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SYNTHESIS AND BIOLOGICAL ACTIVITY STUDIES ON 1-IMIDAZOLE-1-YL-METHYL-4-PHENYLPIPRAZINE

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APPROVAL

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ABSTRACT

Imidazoles and their derivatives are among the most important and well-known heterocyclic molecules in medicinal chemistry. Due to their special structural properties, these molecules display a wide range of important pharmacological or biological activities, and are extensively studied and applied to drug discovery by pharmaceutical companies.

Using imidazole as the core structure, a new Mannich base containing piperazine derivative was synthesized in a fast and efficient manner. This molecule is extensively characterized by ^{1}H-NMR, FT-IR spectroscopy, and ESI-MS analysis. The antibacterial activity of the newly synthesized Mannich base was also tested by disk diffusion method.

Keyword: Imidazole, heterocycles, Mannich base, piprazine, antimicrobial activity.

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LIST OF ABBREVIATIONS

FT-IR	Fourier Transform Infra-red
^{1}H NMR	Proton Nuclear Magnetic Resonance
TLC	Thin Layer Chromatography
ESI-MS	Electrospray mass spectroscopy
MCR	Multi component reaction
WHO	World health organization
$CDCl_3$	Deuterated chloroform
Rf	Retention factor
HIV	Human immunodeficiency virus
UV	Ultraviolet-visible

1. INTRODUCTION

In organic chemistry, the synthesis of fused heterocycles with various construction units plays a major role. Due to their respective structural units, the fused heterocyclic molecules have unique characteristics and receive additional attributes due to electronic environmental changes. In particular, heterocyclic derivatives containing imidazoles are characterized by high biological activity and lower toxicity.

The synthesis of small-molecule imidazole-skeleton has been of interest by pharmacological chemists and organic synthesis researchers, because imidazoles have great pharmacological and biological activity and contribute to the synthesis of many biological entities. The use of this heterocyclic skeleton is a simple and effective method.

The Mannich reaction is a common reaction for the development of new medicines and a primary synthesis of biological active ingredients. The Mannich base exhibits a wide variety of biologic activities, including antioxidation, inflammatory, antifungal, antibacterial, and anticancer. In addition, these new applications have recently received considerable attention due to their vast range of biological, industrial, and synthetic applications.

The main challenge in today's medical discovery was the design and development of new antimicrobial agents with increased efficacy and minimal side effects, in particular as a result of a quick increase in multidrug-resistant microbes.

For all these reasons, in this study we demonstrated the synthesis, structure and biological activities of 1-imidazole-1-yl-1-methyl-4-phenylpiprazine. This molecule is characterized by 1H-NMR, FT-IR, ESI-MS, TLC and melting point analyis. The preliminary antimicrobial activity of this molecule was also studied.

2. LITERATURE REVIEW

2.1. Imidazole

Heterocycles are important pharmacophores that can be used to create specialized chemical structures with pharmacological properties. Different therapeutic agents contain five-membered heterocyclic molecules that contain oxygen, nitrogen, and sulphur, which have an important role in the synthesis of chemical structures possessing pharmacological activities (Akhtar et al., 2017).

An important group of biologically active molecules is formed by azoles. Heterocyclic, azole-based molecules such as imidazole, pyrazole, benzimidazole, and triazole often demonstrate a number of biological activities. Among these, antioxidants (Kálai et al., 2009), anti-inflammatory (Achar et al., 2010), anticancer (Tong et al., 2009), anthelmintic (Valdez et al., 2002), antiviral (Li et al., 2006), antihistaminic (Iemura et al., 1986), and antiproliferative (Sann et al., 20016) Consequently, these characteristics of azole heterocycles have prompted many pharmaceutical companies to investigate their health activities.

In the 1840s, (Debus, 1858) discovered several imidazole derivatives. They were formed by glyoxal and formaldehyde in ammonia. However, in 1858, Heinrich Debus was the first person to synthesize Imidazole. (Kleeman et al., 1999) indicated that Imidazole was the main ingredient of some important molecules in human organisms, such as Vit-B1, biotin, DNA, purines, histamine and histidine. It is important in the structure of many natural or synthetic drug molecules, such as metronidazole, cimetidine, and azomycin. (Kleeman et al., 1999). (Amita et al., 2013) mentioned some of its pharmacological activities, such as: antifungal, analgesic, antibacterial, anticancer, anti-HIV and antitubercular activities. (Vessally, 2017). It was suggested that imidazole was one of the most important molecules in medicinal chemistry.

The imidazole (1,3-diaza-2,4-cyclopentadiene) is an organic molecule with the formula C3H4N2. It is classified as a diazole and as an alkaloid (Kharb and Shahar, 2011). It is a planer five-member heterocyclic ring with 2N and 3C atoms (figure 1). According to (Shalini et al., 2010), the nitrogen atoms occupy the 1st and 3rd positions in the ring structure. There are two kinds of lone pairs in this ring. One of them is non-

delocalized. It has a pka equivalent to 7. and the other one is delocalized. It has a pka equivalent to 7. In general, imidazoles have an amine-like arrangement, are a colorless solid, have a boiling point of 268 °C and a melting point of 88 °C.

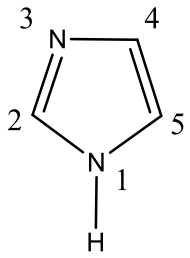


Figure 1: Structure of imidazole

2.1.1. Acid Base Properties of Imidazole

Imidazoles are characterized by their ability to be both basic and acidic because they exhibit amphoteric behavior in nature. In view of the fact that imidazoles contain nitrogen like pyridine and are capable of nucleophilic and electrical attacks (Finar, 2009). However, imidazoles can be basic more than pyridine. The increased base is the result of the constant resonance of the positive charge of the conjugated acid.

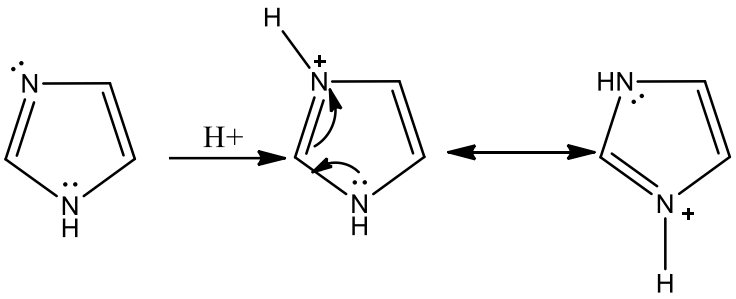


Figure 2: Imidazole in an acid condition

By a strong base, imidazole removes the hydrogen from the nitrogen atom at position 1, thus exhibiting an acidic behavior.

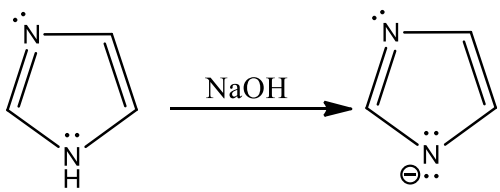


Figure 3: Imidazole in the base condition

2.1.2. Reactivity of Imidazole

The electron density map is still the best tool for understanding imidazole behavior in various reactions.

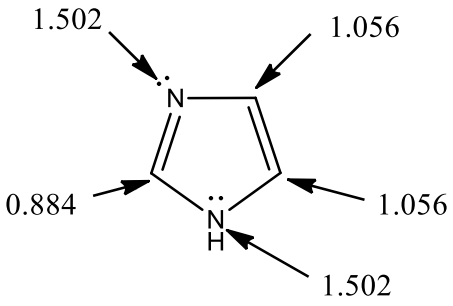


Figure 4: π electron density map of imidazole

Because the fourth and fifth carbon atoms of imidazole are rich in electrons, and the nitrogen atom in imidazole has high electronegativity, so the second carbon atom lacks electrons.

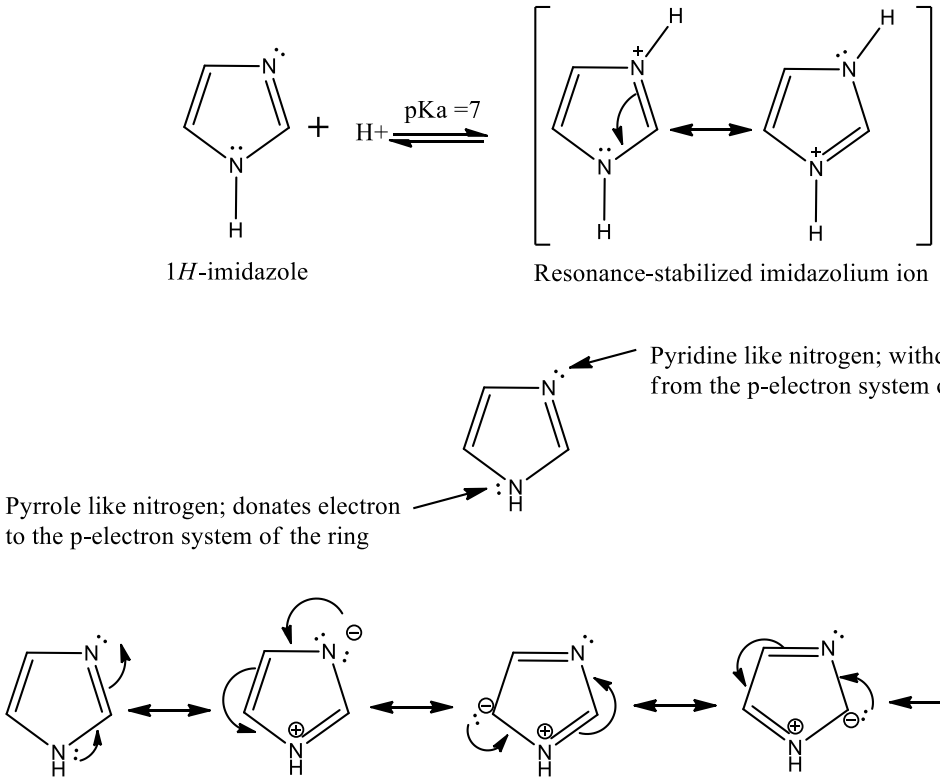


Figure 5: Aromatic resonance of imidazole

A new molecule of 2,4,5-triphenyl substituted imidazole derivatives has been developed and synthesized by Jain et al., (2010) for investigation of antibacterial activities which showed activity against *E. coli, B. subtilus and S. aurius* (figure 6).

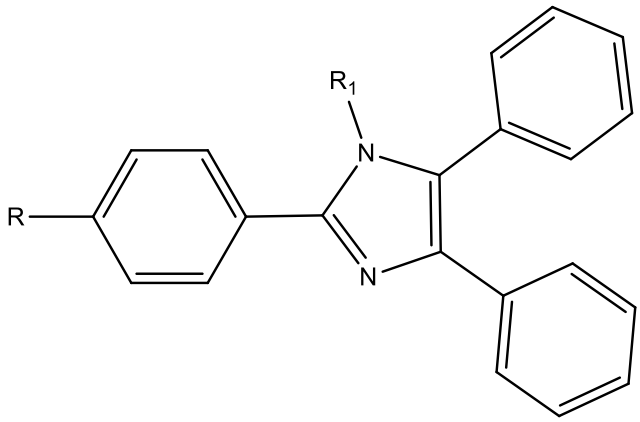


Figure 6: 2,4,5-substituted-tridiphenyl- imidazole derivative showing antibacterial activity

Also, a new novel of N-alkyl imidazole derivatives was synthesized by Khabnadideh et al., (2003) for investigation of anti-bacterial activities. As a result of this study, the greater the number of carbons added to the molecule, the greater the effect of the antibiotic against bacteria (figure 7).

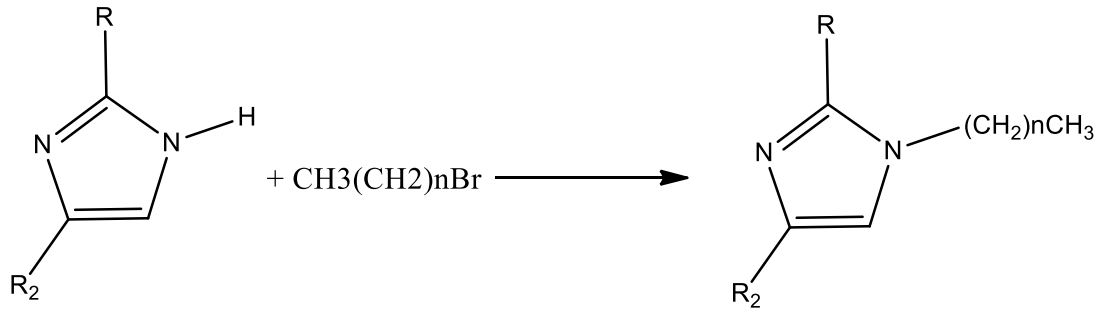


Figure 7: Synthesis of alkyl imidazole showing antibacterial activities

A new molecule of 4,5-diphenyl-1-phenylmethy substituted imidazole derivatives has been synthesized by Ucucu et al. (2001) for investigation of analgesic activities (figure 8).

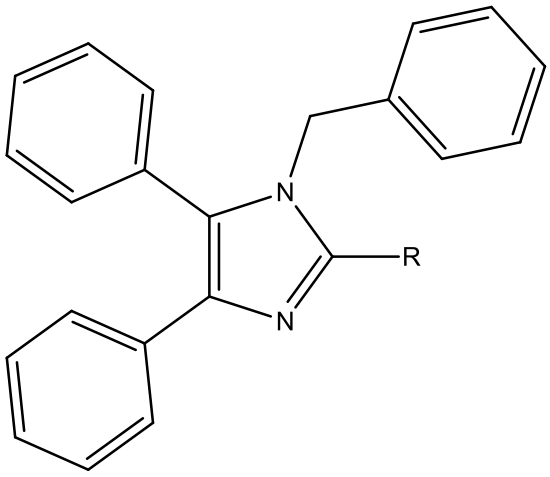
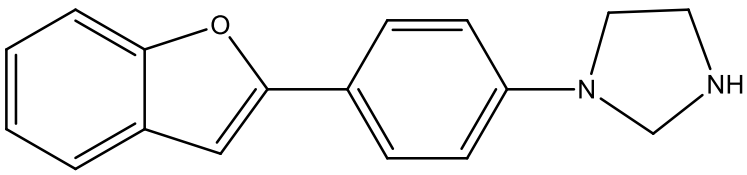


Figure 8: 4,5- diphenyl- 1-phenylmethy substituted of -imidazole showing analgesic activities.

A new molecule of 1-(4-(benzofuran-2-yl) phenyl) substituted imidazole derivatives has been developed and synthesized by Yang et al., (2012) for investigation of anti-cancer activity. These were the most active molecules in the sequence, according to anti-cancer activity screening findings. Cisplatin was utilized as a standard. Figure 9

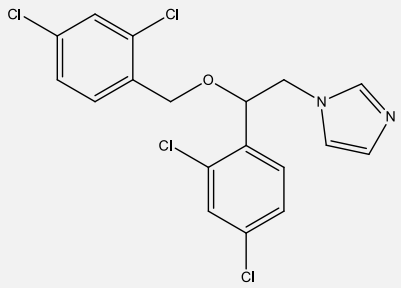
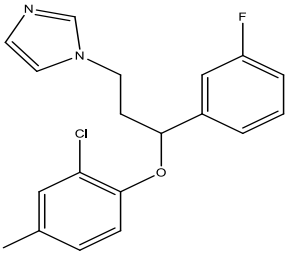
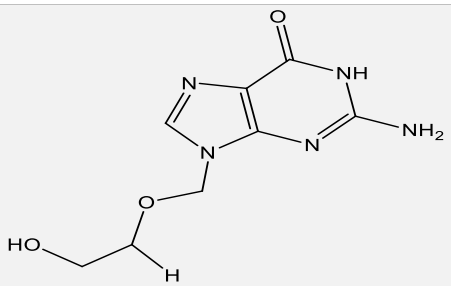
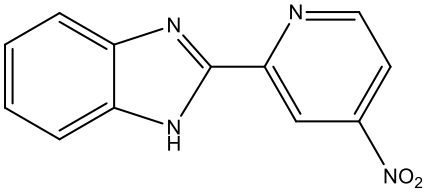


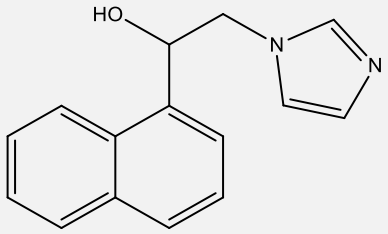
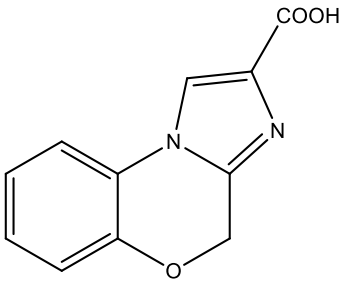
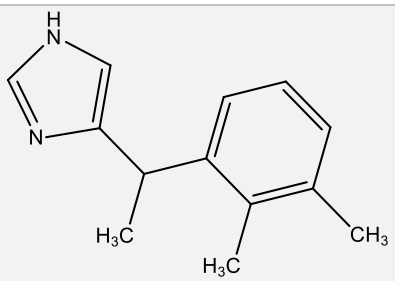
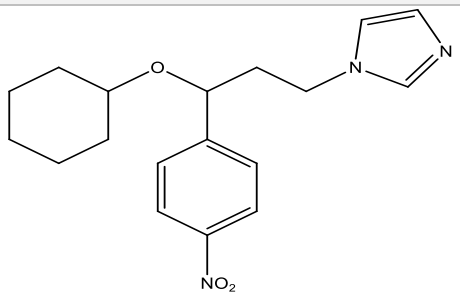
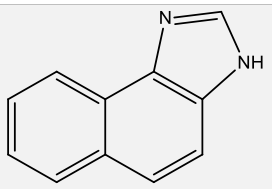
1-(4-(benzofuran-2-yl)phenyl)imidazolidine

Figure 9: 1-(4-(benzofuran-2-yl)phenyl) imidazole showing anticancer activities

Table 1, shows some examples of prepared imidazole compounds that are used in the medical field against many microorganisms, bacteria and fungi. Also, against carcinogenic cells.

Table 1: Substituted molecules of imidazole in relation to their clinical uses.

No	Structures of Molecules	Uses	References
1.	 **1-(2-((2,4-dichlorobenzyl)oxy)-2-(2,4-dichlorophenyl)ethyl)-1*H*-imidazole**	Antibacterial activities	Silvestri et al,2004
2.	 **1-(3-(2,4-dichlorophenoxy)-3-(3-fluorophenyl)propyl)-1*H*-imidazole**	Antibacterial activities	Moraski et al, 2011
3	 **2-amino-9-((2-hydroxyethoxy)methyl)-1*H*-purin-6(9*H*)-one**	Act as antiviral [Active against HSV-1 and HSV-2]	Bochis et al,1981
4	 **2-(4-nitropyridin-2-yl)-1*H*-benzo[*d*]imidazole**	Anti-trichomonal agent	Butler et al,1967

5	 **2-(1*H*-imidazol-1-yl)-1-(naphthalen-1-yl)ethanol**	Anti Epileptic	Mishra & Ganguly ,2012
6	 **4*H*-benzo[*b*]imidazo[1,2-*d*][1,4]oxazine-2-carboxylic acid**	Allergic Activities	Kaminski et al,1987
7	 **4-(1-(2,3-dimethylphenyl)ethyl)-1*H*-imidazole**	Analgesic Inflammatory activities	Boryski et al,1991
8	 **1-(3-(cyclohexyloxy)-3-(4-nitrophenyl)propyl)-1*H*-imidazole**	Antimicrobial agent [Active against C. albican	Silvestri et al,2004
9	 **3*H*-naphtho[1,2-*d*]imidazole**	Analgesic Inflammatory activities	Golankiewicz et al, 1995

2.2. Piprazine

Piperazines are heterogeneous organic cyclic molecules characterized by pharmacological properties and consist of six atoms (four carbon atoms and two nitrogen atoms opposite in the ring). They were first introduced as anthelmintics in 1953 (Brater et al., 2000), and due to their pharmacological activity, they have been used in many medical fields, especially in the pharmaceutical industry. (Zhang and Xiao, 2011).

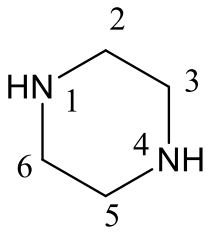


Figure 10: piperazine structure

Piperazine is a unique heterocyclic constituent of several biologically active molecules. Due to the polarity of the piperazine ring according to nitrogen atoms, it is considered a bioactive molecule and enhances favorable interaction with macromolecules (Singh et al., 2011).

Furthermore, to modify the acid-base balance constant and the drug lipid water partition coefficient, piperazines can form ionic or hydrogen bonds because the biological activities are due to the alkalinity of the molecule, modification of chemical and physical properties, and solubility in water (Foye et al.,1995)

Piperazine can be classified among the synthetic heterocyclic organic molecules that can be used in the food and pharmaceutical industry (Mermer et al., 2016). For example, the nucleus of piperazine can be found in many critical drugs such as lomefloxacin, norfloxacin, ciprofloxacin, pefloxacin, and ofloxacin, which can be used as antimicrobial agents against many infections that can be caused by gram negative bacteria (Mermer et al., 2016).

The use of drugs containing piperazine in the medical field, especially in the pharmaceutical industry, includes antihistamines, anti-migraines, anti-bacterials, and other biological activities. This is due to the hydrogen atoms found in the piperazine ring. (Singh et al., 2015). These atoms maintain the pKa value, thus increasing the solubility of the drug in water (Lacivita et al., 2009). Therefore, medical chemistry aims to design drugs, taking into account the maintenance of pharmacokinetic and pharmacodynamic properties as well as their chemical and physical properties. (Maia et al., 2012).

According to the study by Rathi et al (2016), piperazines have a wide range of therapeutic purposes due to their pharmacological efficacy and the possibility of developing their structure. Throughout both the N-substutution of piperazine via different groups of alkyl, Bihan et al., (1999) investigated and synthesized a new novel of diabetic compounds. As it is shown in (figure 11).

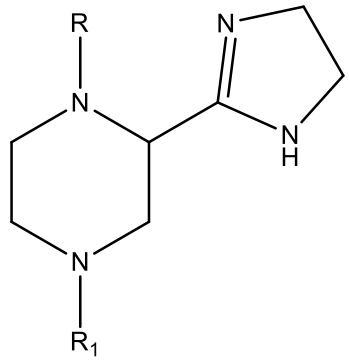


Figure 11: Imidazoline ring showing anti-diabetic activities

A novel of imidazole molecules substituted with piperazine derivatives has been developed and synthesized by Gan et al., (2010) for investigation of antibacterial and antifungal activities which showed significant to moderate activities (figure 12).

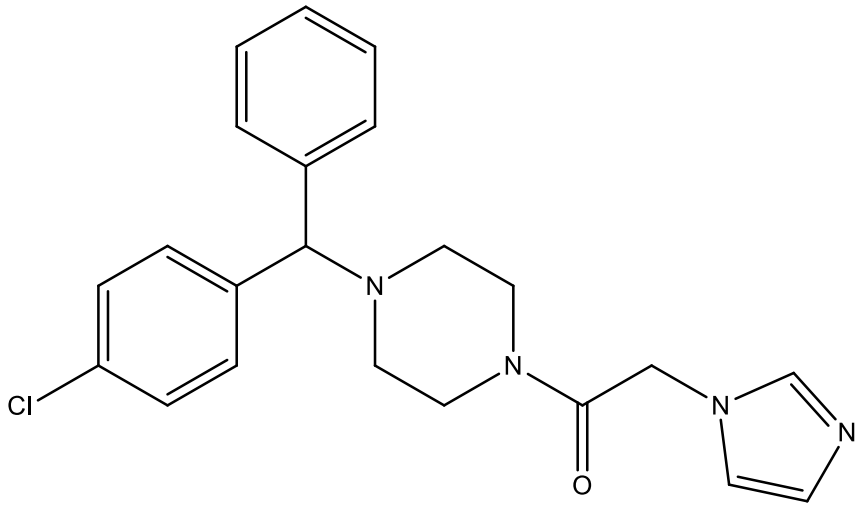


Figure 12: Azole-containing piperazine derivatives

(Tran et al., 2007) indicated Ranolazine drug has a piperazine moiety and can be used as an Antanginal.

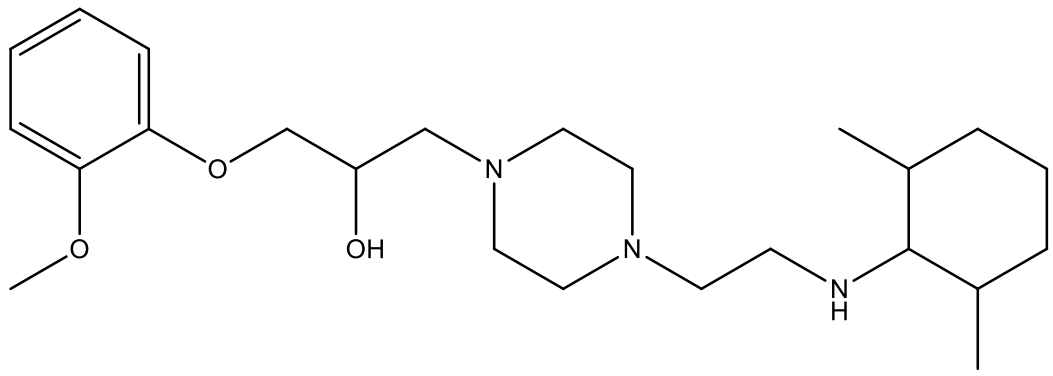


Figure 13: Ranolazine with antianginals activity

As shown in figure 15, Nikolava & Danchev (2008) reported N-methyl piperazine is used for the preparation of anti-HIV agents.

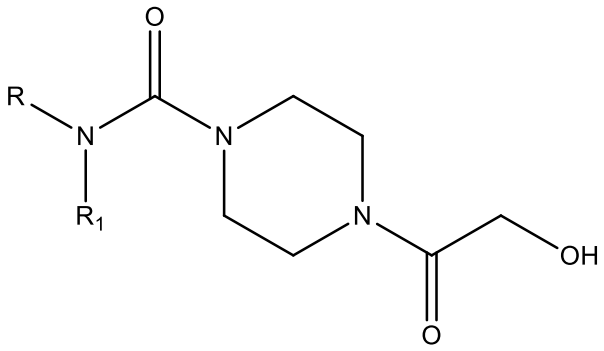


Figure 14: Piperazine containing active carbamates with anti-HIV activity.

However, there is little knowledge about the use of the N-phenylpiperazine subunit in medicinal products. One of the rare examples pertinent to N-phenylpiperazine was given by (LopezRodriguez et al., 2002) where they used this molecule for the purposes of targeting central nervous system targets such as dopaminergic, adrenergic, and serotonergic receptors. (Lopez-Rodriguez et al., 2002).

2.3. Mannich base

Mannich bases can be defined as the β-amino ketones that carry the molecules and are the end products of the Mannich reaction (Wiley & Sons, 1985; Belinelo et al., 2002). Synthetic chemists in the past few decades have focused their attention on the heterocyclic bases of Mannich. Because they are highly reactive, they have developed drug molecules with different biological activities, such as anticancer, antiviral, antibacterial, antimalarial, antihistamine, and anti-inflammatory activities. (Sanghani & Ganatra, 2010).

Because of the nitrogen atoms that are found in natural products and drug products through the Mannich reaction, this reaction has been widely used. (Zhao et al., 2012).

Amino alcohols and heterocyclic cycles can be synthesized using Mannich bases as readily available intermediates and easily obtainable. Mannich bases are considered as prodrugs for the synthesis of many medical products because of their water-soluble property. (Sanghani & Ganatra, 2010).

1,3,4-thiadiazole has been synthesized by Charanjeet (2019), which has an inhibitory effect on infections of microbial origin. The two molecules showed the highest activity against *S. aureus*, while the other two molecules were highly active against *B.subtilis*. It revealed that one molecule exhibited excellent activity against both bacteria.

N – substituted of acetamide of Mannich base, when placed in a reaction vessel containing acetamide solution with phenol and pyridine carboxaldehyde, several molecules were synthesized. Moydeen et al. (2013) performed all tests for all synthesized components, especially the antifungal activities against Candida albicans, as well as the antibacterial activities against pathogenic bacteria. In the end, the results of both compontes showed biological activites against certain microorganisms.

The basic N-Mannich derivatives of amines, amides, imides, urea and hydantoin derivatives have been utilized as potentially useful prodrugs. The group that binds to the parent amine through the Mannich reaction increases the molecule's lipophilicity at physiological pH by reducing protons, and proton restriction enhances uptake through biofilms. (Bundgaard and Johnson, 1980).

Some examples of Mannich base which are clinically beneficial consist of aminoalkyl chains like atropine, cocaine, ranitidine, fluoxetine, biperiden, trihexyphenidyl, ethacrynic acid, and procyclidine as shown in Figure 15. (Suman, 2014).

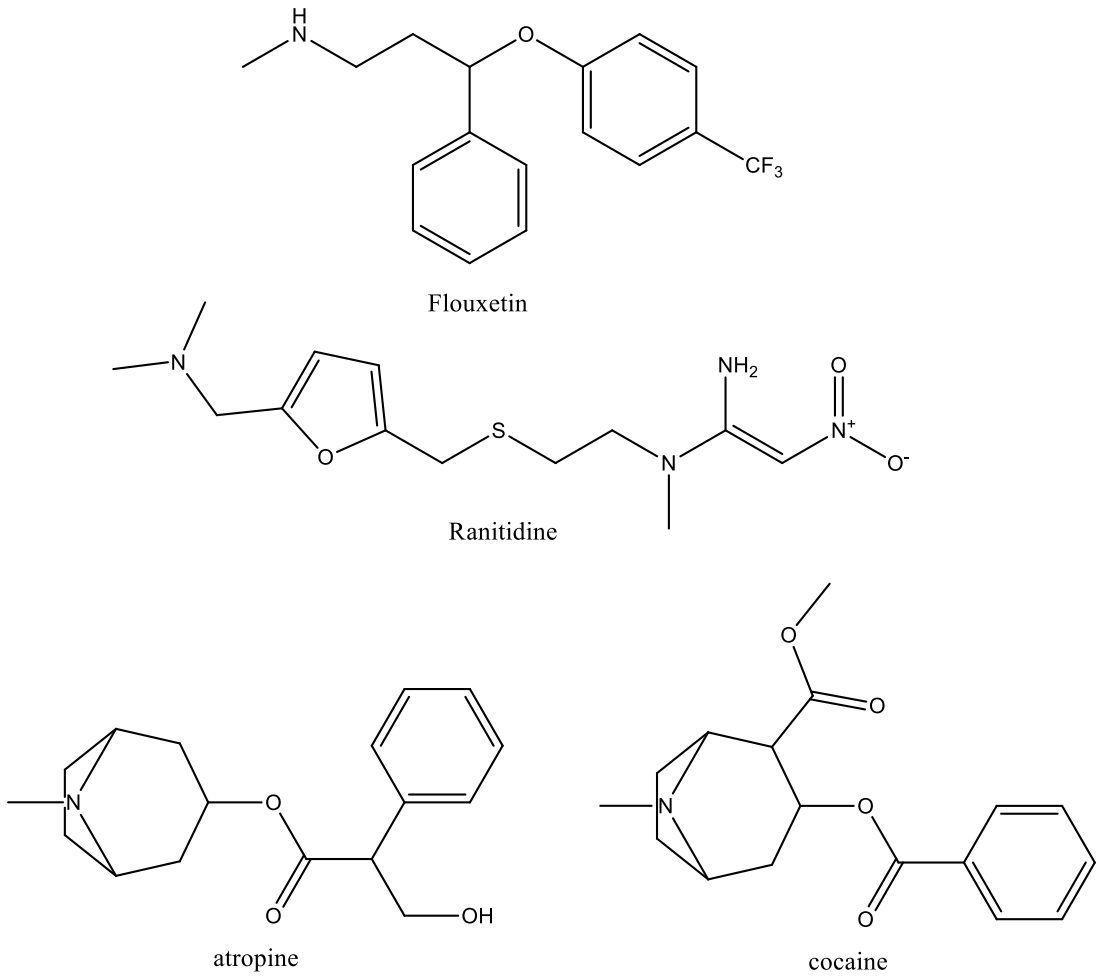


Figure 15: Mannich base examples

2.4. Mannich Reaction

The Mannich reaction is a carbon-carbon bond forming nucleophilic addition reaction and is a key step in the synthesis of a wide variety of natural products and pharmaceuticals (Bala et al., 2014). It is a multicomponent reaction which involves at least three components in a one-pot process reaction to give a single product (Alizadeh & Ghanbaripour, 2014). From this definition, the Mannich reaction is an organic MCR reaction which consists of an amino alkylation of an acidic proton placed next to a carbonyl functional group with formaldehyde and ammonia or any primary or secondary amine. The general principles and components of the Mannich reaction are shown in figure 16.

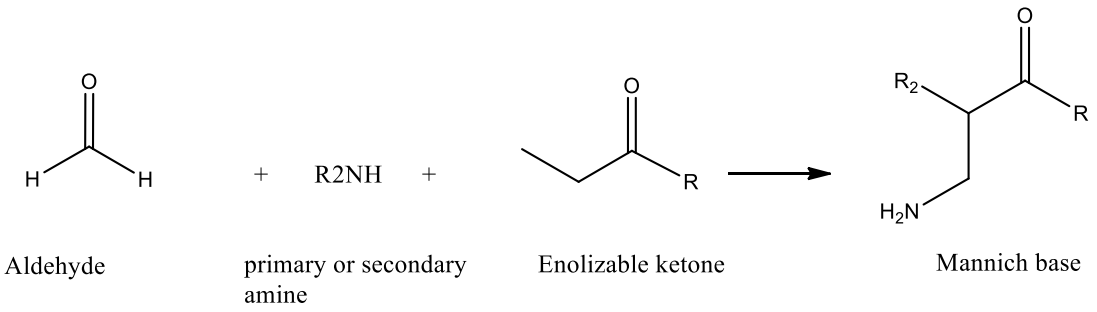


Figure 16: General mechanism of mannich reaction

The Mannich reaction is a nucleophilic addition reaction which involves the condensation of a molecule with active hydrogen (s) with an amine (primary or secondary) and formaldehyde (any aldehyde) (Bala et al., 2014). The first part of this mechanism of the Mannich reaction is the generation of iminium ions as an intermediate product of the nucleophilic addition reaction. The primary or secondary amine or ammonia attacks the carbonyl carbon of an aldehyde to give the added product. The second part is the attack of the iminium ion on a molecule containing active hydrogen, to give the substitution product. So, for this reaction, we require an amine, a nonenolizable aldehyde and a molecule containing active hydrogen. Initially, most commonly, formaldehyde was used as a non-enolizable aldehyde. But later, various substituted benzaldehyde or other aromatic aldehydes were also found to be suitable for the reaction. (Prasun & Wakode, 2013). If the active hydrogen is attached to the nitrogen of the molecule, then the product is called N-mannich base as the immine group is attached to this hydrogen only. Mechanism of reaction shown in figure 17.

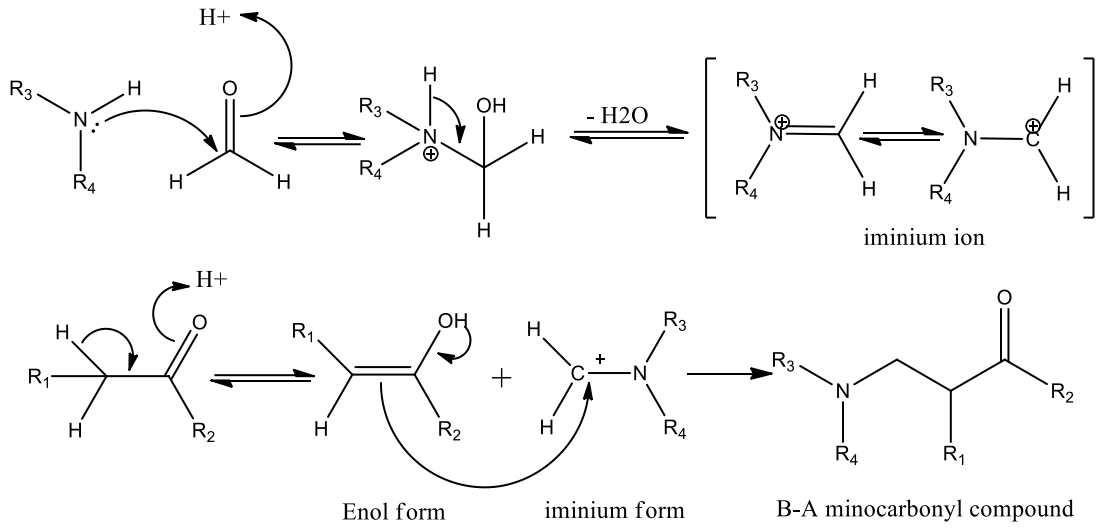


Figure 17: Mannich reaction Mechanism

However, HUSAIN et al., (2010) reported that Mannich reaction proceeds at the 2-position of 4,6diacetyl resorcinol and several new such molecules have been prepared using this method using various amine components and the products have been evaluated for their antibacterial and antifungal activities. It is well known that the amino moiety is an important structural fragment present in numerous clinical drugs. This moiety could form hydrogen bonds, coordinate with metal ions, accept protons and/or perform quaternization. These processes are not only beneficial in the regulation of the physicochemical properties of desired molecules, but also helpful in interacting with various enzymes and receptors in the biological system. Therefore, the-CH2–NH-amino moiety acts as a bioisostere of the-CH2–CH2-linker into target molecules. It is believed that this N-Mannich functional group could influence bioactivities through the enhancement of cell permeability (Zhang et al., 2016).

The Mannich reaction occupies an important position in the field of organic synthesis and has been one of the most important reactions in organic chemistry. The presence of heterocyclic rings in drugs constitutes a part of the pharmacophore. (Sanghani & Ganatra, 2010). However, it is considered as a unique strategy leading to the formation of various bioactive molecules. These properties are due to the components used in the manic reaction, which include low energy consumption, minimum waste production, facile execution, high selectivity and productivity. (Azizi et al., 2015). (Majumder et al., 2014).

2.5. Disk Diffusion Technique.

The disk diffusion technique is considered as the best technique for the AST test, but because of the disadvantages of this technique, microbiologists have tried to create a new technique for determining bacterial sensitivity to antimicrobial drugs that is simpler, more practical, and more developed, and that can be used on a regular basis, the disk diffusion technique. In 1956, M. Kirby and his colleagues at the Washington School of Medicine proposed a single standardized disk technique for AST, which is now known as the Kirby-Baurer disk diffusion technique.

WHO created a committee in 1961 to establish guiding principles for this technique. The Disk diffusion technique is considered as the most frequently used AST technique in clinical microbiology laboratories across the world for routine and regular testing because it does not require any additional equipment. It is the most practical and accessible test (Kahlmeter, et al.,2006).

The antimicrobial agents are injected into small discs with known concentrations and placed on the surface of Mueller-Hinton agar which is inoculated with (0.5 McFarland) of the isolated strains such as E. coli and Staphylococcus aureus. After 24 hours of incubation under 37 °C, the injected discs diffuse into the agar and form an "inhibition zone". The inhibition zone diameter was measured on a millimetric scale for each disk. In terms of resistance/susceptibility, this technique is called disk diffusion technique. Figure 18 (Bayot, Bragg, 2020)

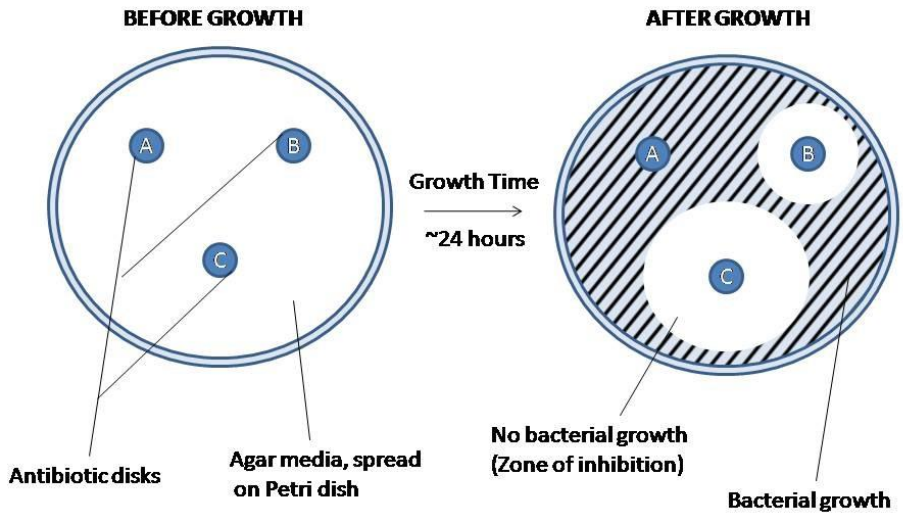


Figure 18: Disk diffusion technique before and after bacterial growth

3. MATERIALS AND METHODS

3.1 Materials

All reactions were carried out using standard laboratory equipment and standard laboratory glassware. The starting materials, phenyl piperazine, imidazole, ethanol, and formaldehyde, used in this study were obtained from Sigma Aldrich Chemical Company and were used without further purification.

3.2. Thin Layer Chromatography Method

3.2.1. Material

The Thin layer chromatography (TLC) was used to monitor the progression of the reaction carried out on Silica gel/TLC-plates (DC-AlufplienKieselgel, Germany) and the solvents used were ethyl acetate, n-hexane, and methanol. The Silica gel plate was detected under UV-light (254 nm).

Three different mobile phases were prepared and used, with different ratios as follows;

M-1/ n-hexane: ethyl acetate (3:1 v/v)

M-2/ ethyl acetate: methanol (9:1 v/v)

M-3/ ethyl acetate: hexane (4:6 v/v)

3.2.2. Method

The mobile phase (solvents) was poured into the TLC chamber to a depth of about 0.5 cm. The chambers were covered with watch glass, gently swirled and allowed to stand while assembling the plates. TLC plates were cut horizontally into plates of about 5 cm tall by different widths and a pencil was used to draw a line across the plate at 0.5 cm from the bottom of the plate. Imidazole, the starting material of the molecule, was dissolved in chloroform. The product was dissolved in chloroform too. Spots were made on the plate with the aid of a microcapillary and gently placed in the TLC chamber, covered with watch glass and left undisturbed. The plate was allowed to develop. Once the solvent front was about half a centimeter just under the top of the plate, the plate was removed, and the solvent front was marked with a pencil and left to dry. The spots were viewed under UV light at 254 nm and Rf values were calculated.

3.3. Melting Point

The melting point of the molecules was recorded on the Mettler Toledo FP900 Thermosystem digital melting point apparatus and the values are uncorrected.

3.4. Spectroscopy

Fourier Transform Infra-Red (FT-IR) The FT-IR spectra of the products were recorded on Perkin Elmer Spectrum 100 shown in wave numbers (cm^{-1}) at the Central Laboratory, Ankara University, Turkey, Faculty of Pharmacy.

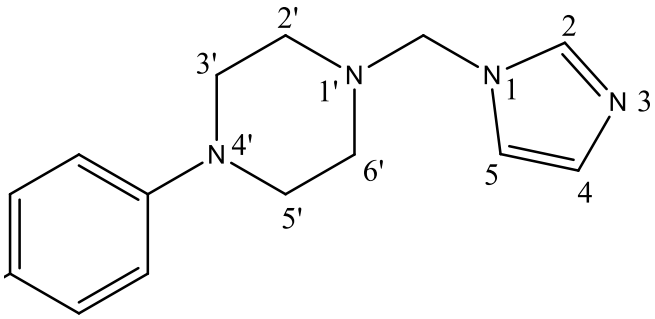
Proton Nuclear Magnetic Resonance (^{1}H-NMR) The ^{1}H-NMR spectra of the products were examined on NMR device of Mercury Varian 400 MHz where tetramethyl silane was used as a standard solution at the Central Laboratory, Ankara University, Turkey, Faculty of Pharmacy.

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.

3.5. Experimental

3.5.1. Synthesis of Molecule

1-IMIDAZOLE-1-YL-METHYL-4-PHENYL-PIPRAZINE



Reflux

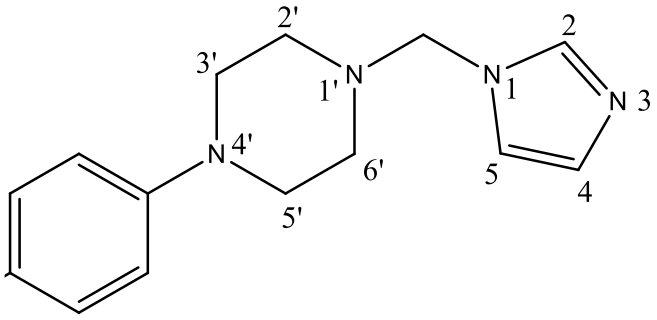
300 mg of imidazole and 0.8 ml of phenyl piprazine were dissolved in 8 mL of ethanol in a 50 ml round bottom flask. 0.2 ml of 37% (w/v) formaldehyde solution was mixed with 2 ml of ethanol and then poured into the reaction mixture. The solution was refluxed in a water bath for 60 min. After completion, the mixture was poured into crushed ice, then extracted with chloroform upon which a precipitate was formed, which was subsequently washed with ethanol to yield a crude product and allowed to dry at room temperature. The reactions were monitored by TLC and the resulting precipitate was purified by recrystallization with ethanol.

3.5.2. Microbiology

10mg/ml of the synthesized molecule is injected into small discs and placed on the surface of Mueller-Hinton agar which is inoculated with (0.5 McFarland) of the isolated strains such as Staphylococcus aureus, *E. coli*, and *Pseudomonas aeruginosa*. After 24 hours of incubation under 37 °C, the injected discs diffuse into the agar and form a zone called "inhibition zone". The inhibition zone diameter was measured on a millimetric scale for each disk. In terms of resistance/susceptibility, this technique is called the disk diffusion technique.

4.RESULT AND DISCUSSION

4.1. Result



Reflux

-White crystalline solid was obtained with a yield of 40.5% and a melting point of (102.6-106.1) °C.

− TLC in the M1, M2, and M3 mobile phases gave Rf values of 0.2, 0.48, 0.23 respectively.

− **Fourier Transforms Infrared (FT-IR) spectroscopy (□max):** FT-IR showed stretches at 2817.9 cm-1 (C-H stretch) and around 1597 cm-1 (C=C stretch) and around 1576.7 cm-1 (C=N)stretch .

− **Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR, CDCl3; ppm) δ:** 6.8-7.4 (m; 8H; Aromatic-H), 3.1-3.2 (4H; H3`-H5`);2.7-2.8 (4H; H2` -H6`) ppm , (2H;CH2) ,2.7-2.9

-**Mass spectroscopy (ESI-MS) base peak is** at m/z 163 amu.

Antimicrobial Results

The target molecule that was synthesized via Mannich reaction was tested at the clinical microbiology laboratory of Near East University. The disk diffusion technique was done for investigation of biological activity. Table 2 shows antimicrobial activity against some bacterial spp.

Table 2: Antimicrobial results

molecule	**Zone of Inhibition (mm)**			
	S.aureus [1]	**P. aeruginosa**[2]	***E.coli*** [2]	***Candida albicans***[3]
	8 mm	6 mm	17 mm	0 mm
Positive Controls[1,2,3,]	40 mm	40 mm	40 mm	15 mm

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

4.2. Discussion

Newly synthesized, Mannich bases of 1-imidazole-1-yl-methyl-4-phenyl-piperazine under reflux condition, Piperazine derivative has been synthesized as shown in figure 19 below. The synthesized molecule was characterized by Fourier Transform Infra-Red (FT-IR) a, Proton Nuclear Magnetic Resonance Spectroscopy ([1]H-NMR) and Mass spectroscopy (ESI-MS) Thin layer chromatography and melting point were used to check the purity and also to cross reference to the previously synthesized molecules. As shown in table 3.

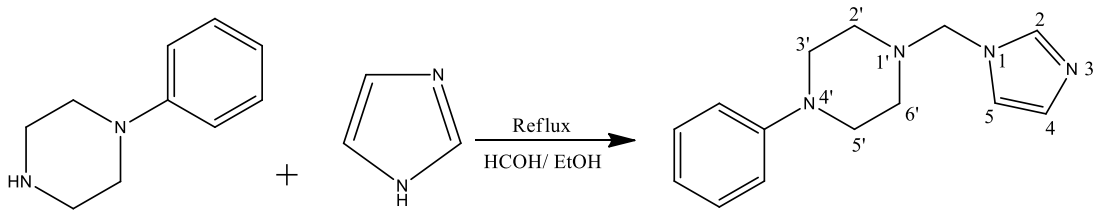
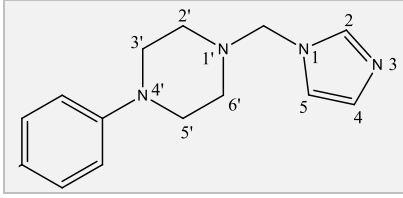


Figure 19: Chemical structure of 1-imidazole-1-yl-methyl-4-phenyl-piperazine

Table 3: characterization of synthesis molecule

Structure of molecule	Melting point	Molecular formula	Molecular weight	Percentage yield
	(102.6-106.1) °C	$C_{14}H_{18}N_4$	242 g/mol.	40.5 % Moderate yield

The FT-IR spectra of molecule show the absence of N-H stretch, which indicates that the reaction has actually taken place at first nitrogen atom of imidazole. Around 2817.9 cm^{-1} C-H stretch was observed in FT-IR spectra. Also, around 1597 cm-1 (C=C stretch) and around 1576.7 cm-1 (C=N stretch) as expected. The FT-IR spectra of the molecules synthesized are shown in Fig 20.

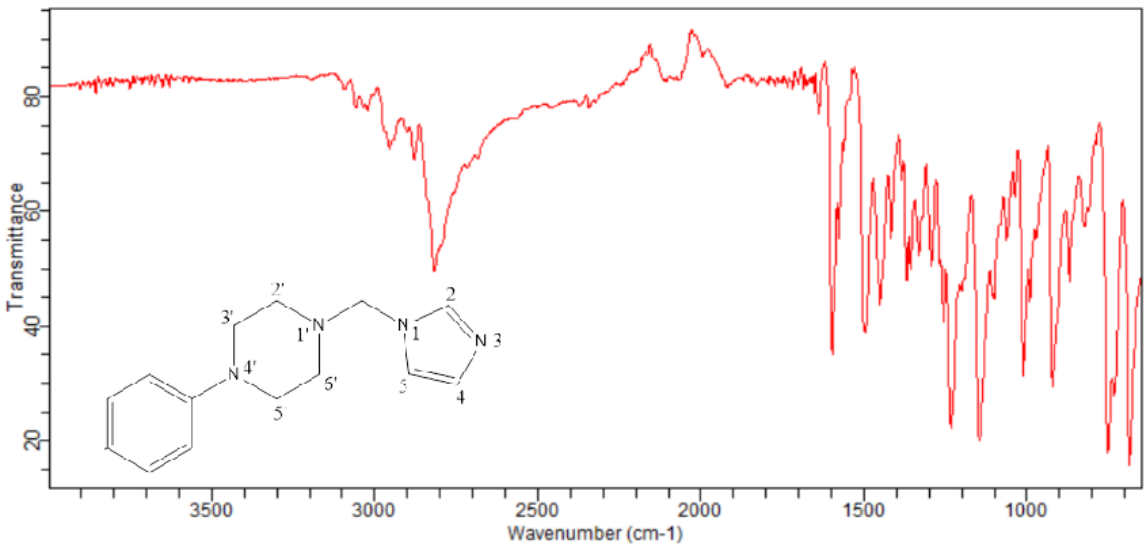


Figure 20: FT-IR Spectrum of 1-imidazole-1-yl-methyl-4-phenyl-piperazine

^{1}H-NMR spectra of molecules in $CDCl_3$ show peaks at expected chemical shifts values. In spectrum, relative to the starting materials, there is an additional Investigations of ^{1}H-NMR spectra reveal the presence of aromatic peaks as multiples between 6.8 to 7.4 ppm. The piperazine protons (H3`-H5`) and (H2` -H6`) were seen as triplets at 3.1-3.2 and 2.7-2.9 ppm respectively. appearance of CH2 peak has overlapped with piprazine signal at 2.7-2.8 ppm and it is confirmed by integral value.

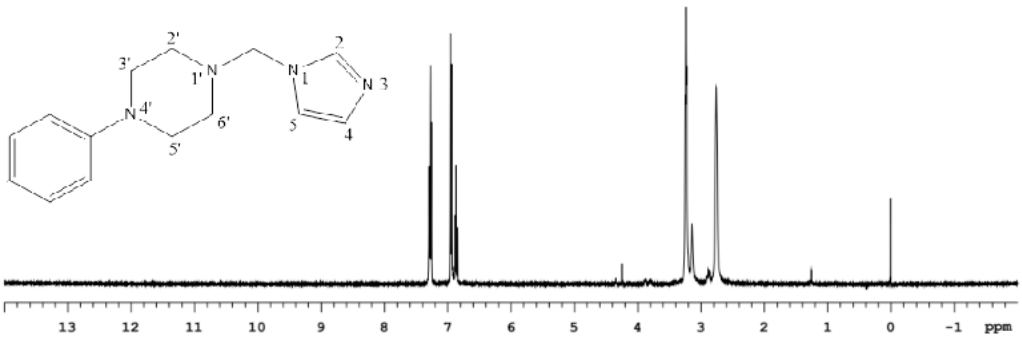


Figure 21: 1H NMR spectrum of 1-imidazole-1-yl-methyl-4-phenyl-piperazine

Mass spectroscopy analysis mass spectra at the electron energy of 70 eV are presented in order to elucidate the most important fragmentation pathways. The molecular ion is not observed, the base peak is due to the fragmented ion which is loss of phenyl ring, The 164 +ion for C8N4H13 +. In the case of the ionization mass spectrum of imidazole (Figure 22).

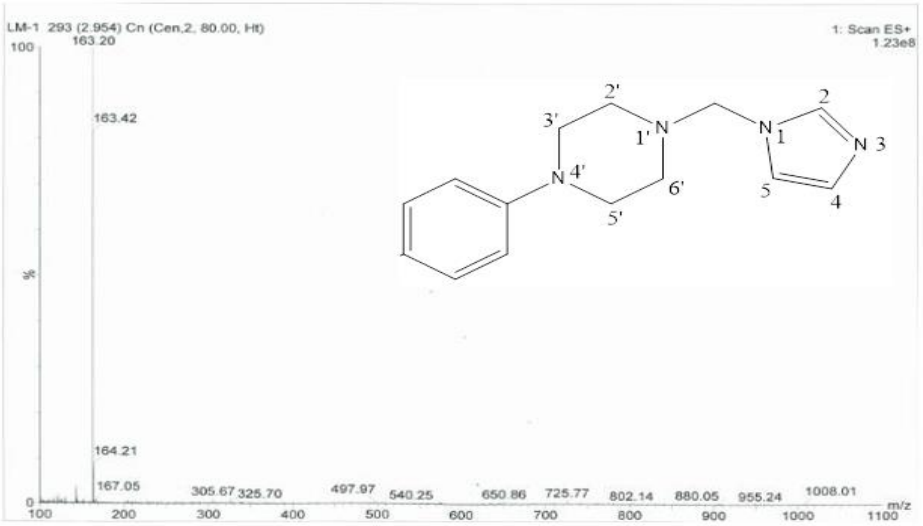


Figure 22: Mass spectroscopy (ESI-MS) of 1-imidazole-1-yl-methyl-4-phenyl-piperazine

5. CONCLUSION

This study focuses on the synthesis and characterization of an imidazole derivative as antimicrobial agents. The imidazole structure has been chosen due to it is different substitution possibilities that may be biologically active.

The antimicrobial activity of the molecule was examined by using Disk diffusion technique in a 24 h incubation period under 37 °C. The inhibition zone showed low activity against *S.aureus, E.coli and P. aeruginosa*.

This molecule was made with low activity but is promising new derivatives of imidazole could be made and further studied due to different substitution possibilities that could be biologically active.

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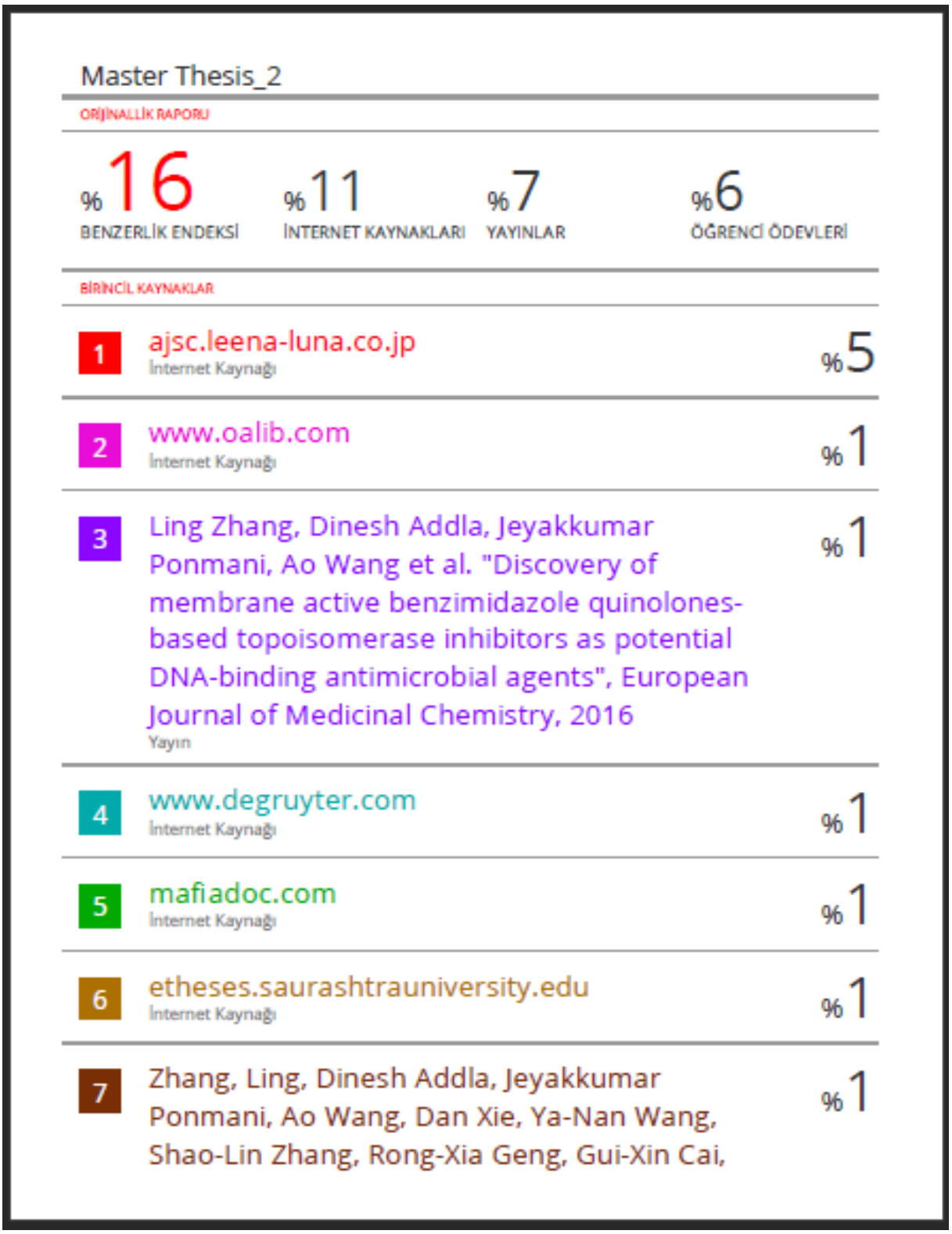
PLAGIARISM REPORT



Master Thesis_2

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