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SYNTHESIS AND CHARACTERIZATION OF 2-FLUORO PHENYLPIPERAZINE SUBSTITUTED IMIDAZOLE

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Advisor

Assist. Prof. Dr. Banu KEŞANLI

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APPROVAL

A thesis presented to the Institute of Health Sciences of Near East University, in partial accomplishment of the requirement for the degree of Masters in Pharmaceutical Chemistry. The thesis defense was online and all the jury members declared their approval as recorded.

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To my parents

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ABSTRACT

Imidazole core is a useful aromatic heterocyclic compound in the heterocyclic aspect of medicinal chemistry that normally is found in products which exist naturally and also found in synthetic molecules which have bioactivity. Its by-products are famous for exhibiting many different biological activities according to the literature.

As an attractive binding site, imidazole possesses the ability to react, in the human biological system, with various cations and anions and also with biomolecules via several reactions thus exhibiting extensive biological activities. This has created avenues for broad analysis of imidazole- based analogues in recent drug discovery and developments as antiviral, anti-inflammatory, anti-allergic, anti-neuropathic, anti-parasitic, antihypertensive, anticancer, antifungal, antibacterial, anti-tubercular, antidepressant, anti-obesity and analgesic.

In this present work, I have created a schematic and well organized proposal for creating Mannich base of 2-fluoro phenyl piperazine substituted imidazole having a piperazine cluster at the first position of the ring was synthesized using a classic Mannich reaction.
The reaction was monitored by TLC and melting point determination, whereas the compound's chemical structures of were gotten by using FT-IR and ^{1}H-NMR, and also ESI-MS analysis. The antimicrobial activity of synthesized molecule was tested via disk diffusion method.

Keywords: Imidazole, Mannich reaction, Reflux Synthesis, antimicrobial

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LIST OF ABBREVIATIONS

FT-IR	Fourier Transform-Infrared
NMR	Nuclear Magnetic Resonance
UV-Vis	Ultraviolet - visible
TLC	Thin Layer Chromatography
MW	Microwave
MAOS	Microwave-Assisted Organic Synthesis
RF	Retention factor
AMU	Atomic Mass Unit

1.INTRODUCTION

The imidazole ring is found in many natural products, especially alkaloids. This ring system is comprised in a number of useful biological building blocks such as, histidine, histamine, purine and nucleic acid. Due to its polarity and ionizability, imidazole compounds enhances the lead molecules' pharmacokinetic properties and is therefore utilized as an antidote for improving solubility of proposed poorly soluble lead molecules as well as their bioavailability parameters. Imidazole has been widely studied by scientists for its biological and pharmaceutical uses. In recent years it has become an important part of many pharmaceuticals. Many fungicides, antifungal, nitro-imidazole series of antibiotics, antihypertensive, antiprotozoal and the sedative midazolam medications contain synthetic imidazoles present in them.(Rich et al., 1997).

Imidazoles have a wide range of antifungal activity- it kills by preventing them from synthesizing ergosterol, the primary sterol found in the cell membrane of the fungi.

Almost one billion people are adjudged to be infected by fungal infections of skin, nail and hair, and over 150 million people are estimated to suffer from major fungal diseases that cause a tremendous effect on their health and lives or sometimes cause death. Despite the fact that mortality resulting from fungal diseases can be avoided, public health authorities still fail to look into this topic. (Bongomin et al., 2017)

This research seeks to further understand n- substituted imidazoles and their reaction with 2-fluoro phenylpiperazine and their role in the pharmaceutical and biological industry.

In this research study, imidazole and 2- fluoro-phenylpiperazine were reacted together through Mannich reaction under reflux conditions to synthesize 1-(2-Fluoro- phenyl) -4-imidazole-1-ylmethyl – piperazine.

The compound formed is characterized using ESI-MS, proton nuclear magnetic resonance (^{1}H – NMR) as well as Fourier transform infra- red (FT-IR). Melting point and thin layer chromatography (TLC) used to determine the purity. Preliminary antimicrobial activity was studied by using disk diffusion technique.

2. LITERATURE REVIEW

Pharmaceutical chemistry is defined as the field of chemistry concerned with the discovery, development, interpretation and the identification of mechanism of action of biologically active compounds, at the molecular level(Faruk Khan et al., 2011). Medicinal chemistry is very useful in the field of pharmaceutical science. This is due to its focus on determining the influence of chemical structure of molecules on biological activity.

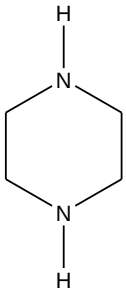
Six- membered, two nitrogen– containing, heterocyclic ring are major components of various biologically active synthetic compounds. Nitrogen- containing heterocyclics are very useful in medicinal chemistry as a result of their biological activity, two of such compounds being imidazole and piperazine.

2.1. Piperazine

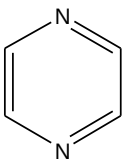
Piperazine is a part of heterocyclic chemstry comprsed of a saturated heterocyclic compound. It contains two nitrogen atoms at para positions to each other (named 1,4 – hexahydropyrazine).

The connecting part is a group of C-H-N, while the functionality of the terminal amine, chain of carbon, and the connecting group's nitrogen atom all make up the moiety of piperazine (Vibhor K Jain et al, 2011). Piperazines (cyclizines) are typically classified as derivatives of ethylenediamine or as cyclic ethylenediamines; they are a wide-ranging group of chemical compounds with several useful medicinal characteristics. The dinotrogen component it contains has been a constant part of a deluge of drugs. Piperazine shares similarity with piperidine in its chemical behavior; its first usage in medicine was as a solvent for uric acid. The first modification of piperazine that possessed broad spectrum activity was first introduced in 1950s. It found use against ascarides, small strongyles and pinworms; however it showed no effect when tested against the larger strongyles (Merck index, 11^{th} edition).

Piperazine and pyrazine amongst other six membered heterocyclic structures have shown numerous characteristics, such as antipsychotic, antidiabetic, antituberculosis, anthelmenitics, antianginals, anti-cancer, analgesic, antidepressant, antihistamines, hypolipidemic and flavouring agent. Medicinal chemists have been able to formulate a lot of new medicinal agents by using these drugs.



Piperazine

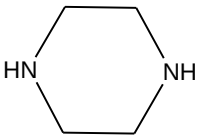


Pyrazine

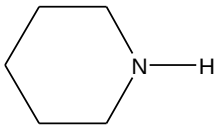
Figure 2.1: Examples of six- membered heterocyclics

2.1.1. Physical properties Of Piperazine

The structure of piperazine molecule comprises a symmetrical hexa- organic compound containing two nitrogen atoms at opposite positions; chemical formula $C_4H_{10}N_2$. The name piperazine originated as a result of the chemical similarity between piperazine and piperidine. The extra nitrogen atom on piperazine is what created the -az- attachment which is added to "piperazine" chemical name. It is, however, note-worthy to mention the fact that piperazines are not formed from the piper genus genre of plants.



Piperazine



Piperidine

The physical properties of piperazine include their occurrence as tiny unruly crystals which are basic and have a saline taste, readily absorbs water and carbon dioxide. It has a pK_b of 5.35 and 9.73 at 25^oc making it a weak base; having a pH range of 10.8 – 11.8 in a 10% aqueous solution. It dissolves readily in water as well as ethylene glycol, but not soluble in diethyl ether.

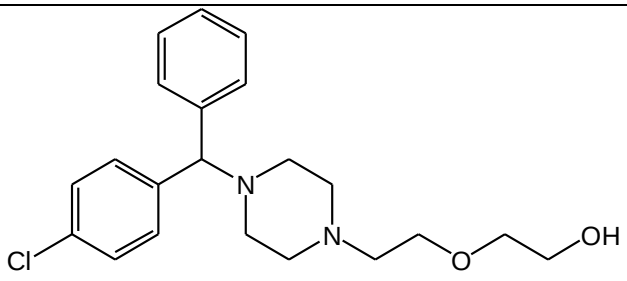
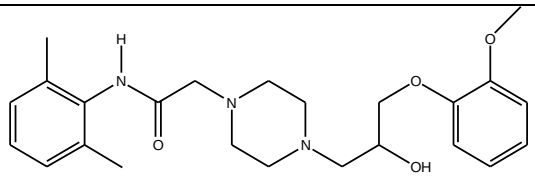
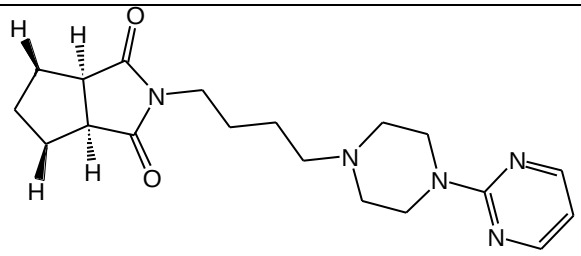
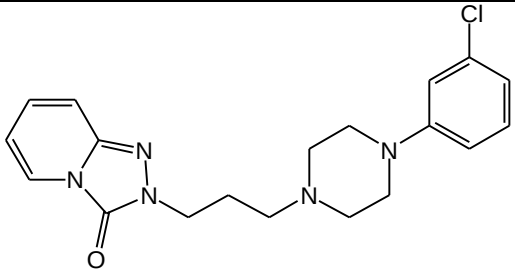
Several piperazine derivatives can be found naturally, however by itself, it can be synthesized via reacting 1, 2 – dichloroethane with alcoholic ammonia. It can also be synthesized by the reacting sodium and ethylene glycol on ethylene diamine hydrochloride, or by reduction of pyrazine with sodium in ethanol. Piperazine is industrially found as the hexahydrate, $C_4H_{10}N_2$. $6H_2O$, having a melting and boiling point of 44 °C and 125–130 °C respectively.

2.1.2. Piperazine ring containing drugs

Piperazine as a drug was first introduced to medicine for its use as a solvent for uric acid. It was then introduced as an anti-helmintic in 1953; a large number of piperazine compounds have anthelmintic action. Their mode of action is generally by paralyzing parasites, and by so doing, allows the host body to easily expel the organism invading.

Upon absorption into the body, the piperazine drug becomes partly oxidized and the other part is eliminated unchanged

Many piperazine derivatives are notable successful drugs, including:

Name	**Chemical Structure**	**Uses**
Meclozine	 2-(2-{4-[(4-Chloro-phenyl)-phenyl-methyl]-piperazin-1-yl}-ethoxy)-ethanol	Anti-histamine; used for preventing and treating nausea, vomiting, and dizziness which is associated with motion sickness.
Ranolazine	 *N*-(2,6-Dimethyl-phenyl)-2-{4-[2-hydroxy-3-(2-methoxy-phenoxy)-propyl]-piperazin-1-yl}-acetamide	Anti - anginal; used to treat heart related chest pain.
Tandospirone	 2-[4-(4-Pyrimidin-2-yl-piperazin-1-yl)-butyl]-tetrahydro-cyclopenta[*c*]pyrrole-1,3-dione	Anti- depressant; used as a treatment for anxiety and depressive disorders,
Trazodone	 2-{3-[4-(3-Chloro-phenyl)-piperazin-1-yl]-propyl}-2*H*-[1,2,4]triazolo[4,3-*a*]pyridin-3-one	Anti- depressant; used for the medical treatment of depression.

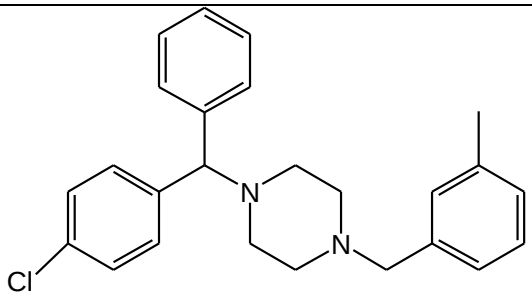
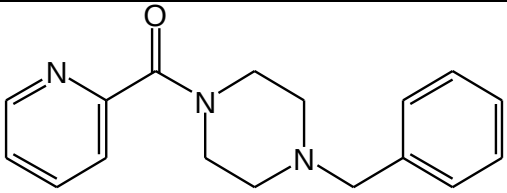
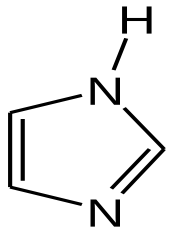
Vilazodone	 1-[(4-Chloro-phenyl)-phenyl-methyl]-4-(3-methyl-benzyl)-piperazine	Anti-depressant; used to treat major depressive disorder.
Piberaline	 (4-Benzyl-piperazin-1-yl)-pyridin-2-yl-methanone	Anti- depressant

Figure 2.2: Piperazine ring containing drugs

2.2. Imidazole

Imidazole molecule is also known as 1,3-diaza-2,4-cyclopentadiene. It has a geometry that is planar, comprising a penta - heteroaromatic molecule containing three carbon as well as two nitrogen atoms at meta position to each other (1,3). On discovery, it was initially named gluoxaline because it was first synthesized with glyoxal and ammonia.



1*H*-Imidazole

Figure 2.3: Structure of imidazole

Imidazole is amphoteric in nature (possessing both acidic and basic properties), making it susceptible to electrophilic as well as nucleophilic attack. The ionization constant for imidazoles stand at 14.5 meaning that it's acidity is more than alcohols but less than that of carboxylic acids, imides and phenols. It is highly stable to several thermal, acidic, basic, oxidation and reduction reaction conditions. Its hydrogen bond exists as wide and intramolecular. This 5 membered heterocyclic exists in two equivalent forms of tautomers,

this is as a result of its hydrogen atom having the ability to bond on either of the two nitrogen atoms.

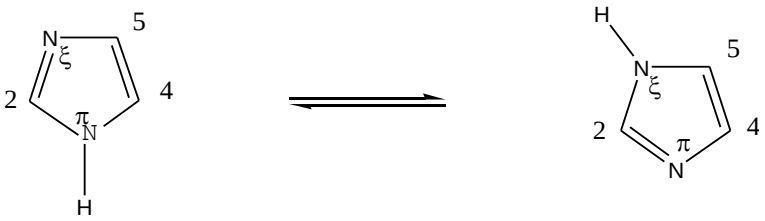


Figure 2.4: Tautomerism of imidazole at position one and three

Imidazole is termed under the classification of aromatic because the π-electrons exist and it does so as a sextet in the ring, The π-electrons in the imidazole ring comprises one electron pair donated from the protonated nitrogen atom and the remaining four electrons each donated by the atoms in the ring.

Below are some examples of imidazole's resonance structure. (Romero et al., 2014)

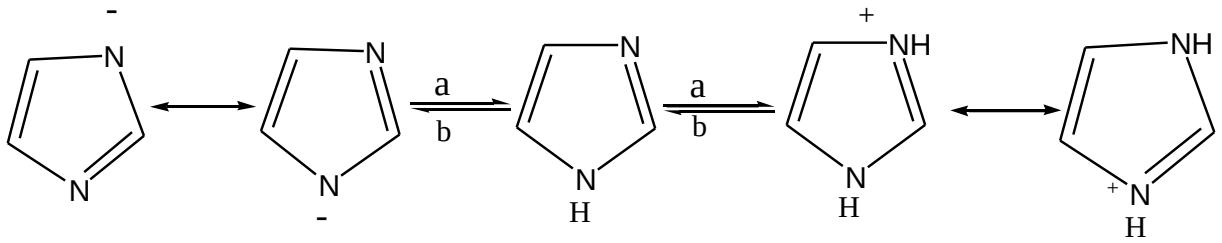
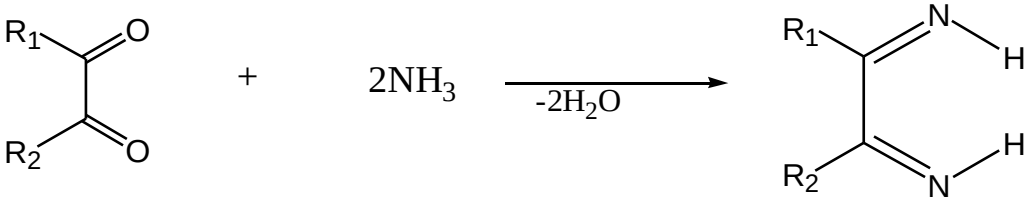


Figure 2.5: Resonance structures of imidazole

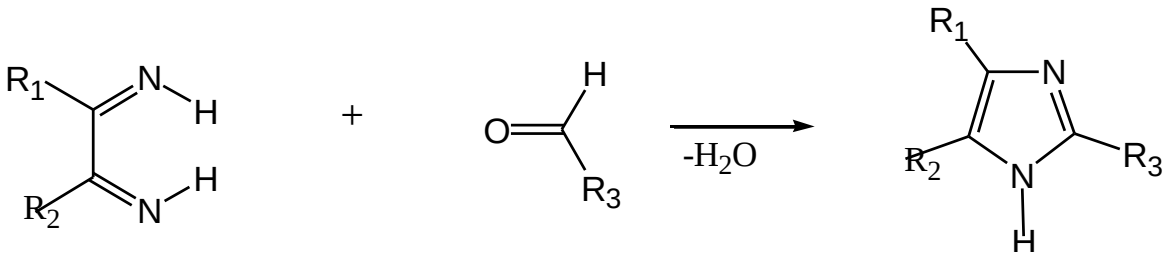
2.2.1. Synthesis of Imidazole

A method available for producing imidazoles commercially is the synthesis by Debus – Radziszewski. This is an organic reaction involving a dicarbonyl, an aldehyde and ammonia. The dicarbonyl component is commonly glyoxal, but can also include several 1,2 – diketones and ketoaldehydes.

The reaction usually occurs in two stages. The dicarbonyl condenses with ammonia to give a diimine in the first stage, (expressed by the unusual orientation of N-H groups):



Aldehyde reacts with diimine in the second stage via condensation reaction. Heinrich Debus and Bronislaw Leonard Radziszewski were the inspiration for the name of this reaction.



The general method is modified by replacing an equivalent of ammonia by an anime, causing formation of *N*-substituted imidazoles in high yields.

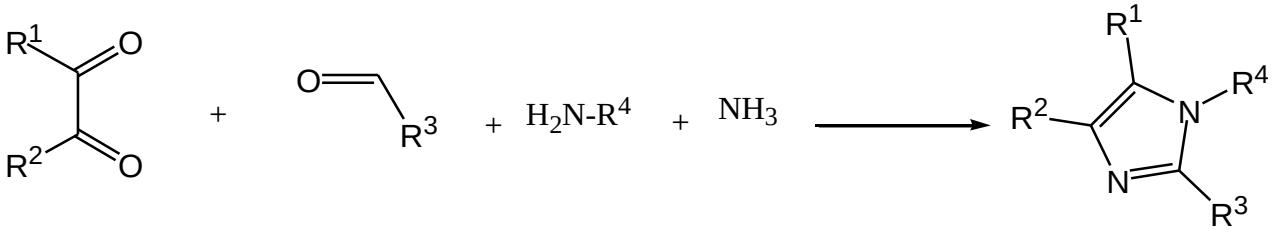


Figure 2.6: Reaction for synthesis of imidazole.

2.3. Pharmacological Activity of Imidazole

Derivatives of Imidazole are of huge interest in pharmaceutical chemistry because they have useful biological activities; several numbers of these compounds have been selected as candidates for drug development and they have garnered the attention of several research groups.

The chemistry of imidazoles has significant usage due to the fact that their ring systems are found in various biologically active compounds. Turner, Huebner, and Scholz synthesized several 4- disubstituted aminomethyl imidazoles via a multistep process. And these imidazole derivatives are known be antihistaminic, while the rest imitated histamine(Stocker et al., 1970).

Judging from the literature surveys, imidazole derivatives have been known to have various pharmacological activities (Asadi et al., 2017)

(i) Antibacterial activity
(ii) Anticancer activity
(iii) Anti-tubercular activity
(iv) Analgesic activity

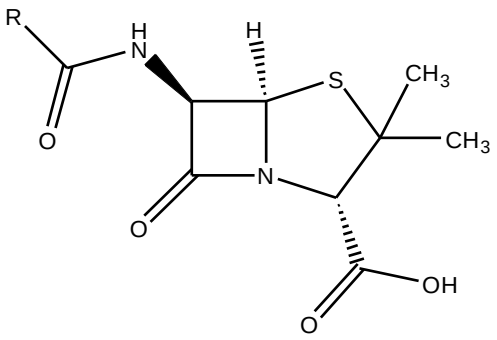
(v) Anti-HIV activity.

(vi) Antifungal

2.3.1. Antibacterial Activity

Antibacterial agents are classified as a body of matter that attack pathogenic bacteria. They kill or reduce the metabolic activity of bacteria, thereby minimizing their effects as pathogens in the biological environments.

Ever since the discovery of penicillin in the 1920s, antimicrobial chemotherapy has become an indispensable part of health care due to their function as treatment for human infectious diseases.



Where R is the variable group

Figure 2.7: Chemical structure of Penicillin.

Numerous antimicrobial agents have been developed over the years and a large variety is currently available for clinical use. However, due to the incessant development of agents make it difficult for pharmacists to keep up with progress in the field. The large amount of antimicrobial agents available make it difficult to decide the right agent to incorporate in routine and specialized susceptibility testing.(Actor et al., 1980)

Compounds with small molecules are typically the most frequently utilized agents during antibacterial therapy(Li et al., 2020). Heterocyclic compounds such as guanidines which are gotten from carbazoles have shown good antibacterial activity because of their azole ring. Their metal ions play a major role in their antibacterial activity. An example of heterocyclic metal ions in antibacterial therapy is the N- heterocyclic carbene complexes of silver which serve as agents against a broad spectrum of bacteria.

Recently though, antibiotic resistance has become an escalating world- wide problem as a result of the fact that the diminishing molecules are produced by using the same strategy to monitor all the different collection of compounds or to modify already existing antibiotics chemically. Usually gram- negative bacteria, more than gram – positive bacteria are especially prone to resisting antibiotics due to the fact the outer membrane of the Gram-negative bacteria is typically embedded by a slime layer. This slime layer then conceals the antigens of the cell.

The gram- negative bacteria possesses an outer membrane with peculiar structure and this structure stops some kinds of drugs and antibiotics from gaining entrance into the cell. The implication of this is that the bacteria has developed resistance to drugs and are thus classified as dangerous disease-causing organism.

2.3.1.1. Classification of Bacteria

Broadly speaking, bacteria can have two different types of cell wall, thus classifying them into two groups; the gram- positive and gram- negative bacteria. Their names come from how their cells react to the gram stain. This has also served a perpetual test for the classification of species of bacteria.

Table 2.1: Classification of bacteria

Gram- negative	**Gram - positive**
Escherichia coli *Salmonella* *Shigella* *Enterobacteriaceae* *Pseudomonas* *Moraxella* *Helicobacter* *Stenotrophomonas* *B dellovibrio* *Acetic acid bacteria* *Legionella* etc	*Staphylococcus* (catalase-positive), *Streptococcus* (catalase-negative), coagulase-positive (*S. aureus*), coagulase-negative (*S. epidermidis*, *S. saprophyticus*) species

Bacteria strains easily get resistant to antibiotics, as a result this has motivated researcheers to evaluate novel antibacterial compounds such as imidazole and thiazole derivatives.(Ghasemi et al., 2015). Pathogenic bacteria are the causal agents for several serious diseases and a lot of mortality in many countries. They spread quickly, and the most susceptible to them are immune- compromised people, pregnant mothers, children and the senior citizens. As a result of the progressive resistance of bacteria to the current antibiotics in medicine due to irregular antibiotic consumption, the general health and hygiene of people are at high risk which is why the identification and utilization of novel antibacterial compounds is crucial. Studying the anti-bacterial effects of these compounds have laid emphasis on their power to inhibit the bacteria such as *Enterococcus faecalis, Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Robert et al., 2003).

Ramya v et al were able to synthesize several new derivatives of 5-(nitro/bromo)-styryl-2-benzimidazole. They were then tested for their antimicrobial activity against several pathogens; *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia,* and *Enterococcus faecalis*. They also tested for their anti-fungal activity against *Aspergillus* fumigates *and Candida albicans*. Their result was then compared with ciprofloxacin.

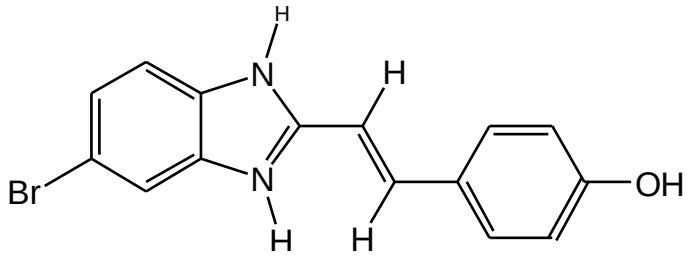
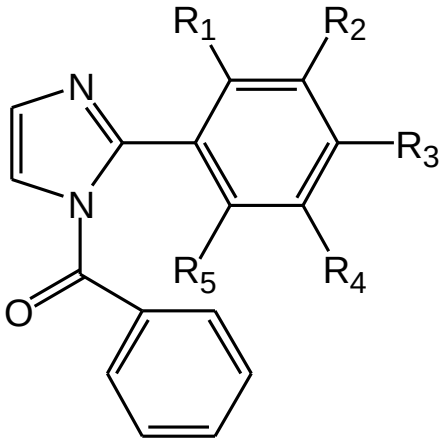


Figure 2.8: Chemical structure of 5-(nitro/bromo)-styryl-2-benzimidazole having anti-fungal as well as anti-bacterial effect

Deepika Sharma et al also carried out antibacterial activity test against Gram negative, gram positive species, and antifungal activity against fungal species using 2-(substituted phenyl)-1H-imidazole as well as (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl] -menthanone analogues. The result was compared against Norfloxacin which was used as standard. The most potent compound was



For compound 1 R1=Cl, R2=H, R3=H, R4=H, R5=H, X=4-NO2

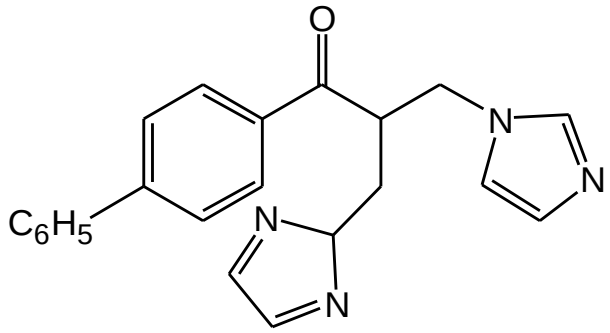
2, R1=COOH, R2=H, R3=H, R4=H, R5=H, X=4-NO2

3, R1=H, R2=H, R3=Cl, R4=H,R5=H,X=2-Br

4 R1=H,R2=H,R3=NO2 ,R4=H,R5=H,X=2-B

Figure 2.9: General chemical structure for (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-menthanone analogues

Daniele Zampieri et al synthesized some derivatives of bis-imidazole and tested their antifungal and anti-mycobacterial activity. The compounds mostly exhibited moderate to good activity against the fungi; *Candida albicans* and *Candida glabrata*. Their result was compared against Miconazole which served as reference drug.



1-Biphenyl-4-yl-3-imidazol-1-yl-2-(2*H*-imidazol-2-ylmethyl)-propan-1-one

Figure 2.10: Chemical structure of bis- imidazole derivatives

Imidazole, 2-methylimidazole and 2-methyl-4-nitroimidazole underwent N-Alkylation according to (S. Khabnadideh et. Al). The alkylation served as synthetic route to effectively create antibacterial agents. Then, they screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Khabnadideh et al., 2003). In alkyl chains, as the number of carbons increases up to nine carbons, the antibacterial effects of 1-alkylimidazole derivatives also increases. When they did substitution on imidazole ring by 2-methyl and 2-methyl-4-nitro groups, the anti-bacterial activity increased.

2.3.2. Anti-Fungal Activity

Antifungal drugs are also known as antimycotics. Medicinal chemists synthesize pharmaceutical fungicide (also known as fungistatic) to prevent and cure mycoses. Examples of such mycoses include ringworm, athlete's foot, candidiasis (thrush), etc. The fungicide also treats serious infections of the body system such as cryptococcal meningitis as well as others. Typically, doctors have to prescribe such drugs before they can be dispensed, but a few can be obtained OTC (over the counter). At the moment, mycoses of the system are mainly treated by using polyenes and azoles. Polyene antibiotics target ergosterol (the major component of fungal membrane), while azole derivatives targets the ergosterol biosynthesis pathway. Ergosterol has a variety of functions in the fungal cell e.g regulating the fluidity, integrity and proper functioning of membrane-bound enzymes like proteins which transport nutrient transport and chitin synthesis.

2.3.2.1. Fungi Infection

Prior to a couple of decades ago, infectious diseases caused by bacteria have been the most feared. Parasites, fungi, prions, worms, helminthes and viruses especially have also been implicated (de Pauw, 2011). Improved methods in controlling bacterial infections resulted in fungi becoming the most perilous pathogens. Fungi such as yeasts and moulds have been ranked in the top ten most commonly separated pathogens among Intensive Care Units patients.(de Pauw, 2011). 7 percent approximately of all feversh episodes that happen to patients during neutropenia can definitely be credited to invasive infections caused by fungi. Hospitals in the USA presently isolate *Candida* a lot more frequently (top four bloodstream isolate) than several historically infamous bacterial pathogens

Fungi belong primarily at the end of biological life even though they can be useful in nutrition by providing us with wine and beer, as well as give taste to cheese and other food.

Their function is to extirpate decaying organic materials such as human cadaver by dissolving them – the big cleansing machines of the world. Fungal growth is signified by the appearance of decay, and once triggered the fungus expands further no thanks to medical intervention. Conversely modern treatment techniques even expedite the growth rate of fungi via negative interferences with the enduring parts of the immune system.

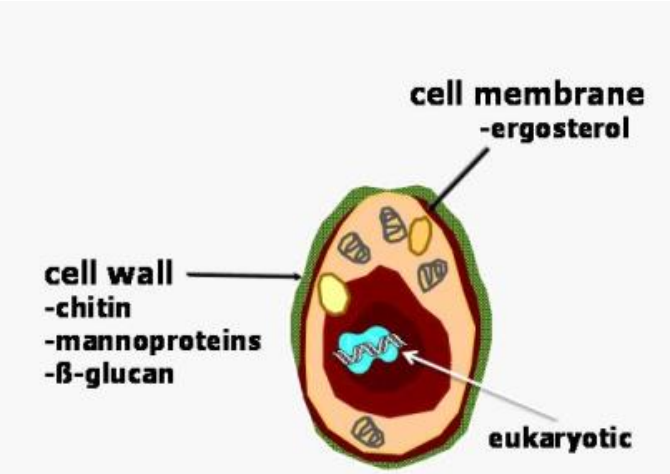


Figure 2.11: The Fungus

2.3.2.2. Fungal Infection in Cancer Patients.

Autopsy examination surveys have been done around several different institutions in Canada, Japan and parts of Europe to analyze how many cancer patients have fungal infections. Leukemic patients and recipients for transplant showed the highest frequency of fungal infections (25 % each). These fungal infections came from 50% *Candida spp* and 30% *Aspergillus spp.* Surveys conducted in different countries displayed sizable irregularity in the frequency of their fungal infections. Regardess, the study definitely confirms that fungal infections are a common problem amongst Leukemic cancer patients(Bodey et al., 1992). Different researchers at their respective institutions have highlighted the high number of candidiasis occurrence recently.

(Bodey, 1966) discovered that Patients with acute leukemia were experiencing an increase in disseminated candidiasis by 7% between 1954 and 1958 to 20 % between the years 1959 and 1964. (Myerwitz et al., 1977) also discovered that out of eighty two patients having acute leukemia who underwent autopsy between the periods of 1963 and 1971, 3% had disseminated candidiasis. Myerwitz also observed between 1972 and 1975 that 33% of 47 patients screened had the disseminated candidiasis

The most commonly occurring of the pathogens are *Candida spp.* and *Aspergillus spp*. Majority of their infections were not considered before the patients died. This is because only few of the patients were given systematic antifungal therapy. There should be more knowledge about the chances of these infections affecting cancer patients in order for higher success rate in managing these infections, as well as better diagnostic methods and greater use of antifungal agents.

Fungal infections especially when invasive pose an incessant and significant threat to human health. They are annually linked to at least1.5 million deaths worldwide. According to literature estimates, invasive *candidiasis* causes 30 – 40% mortality, disseminated *cryptococcosis* and invasive *aspergillosis* supports 20 – 30% mortality rate.

2.3.2.3. Fungal Infections in Aids Patients

Fungal diseases linked to AIDS emerged massively in the 1980s. From the 1970s scientists observed an increase in the frequency of deadly mycoses linked with usage of immunosuppressive medical therapies. This rise has motivated researches geared towards the discovery and development of new antifungal agents. The studies resulted in development of six new agents, divided into two classifications. The agents recently are been licensed to use on humans or undergoing phase 3 clinical trials. Other unofficial classes of novel antifungal agents have been reported but they have not been successfully transitioned into use for therapy after their discovery.

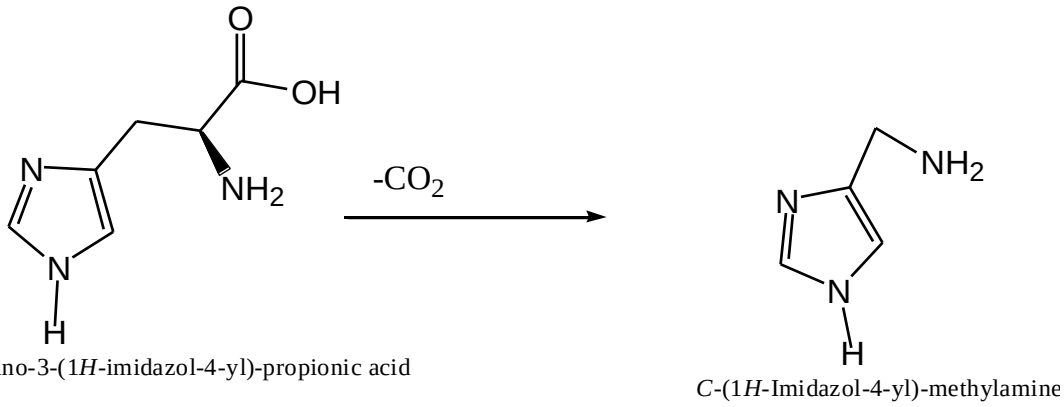
2.3.2.4. Classes of Antifungals

There currently exists four classes of antifungal agents namely; echinocandins, pyrimidine analogs, azoles, and polyenes. These agents are administered either topically, intravenously or orally to treat fungi infections. There exists a fifth class, allylamines, which are only used for treatment of superficial dermathophytic infections

These different antifungals, however, have certain pitfalls in terms of their spectrum of activity, safety, toxicity and pharmacokinetic characteristics. Due to the resistance of emerging strains of fungi to the currently available antifungal agents, there have been great efforts led to make new drugs with new mechanisms of actions which will target the biosynthesis of fungal lipids, proteins and cell wall (Espinel-Ingroff, 2009).

2.4. Biological importance of Imidazole

So many significant biologically active molecules have Imidazole molecule fused into them and the biggest being the amino acid Histidine – imidazole side chain. The Histidine can be found in several proteins and as an enzyme it plays an important part in the structure and binding functions of hemoglobin. Histidine undergoes decarboxylation to form histamine, which is another common biological compound. Histidine is the part of the toxin that causes urticaria, i.e. allergic reactions. The chemical reaction of the decarboxylation of Histidine to histamine is shown below.



2-Amino-3-(1*H*-imidazol-4-yl)-propionic acid

C-(1*H*-Imidazol-4-yl)-methylamine

Figure 2.12: Decarboxylation reaction of Histidine

2.5. Mannich Reaction

Mannich reaction is among the most pivotal type of reaction under basic conditions in organic chemistry . Preparation of beta aminoketones and aldehydes (mannich bases) usually follows the classical method of this reaction. Mannich reaction is defined to be an organic reaction which converts a primary or secondary amine as well as two carbonyl compounds(enolizable and non-enolizable) into a β-amino carbonyl compound (Mannich base) by use of acidic or basic catalyst

It is a very crucial process when synthesizing several many pharmaceutical products and natural products. The derivatives of mannich bases are versatile synthetic intermediates useful in medicinal chemistrry. Example of such bases are 1,3-aminoalcohols or Michael acceptors. The reaction proceeds usually using protic solvents like methanol, ethanol, and acetic acid. The protic solvents are used because of their high concentration of electrophilic iminium ion. The schematic representation of Mannich reaction is shown below.

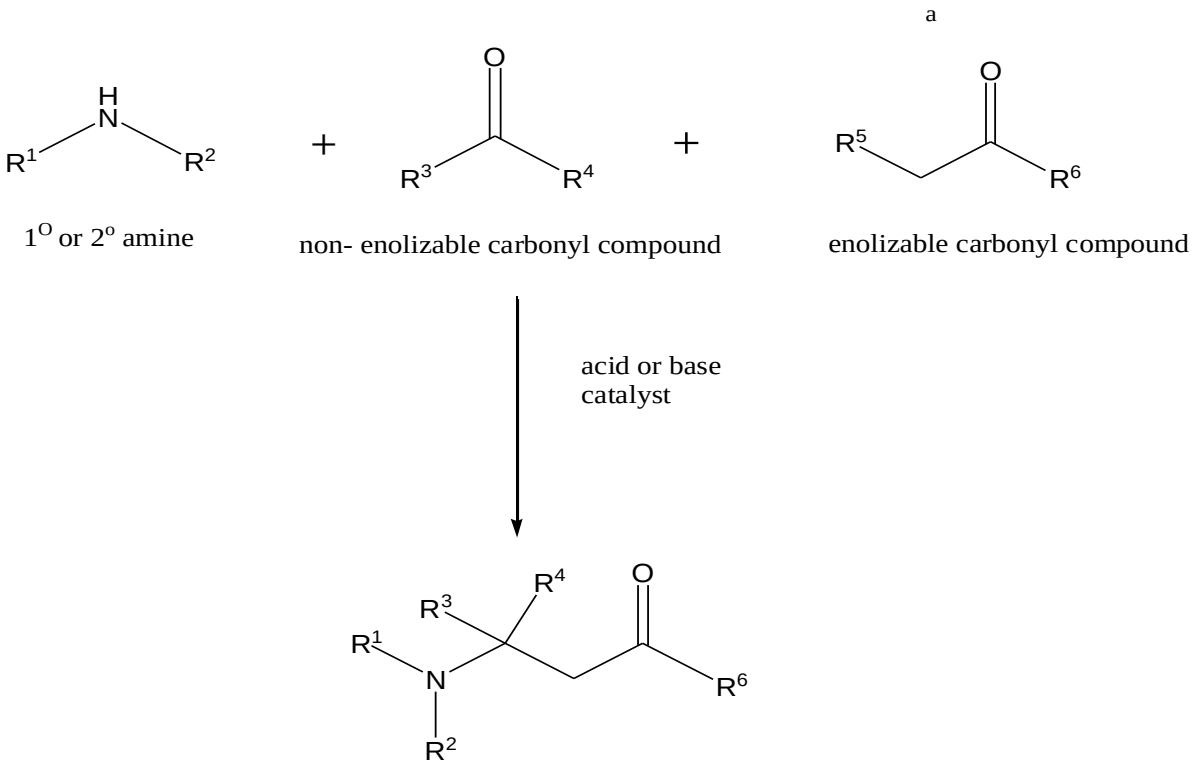


Figure 2.13: Schematic representation of general Mannich reaction

2.5.1. Synthetic Application of Mannich Base

A mannich bases are defined as βeta-amino ketone carrying compound. They are also defined as the end product of a Mannich reaction. Mannich bases function as vital pharmacophores or bioactive "lead" used as active agent and starting material for the synthesis of various drugs. They're highly reactive compounds and easily convert into other compounds, making them indispensable in developing synthetic route in medicinal and pharmaceutical chemistry. A Mannich base is also reportedly anti-inflammatory, antibacterial, antifungal, antiviral and analgesic in characteristics (varma et al.1968). Mannich bases' most useful function is in pharmaceutics. Research has shown that they exhibit excellent antimicrobial, anti-cancer, anti-tubercular and anti-HIV activities. As a side chain they display pronounced antimalarial, anti-inflammatory, analgesic and antimicrobial activities. The nucleus of Imidazole play a major role in drug design of different kinds of drugs e.g oral anti-inflammatory agents, protein kinase inhibitors, angiotensin II receptor antagonists, and fungicides. It is typically found embedded into numerous biologically and medicinally useful substances

Some synthetic applications of Mannich bases are giving below

1. Synthesis of 3,3-[piperazine-1,4-diylbis(methylene))bis(5-chlorobenzo[d]oxazol-2(3H)-one), by reaction of Mannich base (chlorzoxazone), formalin and appropriate secondary amine(piperazine) and methanol as a solvent (Soyer et al., 2013).

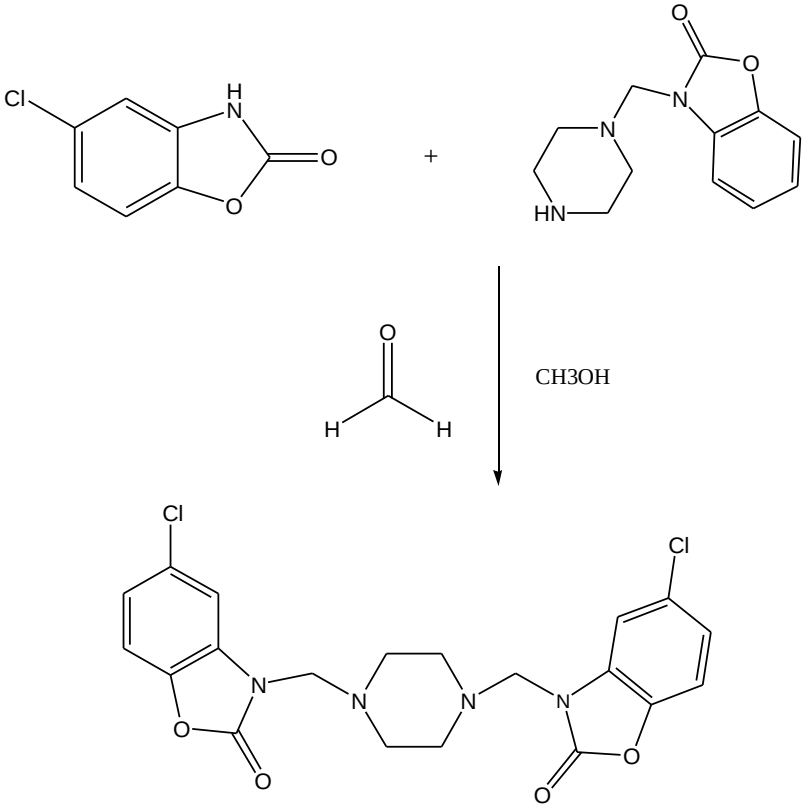


Figure 2.14: Synthesis of a Mannich base, 3,3-[piperazine-1,4-diylbis(methylene))bis(5-chlorobenzo[d]oxazol-2(3H)-one)

Different authors, even as far back as the 19th century have been able to describe Mannich reaction as the amino alkylation of CH- acidic compounds. However, it was Carl Mannich who first realized the expansive gravity of a reaction like this, and through systematic research he was able to expand the chemistry of the reaction into a broad-based method of synthesis (Arend et al., 1998) . Consequently getting named after him, the reaction grew into becoming a very significant kind of reaction in organic chemistry involving Carbon-Carbon bonds.

Practically, aldehydes or ketones which can be enolised function as the CH- acidic substrate when carrying out mannich reactions. A variation of the reaction depicts the carbonyl compounds heated using formaldehyde and an amine hydrochloride using aprotic solvent.

2.5.2. Mechanism of the Mannich Reaction

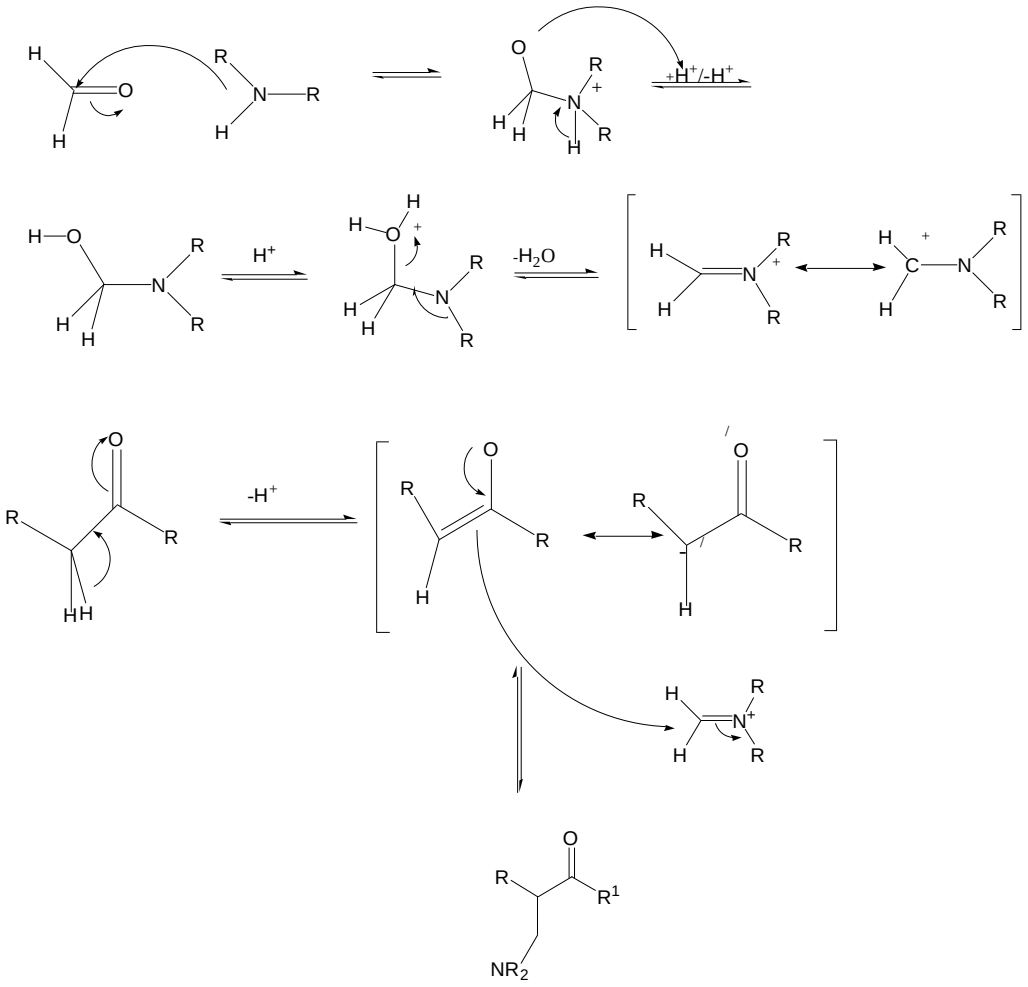


Figure 2.15: Mechanism of Mannich reaction.

2.6. High Temperature Synthesis Method

2.6.1. Reflux Heating Method

The rates of chemical reactions vary greatly. Some reactions are instantaneous, some are slow while others may reach their equilibrium at a very long time. The measure of change in concentration of the reactants or the products per unit time is referred to as reaction rate. The rate of reaction usually increases with increased temperature and vice-versa. Some organic

reactions are extremely slow and take a long time to achieve any noticeable yield. To increase the rate of such reactions, heating is usually explored. However, some organic compounds have low boiling point and may vaporize upon exposure to such heat, thereby inhibiting the reaction to proceed at a satisfactory rate. Therefore heating the mixture under reflux is the solution to overcome this problem.

Figure 2.16: Reflux heating set up

2.6.2. Microwave Heating Method

In research and drug discovery, a major stumbling block usually encountered is synthesis of new chemical entities. Normal strategies of different chemical syntheses are generally used. The burner was invented in 1855 by Robert Bunsen, to serve as a source of energy for heating reaction vessels. It was then replaced by isothermal, hot plate or oil bath (reflux heating), however though, the dangers associated with heating remains constant. Microwave-assisted organic synthesis (MAOS) is now recognized as a new 'principal' in organic synthesis-superior to reflux heating method (Bhupinder et al., 2010). Microwave techniques offer efficient, clean, fast and simple,improved purity and economical (Borkar et al., 2014). It is

also regarded as an important “green chemistry” approach due to its environmentally sound, clean procedures and eco-friendly nature (Budiati et al., 2012).

Figure 2.17: Microwave heating set up

This technique has become popular over the past decade as an instrumental tool for the fast and efficient synthesis of different compounds and become a cutting edge technology across medicinal, pharmaceutical, biotechnological and fine chemical industries (Enamul et al., 2013).

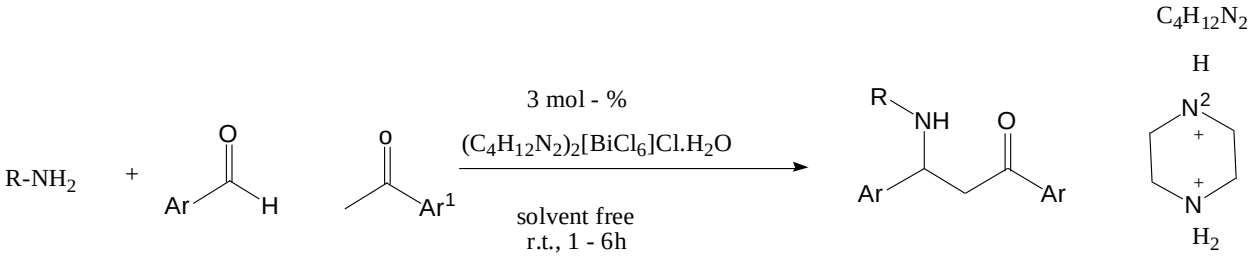
Table 2.2: Difference between reflux and microwave heating methods

	Reflux heating	**Microwave heating**
Heating	by thermal or electric source	by electromagnetic source
Mechanism of heating	by conduction	by energetic coupling, it includes dielectric polarization and conduction
Transfer of energy	Takes place from the wall to the vessel's surface to the reacting mixture and eventually to the reacting species	the core of the reacting mixture is heated directly
Super heating	absence of superheating	super heating occurs (i.e., the temperature of the mixture can rise above its boiling point
Heating selectivity	non-selective heating (i.e., all components of the mixture are heated equally)	Selective heating (i.e., specific components can be heated specifically
Safety	High probability risk	safe, simple and clean procedure

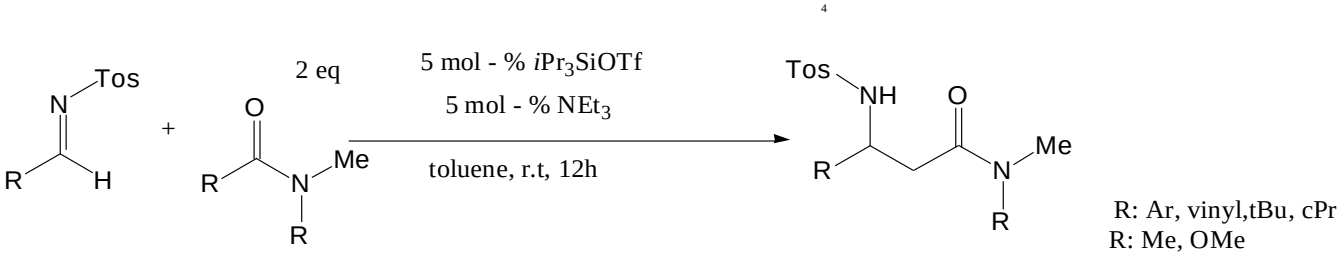
.

2.7. Recent Literature On Mannich Reaction

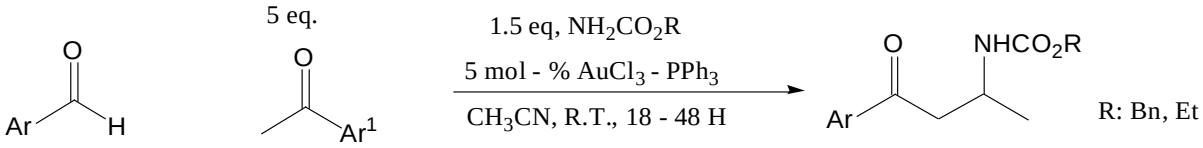
A mild, solvent – free, efficient, one- pot three-Component Mannich Reaction Catalyzed by $(C_4H_{12}N_2)_2[BiCl_6]Cl{\cdot}H_2O$ (Lu et al., 2015)



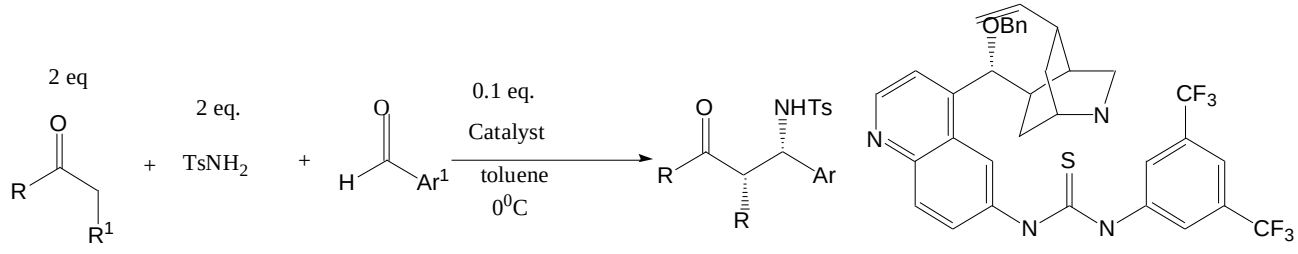
Catalytic Silicon-Mediated Carbon-Carbon Bond-Forming Reactions of Unactivated Amides(Kobayashi et al., 2011)



Transition Metal Salt-Catalyzed Direct Three-Component Mannich Reactions of Aldehydes, Ketones, and Carbamates: Efficient Synthesis of *N*-Protected β-Aryl-β-Amino Ketone Compounds. (Xu et al., 2004)



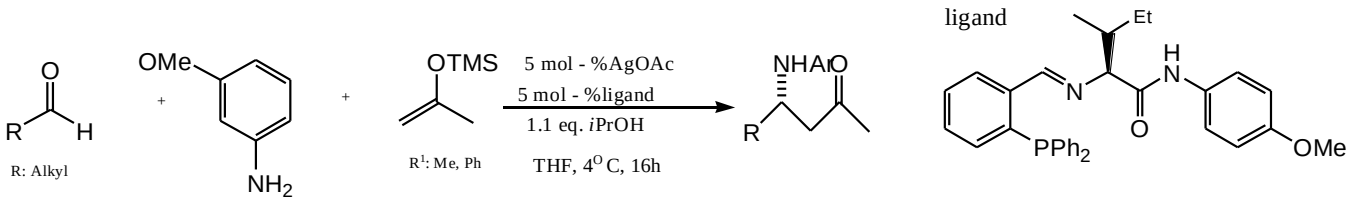
Highly Enantioselective Three-Component Direct Mannich Reactions of Unfunctionalized Ketones Catalyzed by Bifunctional Organocatalysts(Guo & Zhao, 2013)



R: Ar^1
Bn, Et

R^1 : Ph, OBoc

Ag-Catalyzed Asymmetric Mannich Reactions of Enol Ethers with Aryl, Alkyl, Alkenyl, and Alkynyl Imines(Josephsohn et al., 2004)



Disulfonimide-Catalyzed Asymmetric Synthesis of β^3-Amino Esters Directly from *N*-Boc-Amino Sulfones(Wang et al., 2013)

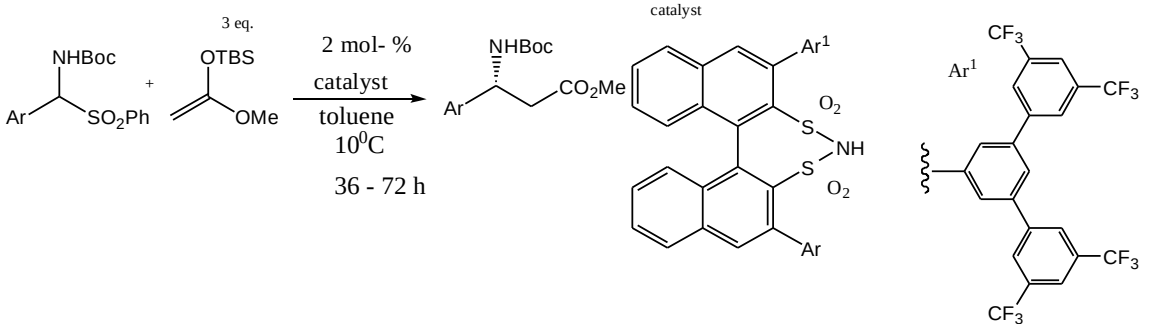


Figure 2.18: Recent Literature on Mannich Reaction

2.8. Mannich Reaction of Imidazole

As earlier stated, imidazoles have quite a significance because of the occurrence of their ring in different kinds of various biologically important compounds. Turner, Huebner, and Scholz using a multistep process, synthesized some 4-disubstituted aminomethyl imidazoles which have been shown to have antihistaminic action. In order to introduce amino methyl group into the imidazole ring, it was crucial to study the one step mannich reaction method (Stocker et al., 1970).

The facile base – catalyzed cyclization of histamine was partially the reason mannich reaction of imidazole was studied.

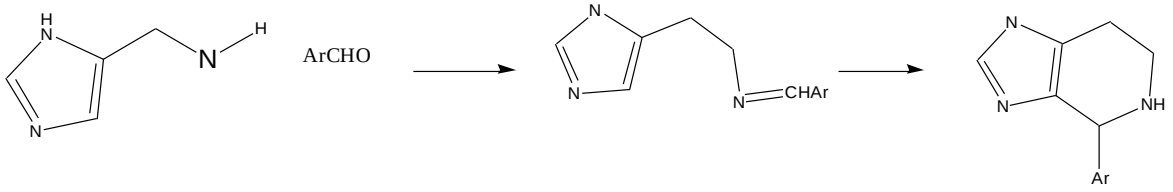


Fig 2.19: Base – catalyzed cyclization of histamine

The imidazole ring, during its mannich reaction, displays reactivity at four sites possibly; positions 1,2, 4 and 5. The basic media forms products that are substituted by carbon and nitrogen. In basic media, the nitrogen substitution process is reversible while carbon substitutions are irreversible, causing the amassing of carbon substituted products over time in the basic media. Position one displays highest reactivity and position three has lowest reactivity. Positions 4 and 5 show more reactivity than position 2. When there are substituents on position 1, that nitrogen would not undergo mannich reaction.

In 1951, Heath, Lawson, and Rimington carried out a research and discovered that substitution takes place on the 5(4) position of the imidazole ring during the mannich reaction of 2-mercapto-4(5)-methylimidazole (Kato et al, 1951). In the product, there was no proof of the substitution site. Kato, Morkawa, and Suzuk P in 1952 also wrote on the reaction of 4(5)-methylimidazole and imidazole when reacted with dimethylamine resulting in a 4 (5) - substituted product

3. MATERALS AND METHODS

3.1. Materials

The starting chemicals that were utilized in this study were 1-(2- fluorophenyl) – piperazine, imidazole, ethanol, formaldehyde solution. The chemicals as purchased from Sigma Aldrich Chemical Company were utilized without any extra purifying or drying. Mettler Toledo FP 900 ThermoSystem was used to measure the melting points device in the laboratory.

3.2. Thin Layer Chromatography (TLC)

3.2.1. Material

In TLC, the plate was made of silica gel/TLC-plates (DC-Alufplien-Kieselgel, Germany) and the solvents used were, benzene, ethyl acetate, hexane and methanol. Silica gel plates were detected under UV- Light (245nm).

Three different mobile phases were prepared with different solvents at different ratio as follows;

M-1: Benzene – Methanol (5:1)

M-2: Benzene – Methanol (9:1)

M-3: Ethylacetate – Hexane (1:2)

3.2.2.Method:

The solvent was transfer into the chamber having a depth just shy of 0.5 cm, it was then gently swirled, and left to sit and during that time, the TLC plate was prepared. TLC plates were cut horizontally into plates of about 6cm long by various widths and a line is drawn across the plate at 0.5 cm counting from the bottom of the plate and also about 0.5cm from the top with the aid of a pencil.

The starting materials and product were dissolved in chloroform and with the aid of a microcapillary spots were made on the TLC plate and the prepared plate was gently placed in the chamber. The plate was allowed to develop until the solvent front was

reached to the previously drawn line about 0.5 cm beneath the top of the plate and the plate was then removed, and then the plate allowed drying. The spots were viewed under
UV light at 254 nm and Rf values calculated.

3.3. Melting Point Determination

The Mettler Toledo FP 900 Thermo System Digital melting point apparatus was used to record the melting point of the compounds.

3.4. Reflux

Reflux reaction was carried out for two hours using a reflux apparatus.

3.5. Spectroscopy

All spectrometric analyses were done at Central Laboratory, Ankara University, Turkey.
A spectrophotometer, Perkin Elmer Spectrum 100, displayed in wave numbers (cm -1) was used to analyze the attenuated reflection of each synthesized molecule.
The proton NMR spectrum was analyzed using a device called Mercury Varian 400 MHz with tetramethylsilane used as a standard solution.
Mass Spectrometry analysis was carried out on Waters Alliance HPLC and ZQ micromass (Waters Corporation, Milford, MA, USA) LC-MS spectrometry as, Electrospray ionization (ESI) in (+) ion mode.

3.6 Experimental

Experimental procedure was short and reaction followed a single step reaction.

3.6.1. Synthesis of Compound

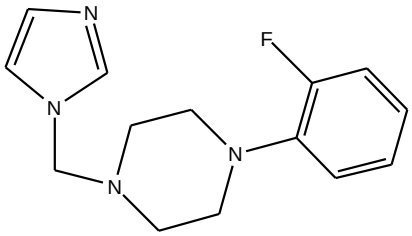


Figure 3.1: Structure of 4- (2- fluorophenyl) 1- methylpiperazine imidazole

0.3g of imidazole was measured into a beaker and dissolved in 10ml ethanol by stirring the solution with a stirring rod. 0.65ml of 1-(2- fluorophenyl) – piperazine was added into the beaker containing dissolved imidazole. 2ml ethanol was mixed with 0.2ml formaldehyde in a separate clean beaker, stirred and poured into the previous beaker then transferred into a 50 ml round bottom flask. It was kept to reflux at high temperature for two hours. After reflux, vacuum filtration was carried out to separate the solid product from the reaction mixture. The solid was recrystallized from ethanol, weighed and analyzed for its melting point and TLC.

3.6.2. Antibacterial activity

10mg of the compound was analyzed for the antibacterial activity, assayed using agar disc – diffusion method. It was injected into small discs and placed on the surface of Mueller – Hinton agar which is inoculated with 0.5 McFarand of the stated strain; *Pseudomonas aeruginosa. Staphylococcus aureus, Escherichia coli,* and *candida albicans.* After incubating for 24 hours under 37^{o}c, the injected discs diffused to the agar and from a zone called "inhibition zone". Inhibition zone diameter was then measured using millimetric scale for each disk.

The principle of this method followed the basis of the rate of diffusion of the antibacterial compound from reservoir disc to the microorganism.

3.6.2.1. Micro- organism tested

Test was carried out for four bacteria (two gram negative and two gram positive) on my sample. *Staphylococcus aureus* was the gram positive micro- organisms, and

gram negative micro-organisms was *Escherichia coli*, and *Pseudomonas aeruginosa.* Test was also done on *candida albicans* fungi.

4. RESULT AND DISCUSSION

4.1. Results

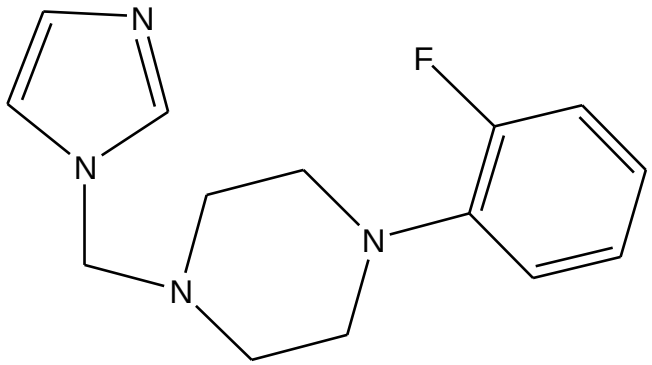


Figure 4.1: Structure of 4- (2- fluorophenyl) 1- methylpiperazine imidazole

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4.1.1. Mannich reaction via reflux

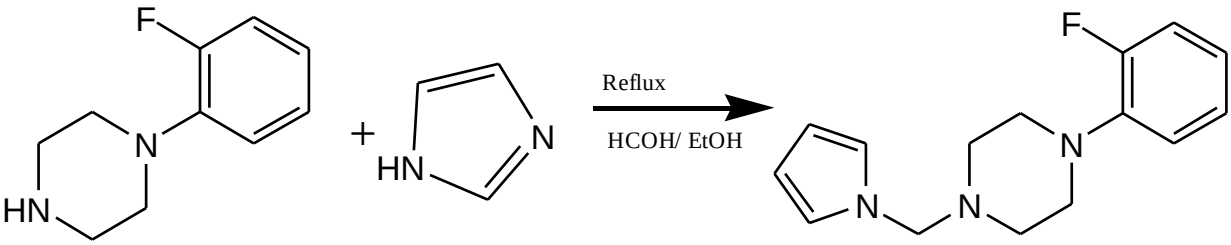


Figure 4.2: Mannich reaction of 4- (2- fluorophenyl) 1- methylpiperazine imidazole

White crystalline solid was obtained with a yield of 21.7% (0.23g) and a melting point of 90^{o} C.

TLC of compound formed gave R_f value of 0.20cm.

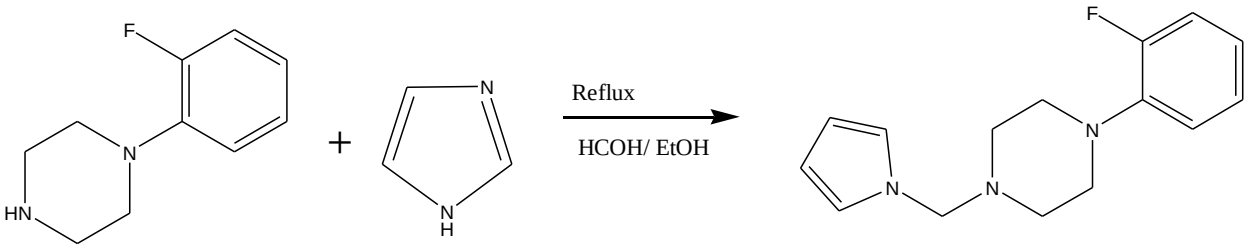
4.1.2. Fourier Transform Infra- red (FT-IR) Spectroscopy (IR V_{max}): FT- IR showed absorption band at 2715 – 3039 cm^{-1} aromatic (C-H stretch), 1231 – 1496 cm^{-1} (C- F stretch).

4.1.3. Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR in $CDCl_3$): ^{1}H NMR showed chemical shift at 7.2 – 7.0 δ (8H, m, Ar- H); 3.3δ (2H, s, N- CH_2 -N); 2.8δ (2H, t, pip H^6- H^7); 3.3δ (4H, t, pip H^5 – H^8)

4.2. DISCUSSION

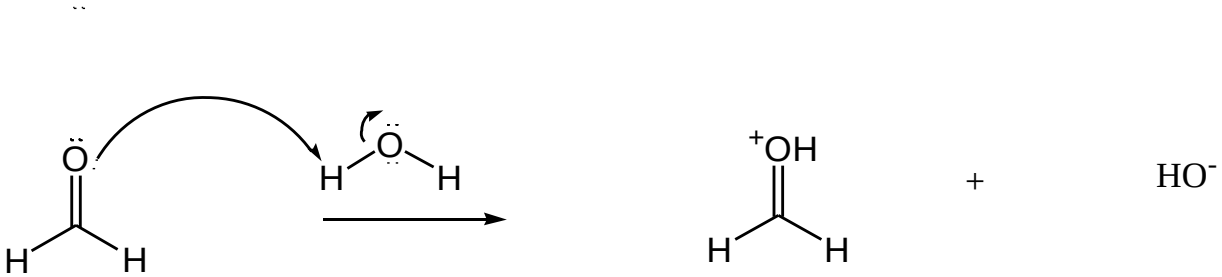
In this research, a piperazine derivative was synthesized via Mannich reaction and was carried out under reflux to gain higher yields.

Piperazine derivative (2-fluorophenyl piperazine) was attached on position 1 of imidazole to produce a Mannich base. The general Mannich reaction for the compound is given below.

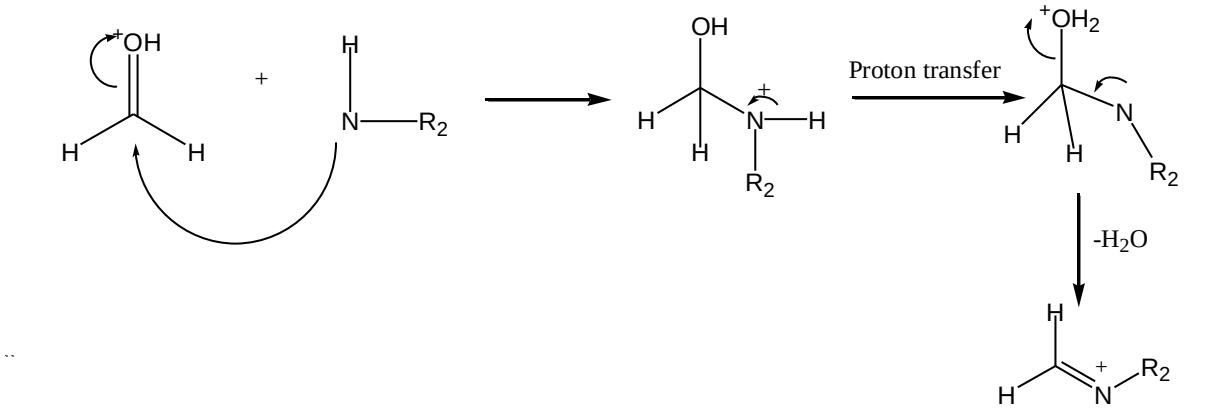


The general reaction mechanism for this reaction follows two major steps; formation of iminium ion and attacking of iminium ion by the substrate (imidazole) nucleus, as a nucleophile.

Formaldehyde in solution, 37% w/v in H2O



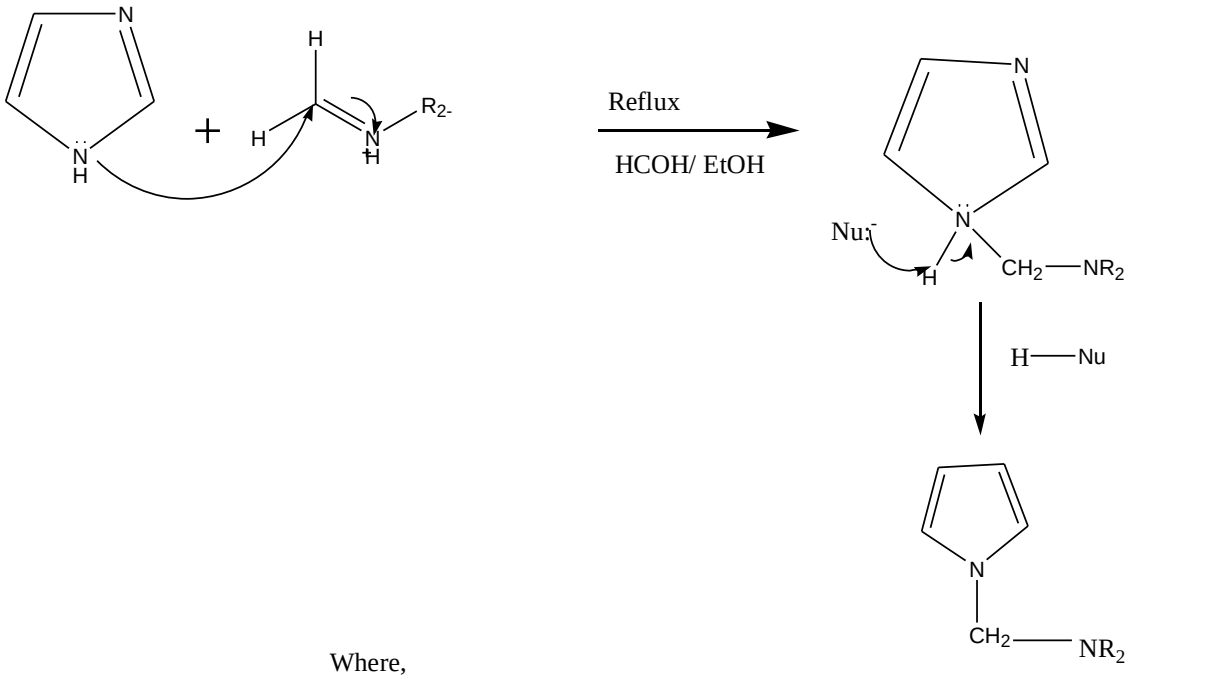
First step: formation of iminium ion



Where,

R_2 = 1-(2 - fluorophenyl) - piperazine

Second step: The substrate attacks the iminium ion and active hydrogen was deprotonated to form the target product.

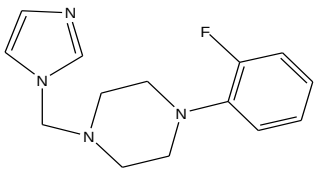


Where,

R_2= 1 -(2- Fluorophenyl) - piperazine

Figure 4.3: Mannich reaction mechanism of imidazole derivative of 1- (2- fluorophenyl) – piperazine

Table 4.1: Summary of the results of compound formed

Structure of compound	Condition/time	Melting point (°C)	Rf Values
 1-(2-Fluoro-phenyl)-4-imidazol-1-ylmethyl-piperazine	Reflux / 2hr	90	0.20cm

The compound synthesized was verified for its structure using Fourier Transform Infra-Red (FT-IR) and Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy. The FT-IR spectrum of synthesized compounds showed absence of (N-H) stretch which is expected to appear at (3100-3550) cm^{-1}, confirming reaction took place at N-1 position of imidazole ringas expected. An absorption band at 2715 – 3039 cm^{-1} aromatic (C-H stretch) and 1231 – 1496 cm^{-1} (C- F stretch) was also observed.

FT-IR spectra of compound synthesized is given in Figure 4.4.

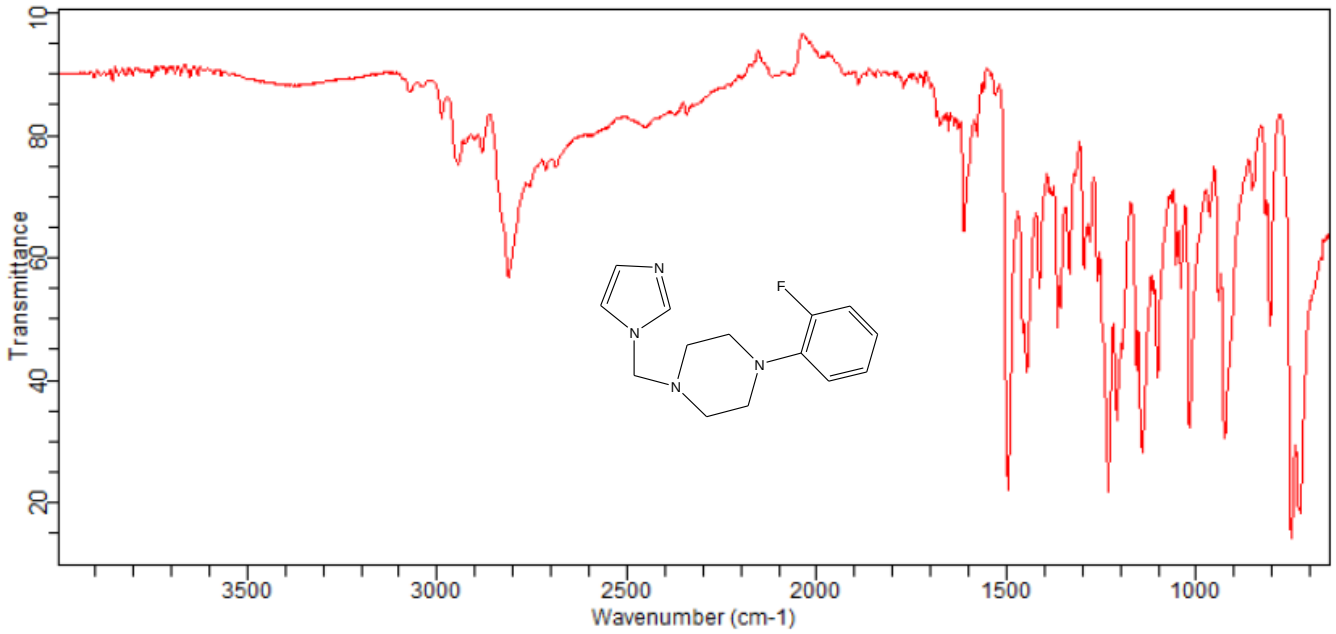


Figure 4.4: FT-IR spectra of 4- (2- fluorophenyl) 1- methylpiperazine imidazole

^{1}H-NMR spectra of my compound in $CDCl_3$ show peaks at expected chemical shifts values.

^{1}H-NMR spectra of compound in CDCl3 are shown in Fig.

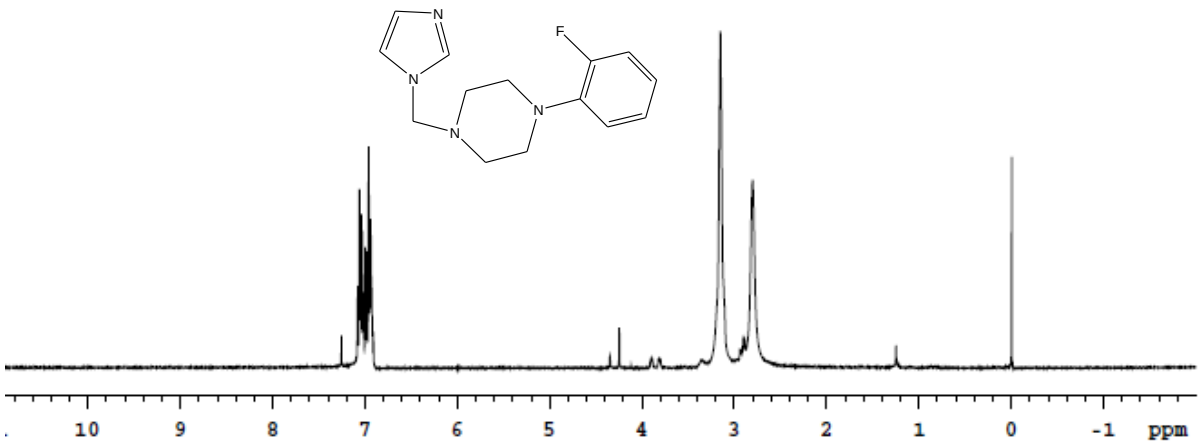


Figure 4.5: ^{1}H-NMR spectra of 4- (2- fluorophenyl) 1- methylpiperazine imidazole

In all spectra, relative to the starting material (imidazole), there is no additional CH_2 (methylene) peak at 4.6 ppm for the target compound. This indicates that the CH_2 signal merged with the piperazine signal at 3.2ppm, as confirmed by the integral values.

Protons of piperazine (H_6 and H_7) and (H_5 and H_8) were observed as triplets at 2.8 and 3.2 ppm respectively. This indicated that less shielded protons (H_6 and H_7) are closer to the piperazine nitrogen next to the electron withdrawing group, benzene, while more shielded protons (H_5 and H_9) are closer to the piperazine nitrogen next to the electron releasing group methylene.

Upon additional inspection, ^{1}H NMR spectra revealed the presence of aromatic peaks as multiplets arising between 6.8 to 7.3 ppm which is expected.

On observation of the ESI-MS spectrum is the absence of the molecular ion, it seems to have fragmented. The base peak, which corresponds to the most intense peak, was noticed at 181. 17 amu, as seen below. Judging from the mass of the base peak, the compound appears to have cleaved at the benzene ring covalent bond. The presence of fluoro- substituent is confirmed by the peak at 183.13 m/z, which is two amu from the base peak.

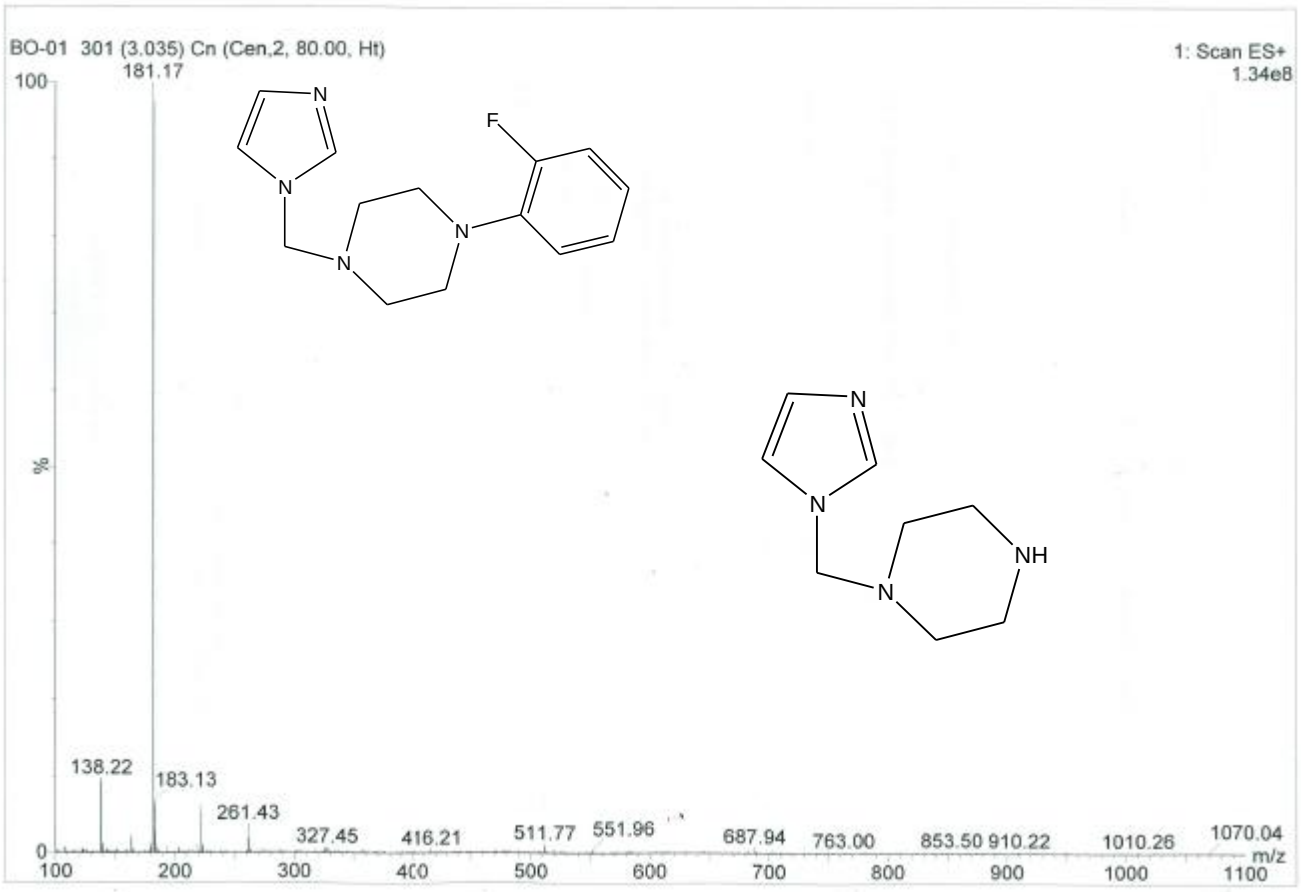


Figure 4.6: ESI-MS spectra of 4- (2- fluorophenyl) 1- methylpiperazine imidazole

4.3. Biological Activity

The target compound that synthesized via Mannich reaction was tested at clinical microbiology laboratory of Near East University; disk diffusion technique was done for investigation of biological activity, the table below shows antimicrobial activity against some bacterial spp.

Table 4.2: Antimicrobial results.

Compound	**Zone of inhibition (mm)**			
	S.aureus[1]	*P.aeruginosa*[2]	*E. coli*[2]	*Candida*

				Albicans[3]
	12mm	6mm	17mm	0mm
Positive Controls[1,2,3,4]	40mm	40mm	40mm	15mm

[1]Linezolid , [2]Meropenem, [3]Amphotericine B

5. CONCLUSION

The Mannich base of 4- (2- fluorophenyl) 1- methylpiperazine imidazole was synthesized in this study using the classical Mannich reaction via reflux method. The procedure was fast and efficient.

Preliminary biological activity of the synthesized compounds was conducted and was found for *E. coli, P.aeruginosa and Stapphilococcus aureus*.

Activity studies apart from anti-bacterial activities are intended to be made in the future since it is possible to do different substitutions at different sites of imidazole structure and different amine groups can also be substituted at hetero-atomic nitrogen in position 1 which could potentially change the biological activities of these types of compounds.

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