \text{J}=8.0 \text{ Hz, Ar-H}), 6.42 (s, 1H, H-2), 6.44 (bd, s, 2H, Ar-H), 6.88 (m, 4H, Ar-H), 7.1 (m, 3H, Ar-H), 7.24 (m, 5H, Ar-H), 7.29 (m, 1H, Ar-H), 10.21 (s, 1H, N-H).

\textbf{\textsuperscript{13}C NMR (100 MHz, CDCl}_3, \text{ δ ppm, Fig. 9):} 168.2 (1C, C=O), 156.4, 144.8, 144.3, 143.02, 135.5, 135.2, 129.8, 129.43, 129.40 (9C, Ar), 128.5, 128.1 (2C, C=C), 127.0, 126.6, 126.1 (9C, Ar), 125.9, 125.0 (4C, Ar), 112.8, 97 (2C, Ar), 59.5, 58.2, 55.1, 33.5, 20.8, 20.1, 14.8 (7C, aliphatic).
### 2.3 Synthesis of N-phenyl-2,6-diphenyl-3-ethylacetato-4-phenylamino-piperidine-3-ene (10)

A solution of 3(phenylamino)-3-methylethylacrylate 8 (1 mmol), aniline 9 (1 mmol) and benzaldehyde 3 (2 mmol) in 5 ml EtOH was stirred for about 4.30 hours in the presence of 0.11 g p-TsOH.H₂O at ambient temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC). After completion of the reaction, the thick white precipitate was filtered off and washed with ethanol (6 ml) to give the pure product 10 with 93% yield and the melting point was recorded as 161 °C-163 °C.

**IR (KBr, cm⁻¹, Fig. 10):** 3249 (N-H), 3061 (C-H, Ar), 2982, 2875 (C-H, aliphatic), 1651 (C=O), 1594, 1592, 1500, 1450 (C=C, aromatic and aliphatic), 1373 (C-O, bending).

**¹H NMR (400 MHz, CDCl₃, δ ppm, Fig. 11):** 1.53 (bd, s, 3H, CH₃), 2.83 (d, 1H, J=15.6 Hz, H-5), 2.95 (d, 1H, J=4.0 Hz, H'-5), 4.41 (bd, s, 1H, OCH₂), 4.53 (bd, s, 1H, OCH₂), 5.21 (bd, s, 1H, H-6), 6.34 (bd, s, 1H, H-2), 6.59 (m, 4H, Ar), 7.13 (bd, s, 5H, Ar), 7.32 (m, 11H, Ar), 10.36 (s, 1H, N-H).

**¹³C NMR (100 MHz, CDCl₃, δ ppm, Fig. 12):** 168.2 (1C, C=O), 156.1, 147.0, 144.0, 142.8, 137.9, 128.9, 128.8 (12C, Ar), 128.6, 128.2 (2C, C=C), 127.1, 126.8, 126.6, 126.4, 126.3, 125.8, 125.7, 116.1, 112.9, 98.2 (12C, Ar), 59.7 (OCH₂), 58.2 (C-2), 55.1 (C-6), 33.6 (C-5), 14.8 (C, CH₃)
2.4 Synthesis of \(N(4\text{-bromophenyl})-2,6\text{-diphenyl-3-ethylcarboxylato-4(4-bromophenylamino)-piperidine-3-ene}\) (13)

A solution of 3(4-bromophenylamino)-3-methylethylacrylate 11 (1 mmol), 4-bromoaniline 12 (1 mmol) and benzaldehyde 3 (2 mmol) in 5 ml EtOH was stirred for about 2.30 hours in the presence of 0.11 g \(p\)-TsOH.H₂O at ambient temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC). After completion of the reaction, the thick white precipitate was filtered off and washed with ethanol (6 ml) to give the pure product 13 with 88% yield and the melting point was recorded as 210 °C-212 °C.

IR (KBr, cm\(^{-1}\), Fig. 13): 3242 (N-H), 3085 (C-H, Ar), 2977, 2912, 2861 (C-H, aliphatic), 1652 (C=O), 1604, 1589, 1493, 1451 (C=C, aromatic and aliphatic), 1373 (C-O, bending).

\(^1\)H NMR (400 MHz, CDCl\(_3\), δ ppm, Fig. 14): 1.47 (bd, s, 3H, CH\(_3\) ), 2.71 (d, 1H, \(J=12\) Hz, H-5), 2.83 (d, 1H, \(J=11.6\) Hz, H’-5), 4.34 (bd, d, 2H, \(J=15.2\) Hz, OCH\(_2\) ), 5.10 (s, 1H, H-6), 5.91 (bd, s, 2H, Ar-H, H-2), 6.39 (bd, s, 3H, Ar-H), 7.16 (m, 14H, Ar-H), 10.24 (s, 1H, N-H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\), δ ppm, Fig. 15): 168.0 (1C, C=O), 155.1, 145.8, 143.0, 142.0, 136.8 (5C, Ar), 132.3, 131.9, 131.5 (8C, Ar), 128.8, 128.3 (2C, C=C), 127.4, 127.2, 126.4, 126.2 (7C, Ar), 119.1, 114.5, 108.3, 98.7 (4C, Ar), 59.9, 58.2, 55.1, 33.4, 14.7 (5C, aliphatic).
2.5 Synthesis of N-phenyl-2,6-di(4-chlorophenyl)-3-ethylcarboxylato-4-phenylamino-piperidine-3-ene (15)

A solution of 3(phenylamino)-3-methylethylacrylate 8 (1 mmol), aniline 9 (1 mmol) and 4-chlorobenzaldehyde 14 (2 mmol) in 5 ml EtOH was stirred for about 3 hours in the presence of 0.11 g p-TsOH.H₂O at ambient temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC). After completion of the reaction, the thick white precipitate was filtered off and washed with ethanol (6 ml) to give the pure product 15 with 92% yield and the melting point was recorded as 190 °C-192 °C.

IR (KBr, cm⁻¹, Fig. 16): 3241 (N-H), 3120 (C-H, Ar), 2983, 2870 (C-H, aliphatic), 1653 (C=O), 1598, 1590, 1490, 1485 (C=C, aromatic and aliphatic), 1370 (C-O, bending).

¹H NMR (400 MHz, CDCl₃, δ ppm, Fig. 17): 1.35 (t, 3H, J=7.2 Hz, CH₃), 1.49 (t, 3H, J=6.8 Hz, CH₃), 2.81 (m, 3H, 2H-5, 1H'-5), 3.05 (dd, 1H, J= 8.0 Hz, 4.0 Hz, H'-5), 4.35 (m, 3H, 2H-OCH₂, 1H-OCH₂'), 4.5 (m, 1H, OCH₂'), 5.06 (t, 1H, J= 5.2 Hz, H-6), 5.14 (bd, s, 1H, H'-6), 5.89 (s, 1H, H-2), 6.42 (s, 1H, H'-2), 6.45 (d, 2H, J=8.0 Hz, Ar-H), 6.51 (d, 2H, J= 8.0 Hz, Ar), 6.57 (d, 2H, J= 8.0 Hz, Ar), 6.69 (t, 1H, J=8.0 Hz, Ar), 6.77 (t, 1H, J=8.0 Hz, Ar), 7.11 (m, 9H, Ar-H), 7.18 (m, 7H, Ar), 7.3 (m, 10H, Ar), 10.30 (s, 1H, N-H), 12.1 (s, 1H, N'-H).

¹³C NMR (100 MHz, CDCl₃, δ ppm, Fig. 18): 170.8 (1C, C=O), 170.0 (1C, C'=O), 155.8, 146.5, 142.4, 140.9, 140.7, 137.7, 132.9, 132.8, 132.1, 129.0, 128.8(14C, Ar), 128.84, 128.81, 128.80, 128.4(4C, 2C=C), 127.8, 125.9, 119.3, 117.5, 116.7, 113.0, 101.6, 97.8 (10 C, Ar), 61.0, 59.9, 57.6, 57.4, 56.1, 54.7, 36.7, 33.7, 14.8, 14.3 (10C, aliphatic).
2.6 Synthesis of \( N \)-phenyl-2,6-di(4-nitrophenyl)-3-ethylcarboxylato-4-phenylamino-piperidine-3-ene (17)

\[
\begin{align*}
\text{NH} & \quad \text{OEt} \\
\text{O} & \quad \text{NH} \\
\text{O} & \quad \text{NH}_2 \\
\text{CHO} & \quad \text{p-TsOH.H}_2\text{O} \\
\text{EtOH, r.t., N} & \quad 12 \text{hrs}
\end{align*}
\]

A solution of 3(phenylamino)-3-methylethylacrylate 8 (1 mmol), aniline 9 (1 mmol) and 4-nitrobenzaldehyde 16 (2 mmol) in 5 ml EtOH was stirred for about 12 hours in the presence of 0.11 g \( p \)-TsOH.\( \text{H}_2\text{O} \) at ambient temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC). After completion of the reaction, the thick pale yellow precipitate was filtered off and washed with ethanol (6 ml) to give the pure product 17 with 90% yield and the melting point was recorded as 175 °C-177 °C.

**IR (KBr, cm\(^{-1}\), Fig. 19):** 3247 (N-H), 3077 (C-H, Ar), 2981, 2855 (C-H, aliphatic), 1651 (C=O), 1595, 1518, 1503 (C=C, aromatic and aliphatic), 1347 (C-O, bending).

**\(^1\)H NMR (400 MHz, CDCl\(_3\), \( \delta \) ppm, Fig. 20):** 1.22 (t, 3H, \( J=8.0 \) Hz, \( \text{CH}_3 \)), 1.31(t, 3H, \( J=8.0 \) Hz, \( \text{CH}_3' \)), 1.48(t, 3H, \( J=8.0 \) Hz, \( \text{CH}_3" \)), 10.32(s, 1H, N-H), 10.72(s, 1H, N'-H), 12.10(bd, s, N"-H).

**\(^{13}\)C NMR (100 MHz, CDCl\(_3\), \( \delta \) ppm, Fig. 21):** 169.5, 167.9, 167.5 (3C, C=O), 156.2, 155.2, 151.6, 149.7, 149.4, 19.27, 147.21, 147.12, 147.07, 146.7, 145.8, 145.7, 137.9, 137.1 (28C, Ar), 129.6, 129.4, 129.3, 129.1, 129.0 (6C, 3 C=C), 128.8, 128.6, 128.1, 127.8, 127.7, 127.6, 127.34, 127.04, 126.31, 125.48, 125.40, 124.2, 124.0, 123.99, 123.74, 123.69, 123.60, 120.8, 120.3, 117.8, 117.7, 112.9, 100.8, 96.8, 96.4 (44C, Ar), 61.3, 61.0, 60.2 (3C, OCH\(_2\)), 60.01, 58.08, 57.32 (3C-2), 56.32, 55.20 (3C-6), 36.7, 36.0, 33.5 (3C-5), 14.7, 14.4, 14.2 (3C, CH\(_3\)).
CHAPTER-3

RESULTS & DISCUSSION
3.1 Characterization of N(4-methoxyphenyl)-2,6-diphenyl-3-ethylacetato-4(4-methoxyphenylamino)-piperidine-3-ene (4)

The compound 4 was synthesized by stirring a solution of 3(4-methoxylphenylamino)-3-methylethylacrylate 1 (1 mmol), 4-methoxyaniline 2 (1 mmol) and benzaldehyde 3 (2 mmol) in ethanol for about 6 hours in the presence of catalytic amount of p-TsOH.H2O (0.11 g) at ambient temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC). After completion of the reaction, the thick white precipitate was filtered off and washed with ethanol to give the pure product 4 with 92% yield and the melting point was recorded as 179 °C-181 °C.

![Chemical Structure of 4]

The IR spectrum (Fig. 4) of the compound 4 showed an absorption band at 3242 cm⁻¹ for N-H moiety. The aromatic C-H stretching was observed at 3060 cm⁻¹. The peaks at 2931 cm⁻¹ and 2835 cm⁻¹ were identified for aliphatic C-H stretching absorption. The intensified peak at 1650 cm⁻¹ was distinctive for C=O functional group. The characteristic peaks at 1611 cm⁻¹, 1595 cm⁻¹, 1509 cm⁻¹ and 1470 cm⁻¹ were distinguished for aliphatic and aromatic C=C bonds. The peak at 1372 cm⁻¹ was indicative for the bending vibration of C-O of ester.

The ¹H NMR spectra (Fig.5) of the compound 4 showed a triplet with the coupling constants J=8.0 Hz for the three protons of esteric -CH₃ at 1.46. The double doublet signals with the coupling constants J=15.2 Hz and J=2.4 Hz were assigned for one proton of H-5 at 2.6. The other one proton of H’-5 was designated as double doublet with the coupling constants J=5.6 Hz and J=5.6 Hz at 2.81. The sharp singlet at 3.66 was attributed for three protons of –OCH₃.
another sharp singlet was distinctive for three protons of –OCH$_3$ at 3.74. The multiplet for one proton of esteric -CH$_2$ was indicative at 4.31. The another multiplet for one proton of esteric –CH$_2$ was ascribed at 4.44. The doublet with the coupling constant $J$=3.2 Hz was identified for one proton of H-6 at 5.06. The doublet with the coupling constant $J$=8.8 Hz was observed for two aromatic protons at 6.2. The downfielded broad singlet for one proton was assigned for H-2 at 6.37. The doublet signal with the coupling constant $J$=8.0 Hz was indicative for two aromatic protons at 6.46. The doublet with the coupling constant $J$=8.8 Hz was attributed for two aromatic protons at 6.61. The another doublet with the coupling constant $J$=9.2 Hz was distinguished for two aromatic protons at 6.67. The signal at 7.20 was characterized as the triplet for three aromatic protons with the coupling constant $J$=8.0 Hz. The five aromatic protons were indicative as triplet with the coupling constant $J$=8.0 Hz at 7.26. The rest two aromatic protons were identified as triplet with the coupling constant $J$=8.0 Hz at 7.30. The distinct sharp singlet for one proton of N-H was distinctive at 10.16.

The $^{13}$C NMR spectra (Fig. 6) of the compound 4 showed a signal at 168.2 for one carbon of C=O. The signals at 157.7, 156.8, 150.8, 144.3, 143.2, 141.5, 130.6 were assigned for seven aromatic carbons. The signals at 128.5 and 128.1 were distinctive for two olefinic carbons. The signals at 127.8, 127.0, 126.7, 126.1, 114.4, 114.0, 97.1 were designative for seventeen aromatic carbons. The upfielded signals at 59.4 and 58.2 were assigned for two –OCH$_3$ carbons. The other aliphatic carbons were identified at 55.6 (1C, C-6), 55.5 (1C, C-2), 55.3 (1C, C-5), 33.5 (1C, CH$_2$), and at 14.7 (1C, CH$_3$).

All the spectral evidences express harmony with the structure of the compound 4 as
Fig. 4: IR Spectrum of compound 4
Fig. 5: $^1$H NMR spectrum of compound 4
Fig. 5a: Extended $^1$H NMR spectrum of compound 4
Fig. 5b: Extended $^1$H NMR spectrum of compound 4
Fig. 5c: Extended $^1$H NMR spectrum of compound 4
Fig. 6: $^{13}$C NMR spectrum of compound 4
Fig. 6a: Extended $^{13}$C NMR spectrum of compound 4
3.2 Characterization of \( N(4\text{-methylphenyl})-2,6\text{-diphenyl-3-ethylacetato-4(4-methylphenylamino)-piperidine-3-ene} \) (7)

The compound 7 was synthesized by stirring a solution of 3(4-methylphenylamino)-3-methylethylacrylate 5 (1 mmol), 4-methylaniline 6 (1 mmol) and benzaldehyde 3 (2 mmol) in ethanol for about 1.30 hours in the presence of catalytic amount of \( p\text{-TsOH.H}_2\text{O} \) (0.11 g) at ambient temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC). After completion of the reaction, the thick white precipitate was filtered off and washed with ethanol to give the pure product 7 with 91% yield and the melting point was recorded as 185 °C-187 °C.

\[
\begin{align*}
\text{NH} & \quad \text{O} \\
\text{OEt} & \quad \text{NH} \\
\end{align*}
\]

The IR spectrum (Fig.7) of the compound 7 showed an absorption band at 3240 cm\(^{-1}\) for N-H moiety. The aromatic C-H stretching was detected at 3026 cm\(^{-1}\). The peaks at 2922 cm\(^{-1}\) and 2861 cm\(^{-1}\) were identified for aliphatic C-H stretching absorption. The intensified peak at 1650 cm\(^{-1}\) was assigned for C=O functional group. The characteristic peaks at 1618 cm\(^{-1}\), 1595 cm\(^{-1}\), 1516 cm\(^{-1}\) and 1500 cm\(^{-1}\) were distinguished for aliphatic and aromatic C=C bonds. The peak at 1372 cm\(^{-1}\) was indicative for the bending vibration of C-O of ester.

The \(^1\)H NMR spectra (Fig. 8) of the compound 7 showed a sharp triplet with the coupling constant \( J = 8.0 \) Hz for the three protons of esteric -CH\(_3\) at 1.45. The singlet three protons of CH\(_3\) were assigned at 2.15. The other singlet for three protons of CH\(_3\) was designated at 2.25. The double doublet with the coupling constants \( J = 1.6 \) Hz and \( J = 1.6 \) Hz was attributed for one proton of H-5 at 2.71. The another double doublet with the coupling constants \( J = 5.6 \) Hz and \( J = 5.6 \) Hz
was ascribed for one proton of H’-5 at 2.83. The two multiplets at 4.33 and at 4.50 were distinctive for two protons of -OCH₂. The doublet with the coupling constant $J=3.6$ Hz was detectable for one benzylic proton of H-6 at 5.11. The doublet with the coupling constant $J=8.0$ Hz was identifiable for two aromatic protons at 6.15. The downfielded singlet for one benzylic proton of H-2 was indicative at 6.42. The broad singlet for two aromatic protons was designated at 6.44. The multiplet at 6.88 was assigned for four identical aromatic protons. The multiplet for three aromatic protons was detected at 7.1. The five aromatic protons showed a multiplet at 7.24. The multiplet at 7.29 was distinctive for one aromatic proton. The distinct sharp singlet for one proton of N-H was at 10.21.

The $^{13}$C NMR spectra (Fig. 9) of the compound 7 showed a signal at 168.2 for one carbon of C=O. The signals at 156.4, 144.8, 144.3, 143.0, 135.5, 135.2, 129.8, 129.43 and 129.40 were assigned for nine aromatic carbons. The signals at 128.5 and 128.1 were detectable for two carbons of C=C. The nine carbons of aromatic ring were detected at 127.0, 126.5 and 126.1. The signals at 125.9 and 125.0 were designated for four carbons of aromatic ring. The rest two carbons of aromatic ring were identified at 112 and 97. The signals at 59.5, 58.2, 55.1, 33.5, 20.8, 20.1 and 14.8 were distinctive for seven aliphatic carbons.

All the spectral evidences express harmony with the structure of the compound 7 as
Fig. 8: $^1$H NMR spectrum of compound 7
Fig. 8a: Extended $^1$H NMR spectrum of compound 7
Fig. 8b: Extended $^1$H NMR spectrum of compound 7
Fig. 8c: Extended $^1$H NMR spectrum of compound 7
Fig. 9: $^{13}$C NMR spectrum of compound 7

INARS, HSQC, $^{13}$C spectrum, FT-R in CDCl$_3$, Sonia, BUET
3.3 Characterization of \(N\)-phenyl-2,6-diphenyl-3-ethylacetato-4-phenylamino-piperidine-3-ene (10)

The compound 10 was synthesized by stirring a solution of 3(phenylamine)-3-methylethylacrylate 8 (1 mmol), aniline 9 (1 mmol) and benzaldehyde 3 (2 mmol) in ethanol for about 4.30 hours in the presence of catalytic amount of \(p\)-TsOH.H\(_2\)O (0.11 g) at ambient temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC). After completion of the reaction, the thick white precipitate was filtered off and washed with ethanol to give the pure product 10 with 93% yield and the melting point was recorded as 161 °C-163 °C.

\[
\begin{align*}
\text{NH} & \\
\text{OEt} & \\
\text{NH} & \\
\text{O} & \\
\text{CHO} & \\
p-\text{TsOH.H}_2\text{O} & \\
\text{EtOH, r.t., } & \\
4.30 \text{ hrs} & \\
8 & + & 9 & + & 2 & 3 & \rightarrow & 10
\end{align*}
\]

The IR spectrum (Fig. 10) of the compound 10 showed an absorption band at 3249 cm\(^{-1}\) for N-H moiety. The aromatic C-H stretching was observed at 3061 cm\(^{-1}\). The peaks at 2982 cm\(^{-1}\) and 2875 cm\(^{-1}\) were identified for aliphatic C-H stretching absorption. The intensified peak at 1651 cm\(^{-1}\) was distinctive for C=O functional group. The characteristic peaks at 1594 cm\(^{-1}\), 1592 cm\(^{-1}\), 1500 cm\(^{-1}\) and 1450 cm\(^{-1}\) were distinguished for aliphatic and aromatic C=C bonds. The peak at 1373 cm\(^{-1}\) was indicative for the bending vibration of C-O of ester.

The \(^1\)H NMR spectra (Fig. 11) of the compound 10 showed a broad singlet for the three protons of esteric -CH\(_3\) at 1.53. The doublet signal with the coupling constant \(J=15.2\) Hz was assigned for one proton of H-5 at 2.83. The other one proton of H’-5 was designated as doublet with the coupling constant \(J=4.0\) Hz at 2.95. A broad singlet at 4.41 was attributed for one proton of –OCH\(_2\). The another –OCH\(_2\) proton was identified at 4.53 as a broad singlet. The broad singlet at
5.21 was designated for one benzylic proton of H-6. The other benzylic proton of H-2 was attributed at 6.34 as a broad singlet. The multiplet at 6.59 was assigned for four aromatic protons. The broad singlet at 7.13 was detected for five aromatic protons. The multiplet at 7.32 was ascribed for eleven aromatic protons. The distinct sharp singlet for one proton of N-H was distinctive at 10.36.

The $^{13}$C NMR spectra (Fig. 12) of the compound 10 showed a signal at 168.2 for one carbon of C=O moiety. The signals at 156.1, 147.0, 144.0, 142.8, 137.9, 128.9 and 128.8 were assigned for twelve aromatic carbons. The signals at 128.6 and 128.2 were designated for two olefinic carbons. The signals at 127.1, 126.8, 126.6, 126.4, 126.3, 125.8, 125.7, 116.1, 112.9 and 98.2 were identified for twelve aromatic carbons. The one –OCH$_2$ carbon was distinctive at 59.7. The signals at 58.2, 55.1, 33.6 and 14.8 were designated for four carbons of C-2, C-6, C-5 and C-CH$_3$ respectively.

All the spectral evidences express harmony with the structure of the compound 10 as

![Compound 10](image-url)
Fig. 10: IR spectrum of compound 10
Fig. 11: $^1$H NMR spectrum of compound 10
Fig. 11a: Extended $^1$H NMR spectrum of compound 10
Fig. 11b: Extended $^1$H NMR spectrum of compound 10
Fig. 12: $^{13}$C NMR spectrum of compound 10
3.4 Characterization of N-(4-bromophenyl)-2,6-diphenyl-3-ethylacetato-4(4-bromophenylamino)-piperidine-3-ene (13)

The compound 13 was synthesized by stirring a solution of 3(4-bromophenylamino)-3-methylethylacrylate 11 (1 mmol), 4-bromoaniline 12 (1 mmol) and benzaldehyde 3 (2 mmol) in ethanol for about 2.30 hours in the presence of catalytic amount of p-TsOH.H₂O (0.11 g) at ambient temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC). After completion of the reaction, the thick white precipitate was filtered off and washed with ethanol to give the pure product 13 with 88% yield and the melting point was recorded as 210 °C-212 °C.

\[
\begin{align*}
\text{Br} & \quad \text{NH} \quad \text{O} \\
\text{NH} \text{O} & \quad \text{NH}_2 \quad \text{CHO} \\
\text{p-TsOH.H}_2\text{O} & \quad \text{EtOH, r.t., N} \\
\text{EtOH, r.t., N} & \quad \text{O} \\
2 \text{hrs} & \quad 13
\end{align*}
\]

The IR spectrum (Fig. 13) of the compound 13 showed an absorption band at 3242 cm⁻¹ for N-H moiety. The aromatic C-H stretching was observed at 3085 cm⁻¹. The peaks at 2977 cm⁻¹, 2912 cm⁻¹ and 2861 cm⁻¹ were identified for aliphatic C-H stretching absorption. The intensified peak at 1652 cm⁻¹ was distinctive for C=O functional group. The characteristic peaks at 1604 cm⁻¹, 1589 cm⁻¹, 1493 cm⁻¹ and 1451 cm⁻¹ were distinguished for aliphatic and aromatic C=C bonds. The peak at 1373 cm⁻¹ was indicative for the bending vibration of C-O of ester.

The \(^1\)H NMR spectra (Fig. 14) of the compound 13 showed a broad singlet for the three protons of esteric -CH₃ at 1.47. The doublet signals with the coupling constant \(J=12.0\) Hz was identified for one proton of H-5 at 2.71. The other one proton of H’-5 was designated as doublet with the coupling constant \(J=11.6\) Hz at 2.83. The broad doublet with the coupling constant \(J=15.2\) Hz at 4.34 was ascribed for two protons of –OCH₂. The singlet for one proton of H-6 was detected at 5.10. The broad singlet at 5.91 was assigned for one proton of H-2 and another one of aromatic
proton merged each other. The broad singlet at 6.39 was detected for three aromatic protons. The multiplet at 7.16 was designated for fourteen aromatic protons. The downfielded sharp singlet for one proton of N-H was distinctive at 10.24.

The $^{13}$C NMR spectra (Fig. 15) of the compound 13 showed a signal at 168.0 for one carbon of C=O moiety. The signals at 155.1, 145.8, 143.0, 142.0 and 136.8 were ascribed for five aromatic carbons. The signals at 132.3, 131.9 and 131.5 were indicative for eight aromatic carbons. The signals at 128.8 and 128.3 were attributable for two olefinic carbons. The signals at 127.4, 127.2, 126.4, 126.2, 119.1, 114.5, 108.3 and 98.7 were identified for eleven aromatic carbons. The signals at 59.9, 58.2, 55.1, 33.4 and 14.7 were designated for five aliphatic carbons of OCH$_2$, C-2, C-6, C-5 and CH$_3$ respectively.

All the spectral evidences express harmony with the structure of the compound 13 as
Fig. 13: IR spectrum of compound 13
Fig. 14: $^1$H NMR spectrum of compound 13
Fig. 14a: Extended $^1$H NMR spectrum of compound 13
Fig. 15: $^{13}$C NMR spectrum of compound 13
3.5 Characterization of \(N\)-phenyl-2,6-di(4-chlorophenyl)-3-ethylacetato-4-phenylamino-piperidine-3-ene (15)

The compound 15 was synthesized by stirring a solution of 3(phenylamino)-3-methylethylacrylate 8 (1 mmol), aniline 9 (1 mmol) and 4-chlorobenzaldehyde 14 (2 mmol) in ethanol for about 3 hours in the presence of catalytic amount of \(p\)-TsOH.H\(_2\)O (0.11 g) at ambient temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC). After completion of the reaction, the thick white precipitate was filtered off and washed with ethanol to give the pure product 15 with 92% yield and the melting point was recorded as 190 °C-192 °C.

\[
\begin{align*}
\text{OEt} & \quad \text{NH} & \quad \text{O} \\
\text{NH} & \quad \text{2} & \quad \text{CHO} \\
\text{p-TsOH.H2O} & \quad \text{EtOH, r.t., N} & \quad \text{O} \\
\text{NH} & \quad \text{Cl} & \quad \text{Cl} \\
\text{1} & \quad \text{2} & \quad \text{3} \\
\text{3 hrs} & \quad \text{Cl} & \quad \text{Cl} \\
\text{8} & \quad \text{9} & \quad \text{14} \\
\text{EtOH} & \quad \text{15} 
\end{align*}
\]

The IR spectrum (Fig. 16) of the compound 15 showed an absorption band at 3241 cm\(^{-1}\) for N-H moiety. The aromatic C-H stretching was observed at 3120 cm\(^{-1}\). The peaks at 2983 cm\(^{-1}\) and 2870 cm\(^{-1}\) were identified for aliphatic C-H stretching absorption. The intensified peak at 1653 cm\(^{-1}\) was distinctive for C=O functional group. The characteristic peaks at 1598 cm\(^{-1}\), 1590 cm\(^{-1}\), 1490 cm\(^{-1}\) and 1485 cm\(^{-1}\) were distinguished for aliphatic and aromatic C=C bonds. The peak at 1370 cm\(^{-1}\) was indicative for the bending vibration of C-O of ester.

The \(^1\)H NMR spectra (Fig. 17) of the compound 15 showed a triplet with the coupling constant \(J=7.2\) Hz at 1.35 for the three protons of esteric -CH\(_3\). The triplet with the coupling constant \(J=6.8\) Hz was assigned for three protons of -CH\(_3\) at 1.49. The multiplet of 2H-5 and 1H-5' was designated at 2.81. The double doublet with the coupling constant \(J=8.0\) Hz and \(J=4.0\) Hz were assigned for one proton of H-5' at 3.05. The multiplet at 4.35 was designated for three protons of 2H-OCH\(_2\) and 1H-OCH\(_2\)' . The single proton of –OCH\(_2\)' was ascribed as multiplet at 4.5. The triplet with the coupling constant \(J=5.2\) Hz was distinctive for one proton of H-6 at 5.06. The
broad singlet of one proton of H-6′ was identified at 5.14. The sharp singlet for one proton of H-2 was assigned for at 5.89. The other singlet for one proton of H-2′ was designated at 6.42. The doublet with the coupling constant $J=8.0$ Hz was distinguished for two aromatic protons at 6.45. The doublet with the coupling constant $J=8.0$ Hz at 6.51 was indicative for two aromatic protons. The doublet with the coupling constant $J=8.0$ Hz at 6.57 was distinguishable for two aromatic protons. The triplet with the coupling constant $J=8.0$ Hz at 6.69 was ascribed for one aromatic proton. The triplet with the coupling constant $J=8.0$ Hz at 6.77 was assignable for one aromatic proton at 6.77. The multiplet for nine aromatic protons was assignable at 7.11. The multiplet at 7.18 was detected for seven aromatic protons. The multiplet for ten aromatic protons was attributed at 7.3. The sharp downfielded singlet for one proton of N-H was distinctive at 10.3. The downfielded sharp singlet for one proton of N′-H was identified at 12.1.

The $^{13}$C NMR spectra (Fig. 18) of the compound 15 showed a signal at 170.8 for one carbon of C=O and 170.0 for one carbon of C′=O. The signals at 155.8, 146.5, 142.4, 140.9, 140.7, 137.7, 132.9, 132.8, 132.1, 129.0 and 128.8 were assigned for fourteen aromatic carbons. The signals at 128.84, 128.81, 128.80 and 128.4 were designated for four carbons of 2C=C. The signals at 127.8, 125.9, 119.3, 117.5, 116.7, 113.0, 101.6, and 97.8 were identified for ten aromatic carbons. The signals were assigned as at 61.0 (OCH$_2$), 59.9 (OC'H$_2$), 57.6 (C-2), 57.4 (C′-2), 56.1 (C-6), 54.7 (C′-6), 36.7 (C-5), 33.7 (C′-5), 14.8 (CH$_3$) and 14.3 (C'H$_3$) for ten aliphatic carbons.

All the spectral evidences express harmony with the isomeric structure of the compound 15a and 15b as
Fig. 16: IR spectrum of compound 15
Fig. 17: $^1$H NMR spectrum of compound 15
Fig. 17a: Extended $^1$H NMR spectrum of compound 15
Fig. 17b: Extended $^1$H NMR spectrum of compound 15
Fig. 18: $^{13}$C NMR spectrum of compound 15
3.6 Characterization of N-phenyl-2,6-di(4-nitrophenyl)-3-ethylacetato-4-phenylamino-piperidine-3-ene (17)

The compound 17 was synthesized by stirring a solution of 3(phenylamino)-3-methylethylacrylate 8 (1 mmol), aniline 9 (1 mmol) and 4-nitrobenzaldehyde 16 (2 mmol) in ethanol for about 12 hours in the presence of catalytic amount of p-TsOH.H₂O (0.11 g) at ambient temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC). After completion of the reaction, the thick white precipitate was filtered off and washed with ethanol to give the pure product 17 with 90% yield and the melting point was recorded as 175 °C-177 °C.

The IR spectrum (Fig. 19) of the compound 17 showed an absorption band at 3247 cm⁻¹ for N-H. The aromatic C-H stretching was observed at 3077 cm⁻¹. The peaks at 2981 cm⁻¹ and 2855 cm⁻¹ were identified for aliphatic C-H stretching absorption. The intensified peak at 1651 cm⁻¹ was identified for C=O functional group. The characteristic peaks at 1595 cm⁻¹, 1518 cm⁻¹ and 1503 cm⁻¹ were distinguished for aliphatic and aromatic C=C bonds. The peak at 1347 cm⁻¹ was indicative for the bending vibration of C-O of ester.

The ¹H NMR spectra (Fig. 20) of the compound 17 showed a three triplets with the coupling constant J=8.0 Hz at 1.22, 1.31 and 1.48 for nine protons of three esteric -CH₃. A large number of aromatic protons were observed at the different aromatic proton region of the spectra but specification was difficult. The three downfielded sharp singlets for three protons of three N-H were identified at 10.32, 10.72 and 12.10.
The $^{13}$C NMR spectra (Fig. 21) of the compound 17 showed the signals at 169.5, 167.9 and 167.5 for three carbons of 3C=O. The signals at 156.2, 155.2, 151.6, 149.7, 149.4, 149.27, 147.21, 147.12, 147.07, 146.7, 145.8, 145.7, 137.9 and 137.1 were assigned for 28 aromatic carbons. The signals at 129.6, 129.4, 129.3, 129.1 and 129.0 were designated for six carbons of 3C=C. The signals at 128.8, 128.6, 128.1, 127.8, 127.7, 127.6, 127.34, 127.04, 126.31, 125.48, 125.40, 124.2, 124.0, 123.90, 123.74, 123.69, 123.60, 120.8, 120.3, 117.8, 117.7, 112.9, 100.8, 96.8 and 96.4 were distinctive for 44 aromatic carbons. The signals at 61.3, 61.0, 60.2 were for three carbons of –OCH$_2$, 60.01, 58.08, 57.32 were for three carbons of 3C-2, 56.32, 55.20 were for three carbons of C-6, 36.7, 36.0, 33.5 were for three carbons of three C-5 and 14.7, 14.4, 14.2 were for three carbons of three -CH$_3$.

All the spectral evidences support in favour of the three isomeric structures of the compound 17 as

![Compound 17a](image1.png)  ![Compound 17b](image2.png)  ![Compound 17c](image3.png)
Fig. 19: IR spectrum of compound 17
Fig. 20: $^1$H NMR spectrum of compound 17
Fig. 20a: Extended $^1$H NMR spectrum of compound 17
Fig. 20b: Extended $^1$H NMR spectrum of compound 17
Fig. 21: $^{13}$C NMR spectrum of compound 17
4.1 Introduction

The evolution and spread of antibiotic resistance, as well as the evolution of new strains of diseases causing agents, is of great concern to the global health community. Our ability to effectively treat disease is dependent on the development of new pharmaceuticals, and one potential source of novel drugs is traditional medicine. This study explores the antibacterial properties of fungal extracts of medicinal plants. Bacteria are responsible for many infectious diseases. The increasing clinical importance of drug resistant bacteria pathogens has lent additional urgency to antibacterial research. The antibacterial screening which is the first stage of antibacterial research is performed to ascertain the susceptibility of various bacteria to any agent. This test measures the ability of each antibacterial agent to inhibit the \textit{in vitro} bacterial growth. This ability may be estimated by any of the following three methods.

- Disc diffusion method
- Serial dilution method
- Bio-autographic method

In serial dilution method the original culture suspension is diluted more one time, in tubes or in appropriate medium. Because of the reduction in the no. of bacteria due to dilution isolation is obtained. When greatly diluted the specimen contains only few organisms of only one species. The cultured obtained is conserved by the spread plate or streak plate method. The disc diffusion technique [194] is a widely accepted \textit{in vitro} investigation for preliminary screening of agents which may possess any antibacterial activity. It is essentially a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic or bactericidal activity can be made by this method [195].

Fungi are responsible for many infectious diseases. Infectious diseases are caused by living organisms called pathogens. World health problems caused by drug resistant fungi are increasing. Invasive fungal infections have not only increased in frequency but also new fungal species have been reported to cause infection, especially in immune compromised patients. Concurrent with the increase in fungal infections, a large variety of antifungal drugs are available with different which give results that correspond to the clinical outcome of spectrum of activity. Our ability to effectively treat disease is dependent on the development of new pharmaceuticals, and one potential source of novel drugs is traditional medicine.
The increasing clinical importance of drug resistant fungal pathogens has lent additional urgency to antifungal research. The antifungal screening which is the first stage of antifungal research is performed to ascertain the susceptibility of various fungi to any agent. This test measures the ability of each antifungal agent to inhibit the in vitro fungal growth. This ability may be estimated by any of the following three methods such as disc diffusion method, serial dilution method and bio-autographic method.

4.2 Principle of Disc Diffusion Method

Solutions of known concentration (µg/ml) of the test samples are made by dissolving measured amount of the samples in definite volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) are then impregnated with known amounts of the test substances using micropipette. Discs containing the test material are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) are used as positive and negative control. These plates are then kept at low temperature (4 °C) for 24 hours to allow maximum diffusion. During this time dried discs absorb water from the surrounding media and then the test materials are dissolved and diffused out of the media. The diffusion occurs according to the physical law that controls the diffusion of molecules through agar gel [196]. As a result, there is a gradual change of test materials concentration in the media surrounding the discs. The plates are then incubated at 37 °C for 24 hours to allow maximum growth of the organisms. If the test materials have any antibacterial activity, it will inhibit the growth of the microorganisms giving a clear, distinct zone called “Zone of Inhibition”. The antibacterial activity of the test agent is determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment is carried out more than once and the mean of the readings is required [197]. In the present study synthesized compounds were tested for antibacterial activity by disc diffusion method.
4.3 Determination of Antimicrobial Activity by the Zone of Inhibition

The antimicrobial potency of the test agents was measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

![Image](https://example.com/image.png)

**Fig. 22:** Inhibition Zone measurement.

In the present study synthesized compounds were tested for antimicrobial activity by disc diffusion method.

4.4 Antimicrobial Screening

4.4.1 Disc Diffusion Methods

The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the NCCLS. The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures as described here.
4.4.2 Materials Used

a) Test Organisms: Both gram positive and gram-negative organisms were taken for the test and they are listed in the Table.

Table 6: List of bacteria used in antibacterial screening

<table>
<thead>
<tr>
<th>Gram positive Bacteria</th>
<th>Gram negative Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td><em>Salmonella typhimurium</em></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td></td>
</tr>
<tr>
<td><em>Citroacterfreundii</em></td>
<td></td>
</tr>
</tbody>
</table>

The fungal strains used for the experiment were collected as pure cultures and two organisms were taken for the test.

Table 7: List of fungi used in antifungal screening

<table>
<thead>
<tr>
<th>Aspergillus niger</th>
<th>Tricoderma harzianum</th>
</tr>
</thead>
</table>

b) Growth Media: The activity was conducted on the Nutrient Agar Media produced from TSA (Tryptone Soya Agar).

Composition:

- Pancreatic digest of casein 15.0 g/L
- Enzymatic digest of soya bean 5.0 g/L
- Sodium chloride 5.0 g/L
- Agar 15.0 g/L
c) **Apparatus Used:**

- **Petri plate**: Plastic plate, which was previously sterilized.
- **Pipette**: Micropipette was used for adding the required concentration of sample to the plates.
- **Blank discs**: Susceptible blank discs were used, which was stored in 20°C to 8°C.
- **Glasswares**: 500 ml conical flask and test tubes were used.
- **Compounds Screened**: All the synthesized compounds.
- **Solvent Used**: Chloroform.
- **Standard Used**: Ciprofloxacin for bacteria and Miconazole for fungi.

d) **Test materials (Synthesized Compounds-13, 15 & 10)**

1) \(N\)(4-bromophenyl)-2,6-diphenyl-3-ethylacetato-4(4-bromophenylamino)-piperidine-3-ene

2) \(N\)-phenyl-2,6-di(4-chlorophenyl)-3-ethylacetato-4-phenylamino-piperidine-3-ene

3) \(N\)-phenyl-2,6-diphenyl-3-ethylacetato-4-phenylamino-piperidine-3-ene

**4.5 Procedure for Performing the Disc Diffusion Test**

Inoculums Preparation

**4.5.1 Growth Method**

The growth method is performed as follows

At least three to five well-isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop.

a) growth was transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth.

b) The broth culture was incubated at 37 °C until it achieved or exceed the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours).

c) The turbidity of the actively growing broth culture was adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard.
4.5.2 Inoculation of Test Plates

a) Media was prepared by adding 40.0 gm of Nutrient agar to 1L of distilled water. Then it was sterilized by autoclaving at 15 lb/inch and at 210 °C temperatures for two hours.

b) Media was cooled to the temperature of approximately 40 °C and microorganisms were inoculated to the media. 25ml was transferred to a petri plate. Two such plates were prepared for each organism. Plates were allowed to cool for 20 minutes.

c) Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.

d) The dried surface of a TSA plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed.

e) The lid may be left the plate for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

4.5.3 Application of Discs to Inoculated Agar Plates

The predetermined battery of antimicrobial discs was dispensed onto the surface of the inoculated agar plate. Each disc was pressed down to ensure complete

a) contact with the agar surface. The discs were placed such a way so that they were no closer than 24 mm from center to center.

b) The plates are inverted and placed in an incubator set to 37 °C within 15 minutes after the discs were applied.
4.5.4 Application of Samples on the discs

a) Crude fungal extract was dissolved in chloroform and diluted to get concentration of 300 µg/disc.

b) Four blank discs were placed in the petriplates. Reference standard Tetracycline was impregnated on one of the discs, and only solvent as a blank was impregnated on one of the discs, and others experimental solutions were impregnated on others discs. Each disc was marked by a marker as a small symbol so that each of the discs could be easily identified. 25µl of solution was injected on each disc.

Fig. 23: Application of Samples on the discs.

4.5.5 Reading Plates and Interpreting Results
The above culture plates were incubated at 37 °C for 24 hours. The zones of inhibition produced by compounds and Ciprofloxacin for bacteria and Miconazole for fungi were recorded in mm and compared.

4.6 Determination of Antimicrobial Activity by Measuring the Zone of Inhibition

4.6.1 Test Samples
The synthesized compounds (13, 15 & 10) for the antimicrobial test are numbered as 15, 16 and 17 on petri plates.
### List of antimicrobial tested compounds’ names

<table>
<thead>
<tr>
<th>Sample code on petri plates</th>
<th>Compound no</th>
<th>Sample / compounds’ names</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>13</td>
<td>$N(4$-bromophenyl)-$2$,$6$-diphenyl-$3$-ethylacetato-$4$($4$-bromophenylamino)-piperidine-$3$-ene</td>
</tr>
<tr>
<td>16</td>
<td>15</td>
<td>$N$-phenyl-$2$,$6$-$di(4$-chlorophenyl)-$3$-ethylacetato-$4$-phenylamino-piperidine-$3$-ene</td>
</tr>
<tr>
<td>17</td>
<td>10</td>
<td>$N$-phenyl-$2$,$6$-diphenyl-$3$-ethylacetato-$4$-phenylamino-piperidine-$3$-ene</td>
</tr>
</tbody>
</table>

### List of antimicrobial tested compounds’ structures

<table>
<thead>
<tr>
<th>Sample code on petri plates</th>
<th>Compound no</th>
<th>Sample / compounds’ structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>13</td>
<td><img src="image1.png" alt="Structure 1" /></td>
</tr>
<tr>
<td>16</td>
<td>15</td>
<td><img src="image2.png" alt="Structure 2" /></td>
</tr>
<tr>
<td>17</td>
<td>10</td>
<td><img src="image3.png" alt="Structure 3" /></td>
</tr>
</tbody>
</table>
4.6.2 Measurement of the Zone of Inhibition

After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale. Synthesized compounds (13, 15 & 10) were tested for antibacterial activity against six bacteria and antifungal activity against two fungi. All compounds were tested at 300 µg/disc concentration.

**Table 8: In Vitro Bactericidal and Fungicidal Profiles of compounds in Terms of Zone of Inhibition**

<table>
<thead>
<tr>
<th>Antimicrobial Screening</th>
<th>13</th>
<th>15</th>
<th>10</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Miconazole</td>
</tr>
<tr>
<td><strong>Gram positive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (+)</td>
<td>9</td>
<td>9</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>Gram negative</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> (-)</td>
<td>10</td>
<td>22</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> (-)</td>
<td>14</td>
<td>15</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> (-)</td>
<td>7</td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em> (-)</td>
<td>10</td>
<td>-</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><em>Citrobacter freundii</em> (-)</td>
<td>11</td>
<td>10</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>9</td>
<td>11</td>
<td>8</td>
<td>28</td>
</tr>
<tr>
<td><em>Tricoderma harzianum</em></td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>25</td>
</tr>
</tbody>
</table>

50 µL dose was used & concentration was 300 µg disc⁻¹. The observed zone of inhibition is indicated by diameters (in mm) and (-) represents No activity.
4.6.3 Column Charts of Antimicrobial Test

Antibacterial activity against *Staphylococcus aureus*

Antibacterial activity against *Bacillus subtilis*

Antibacterial activity against *Escherichia coli*

Antibacterial activity against *Pseudomonas aeruginosa*
Antibacterial activity against *Salmonella typhimurium*  

Antibacterial activity against *Citrobacter freundii*

**Fig. 24:** Column charts of antibacterial activity of synthesized compounds against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium* and *Citrobacter freundii*

Antibacterial activity against *Aspergillus niger*  

Antibacterial activity against *Tricoderma harzianum*

**Fig. 25:** Column charts of antifungal activity of synthesized compounds against *Aspergillus niger* and *Tricoderma harzianum*
4.6.4 Clear Zone of Inhibition on Petri Plates at Sample code 15, 16 & 17 (Synthesized Compound no 13, 15 & 10)

Fig. 26: Zone of inhibition for *Escherichia coli* (bacteria)

Fig. 27: Zone of inhibition for *Salmonella typhimurium* (bacteria)
Fig. 28: Zone of inhibition for *Citrobacter freundii* (bacteria)

Fig. 29: Zone of inhibition for *Bacillus subtilis* (bacteria)
Fig. 30: Zone of inhibition for *Staphylococcus aureus* (bacteria)

Fig. 31: Zone of inhibition for *Pseudomonas aeruginosa* (bacteria)
Fig. 32: Zone of inhibition for *Aspergillus niger* (fungi)

Fig. 33: Zone of inhibition for *Tricoderma harzianum* (fungi)
4.7 Results and discussion of \textit{in vitro} Antimicrobial Screening

The tested compounds showed activities at a dose of 300 $\mu$g disc$^{-1}$ comparable to Ciprofloxacin for bacteria and Miconazole for fungi (standard) at 300 $\mu$g disc$^{-1}$.

The compound 13 showed bactericidal moderate activity against \textit{E. coli}, \textit{B. subtilis}, \textit{S. typhimurium} and \textit{C. freundii}. Noticeably, all the compounds showed the antibacterial activity against \textit{B. subtilis}, \textit{E. coli}, \textit{S. aureus}, \textit{C. freundii}. Among them compound 15 showed very good activity against \textit{B. subtilis}, \textit{E. coli} and \textit{C. freundii}. Compound 10 showed very good activity against \textit{S. aureus}, \textit{B. subtilis} and \textit{C. freundii}. Only compound 15 exhibited no activity against \textit{S. typhimurium}.

Compound 15 showed moderate antifungal activity against \textit{A. niger} but the other compounds exhibited very low activity against \textit{A. niger} and \textit{T. harzianum}. 

CHAPTER 5

SUMMARY
Our objective was to synthesize piperidine derivatives by multi-component one-pot process with the help of \( p \)-toluenesulphonic acid catalyst and study of their biological activity. This one-pot reaction has some important advantages such as the easy workup procedure, simple and relatively available precursors and inexpensive catalyst.

\( p \)-Toluenesulphonic acid was a highly effective and efficient catalyst for the one-pot multicomponent coupling of phenylaminoethylacrylate derivatives, substituted amines and substituted aldehydes in ethanol at ambient temperature to give highly functionalized piperidines in high yields. The piperidine derivatives were characterized with spectral evidences such as IR, \(^1\)H NMR and \(^{13}\)C NMR.

\[
\begin{align*}
\text{R}_1 \text{NH}_2 + \text{O} & \text{Et} \xrightleftharpoons[\text{EtOH, r.t.}]{p\text{-TsOH.H}_2\text{O}} \text{NH} \xrightarrow{} \text{OEt} \\
\text{R}_1 &= \text{-OCH}_3, \text{-CH}_3, \text{-Br, H} \\
\text{R}_2 &= \text{H, Cl, NO}_2
\end{align*}
\]

Scheme 26: Synthesis of piperidine derivatives
Synthesis of Piperidine derivatives

<table>
<thead>
<tr>
<th>SI. No</th>
<th>Starting Material</th>
<th>Product</th>
<th>M.P.(°C)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H₃CO-</td>
<td>NH₂-OEt + 2C₆H₅-OCH₃</td>
<td>178-182</td>
<td>92</td>
</tr>
<tr>
<td>2</td>
<td>C₆H₅-NH-OEt + C₆H₅-CHO</td>
<td>185-187</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C₆H₅-NH-OEt + C₆H₅-CHO</td>
<td>161-163</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>C₆H₅-NH-OEt + C₆H₅-CHO</td>
<td>210-212</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>C₆H₅-NH-OEt + C₆H₅-CHO</td>
<td>190-192</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>C₆H₅-NH-OEt + C₆H₅-CHO</td>
<td>175-177</td>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>
Here are the synthesized compounds 4, 7, 10, 13, 15 and 17.

Fig. 34: Six synthesized piperidine derivatives
Antibacterial and antifungal tests

Piperidine derivatives showed moderate to good antibacterial and antifungal activity against *Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Citrobacter freundii, Aspergillus niger, Tricoderma harzianum*. The results have shown below.

*In Vitro* Bactericidal and Fungicidal Profiles of compounds in Terms of Zone of Inhibition

<table>
<thead>
<tr>
<th>Organism</th>
<th>13</th>
<th>15</th>
<th>10</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Miconazole</td>
</tr>
<tr>
<td><strong>Gram positive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus (+)</em></td>
<td>9</td>
<td>9</td>
<td>12</td>
<td>38</td>
</tr>
<tr>
<td><strong>Gram negative</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis (-)</em></td>
<td>10</td>
<td>22</td>
<td>12</td>
<td>36</td>
</tr>
<tr>
<td><em>Escherichia coli (-)</em></td>
<td>14</td>
<td>15</td>
<td>19</td>
<td>40</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa (-)</em></td>
<td>7</td>
<td>9</td>
<td>7</td>
<td>42</td>
</tr>
<tr>
<td><em>Salmonella typhimurium (-)</em></td>
<td>10</td>
<td>-</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td><em>Citrobacter freundii (-)</em></td>
<td>11</td>
<td>10</td>
<td>15</td>
<td>37</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
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<tr>
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<td>8</td>
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</tr>
<tr>
<td><em>Tricoderma harzianum</em></td>
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<td>7</td>
<td>7</td>
<td>25</td>
</tr>
</tbody>
</table>

50 µL dose was used & concentration was 300 µg disc\(^{-1}\). The observed zone of inhibition is indicated by diameters (in mm) and (-) represents No activity. The zones of inhibition produced by synthesized compounds (13, 15 & 10) were compared against the standard Ciprofloxacin for bacteria and Miconazole for fungi.

Microbial results revealed that the synthesized compounds 13 and 15 exhibited moderate to good antibacterial activity against *Bacillus subtilis, Escherichia coli* and *Citrobacter freundii*. Compound 10 showed good activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Citrobacter freundii*. Moderate fungicidal activity was observed against *Aspergillus niger* and *Tricoderma harzianum*.
CHAPTER-6

POSSIBLE MECHANISM
Scheme 27: Possible mechanism of piperidine derivative
CHAPTER-7

REFERENCES


[119] “Fentanyl Drug Overdose”, CDC Injury Center (29 August 2017), Archived from the original (15 December 2017), Retrieved (14 December 2017).


[164] Leusen, A. M. V., “p-TOLYLSULFONYLMETHYL ISOCYANIDE


