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SYNTHESIS AND CHARACTERIZATION OF AN IMIDAZOL DERIVATIVE AS ANTIMICROBIAL AGENTS

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APPROVAL

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

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ABSTRACT

Medicinal chemistry is a very essential field of contemporary life which study serious diseases around the world. It is dependent on many fields including organic chemistry, molecular biology, immunology, pharmacology, pharmacognosy and medical microbiology. Because of the high risk of antibiotic resistance in many nations by microorganisms, a new molecule with antimicrobial activity was developed, which has an imidazole core structure with a piperazine substituent.

This study presents the design and development of a Mannich base of 4-fluoro phenyl piperazine substituted to imidazole ring on first position via classic Mannich reaction.

The target molecule was characterized by Proton Nuclear Magnetic Resonance Spectroscopy [^{1}H-NMR] and Fourier Transform Infra-Red [FT-IR]. Thin layer chromatography and melting point were done for checking the purity. The FT-IR spectra of the compound show the absence of N-H stretch, which indicates that the reaction has actually taken place at position 1 of imidazole, disk diffusion technique was also done for antimicrobial susceptibility

Disk diffusion technique shows activity to several gram positive and gram negative microorganisms including: *E. coli*, *Pseudomonas spp.* and *Staphylococcus spp.*

Keywords: Imidazole, Mannich Reaction, antimicrobial, disk diffusion method

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic Acid
FT-IR	Fourier Transform Infra-Red
H NMR	Proton Nuclear Magnetic Resonance
TLC	Thin Layer Chromatography
Rf	Retention Factor
amu	Atomic Mass Unit
UV	Ultra Violet
g/mol	Gram per mole
MP	Melting Point
pFPP	para-Fluorophenyl-piperazine
AST	Antimicrobial Susceptibility Test
G +ve	gram positive
G -ve	gram negative
E. coli	*Escherichia coli*
spp	Species
TB	Tuberculosis
MDR	Multi-Drug Resistant
TosMICs	Toluenesulfonylmethylisocyanide
ml	Millilitre
ppm	Parts Per Million
s	Singlet
m	Multiplet
t	Triplet
Pip	Piperazine
Arom	Aromatic

1. INTRODUCTION

Medicinal chemistry has become one of the most important fields of the modern era, as it studies common diseases around the world and how to treat them. It relies on the classical chemistry branches, organic chemistry, especially heterocyclic fields, also molecular biology, pharmacology, microbiology and some fields of physics. Heterocyclic is a substance that is widely present across several important natural compounds, including deoxyribonucleic acid (DNA), purine, histidine, histamine, antibacterial, antifungal, anti-inflammatory, anticancer, antiallergic and antioxidant [1].

In recent decades, a global problem has been observed due to infectious bacterial diseases, as microorganisms have reached a dangerous stage in many countries of the world due to their resistance to antibiotics due to their misuse by the human race, such as beta-lactam, macrolides, quinoline, and other antibiotics. To reduce this global problem, there is a need to develop a new type of molecule with high biological activity, which could be represented by imidazole and its derivatives combined with piperazine for the treatment of bacterial infectious illnesses according to the literature [2].

Imidazole [1,3-diaza-2,4-cyclopentadiene] is a heterocyclic organic molecule. The Imidazole structure consists of a five-member ring and is unsaturated, and this ring consists of two nitrogen atoms and three carbon atoms, with a $C_3H_4N_2$ formula. Imidazole is a white or colourless solid that can dissolve in H_2O, and produces a mild alkaline solution [3]

Piperazine [1,4-Diazacyclohexane] is a heterocyclic organic molecule. The piperazine structure consists of a six-member ring and this ring consists of two nitrogen atoms at opposite sides and four carbon atoms, with a $C_4H_{10}N_2$ formula. Piperazine is a white crystalline solid that can freely dissolve in H_2O [4]

This research study is aimed to synthesize-imidazole derivatives throughout substitution reaction on the first position, which was done with *para*-fluorophenyl-piperazine (pFPP) using reflux technique via Mannich reaction. Characterization of

the molecule was done via Proton Nuclear Magnetic Resonance (^{1}H-NMR), Fourier-Transform Infrared Spectroscopy (FT-IR), also Mass Spectrometry (MS). Thin Layer Chromatography (TLC) and Melting Point (MP) were done to detect the purity of the compound. At the end, disk diffusion technique was done and the result shows that the molecule has promising antimicrobial activity against both gram positive (G +ve) and gram negative (G-ve) bacteria such as such as: *Staphylococcus species*, *Escherichia coli*, and *Pseudomonas species* at low concentrations [10 mg/ml] after 24 - 48h incubation time

2. LITERATURE REVIEW

2.1. Antibiotics and Antimicrobials Deference?

Antimicrobial is a popular term that describes the group of medicines that are often known as antibacterial, antiviral, antifungal, antiprotozoal and anthelmintic, this approach is effective against many bacterial, viral, fungal, protozoan and parasitic infections separately. There are two main types of antimicrobials, in term of inhibiting the growth of microorganisms called bacteriostatic while killing the microorganisms called bactericidal. While antibiotics are synthesized chemicals with small molecule weight with antibacterial properties. [5].

2.1.1. Antimicrobial Susceptibility Test (AST)

The Antimicrobial susceptibility test (AST), is the most significant assay performed in microbiology laboratories to evaluate a specific pathogen's sensitivity to antibiotics that may be employed in therapy. Also, it can be defined as the capacity of antibiotics to certain microorganism species in vitro study which are the reason for causing illness [6].

2.1.2. Antibacterial Agents

For a long time, the conflict has gone on between the human race and the infectious microorganisms that cause many diseases, such as tuberculosis, plague, malaria, acquired immunodeficiency, and most recently, the Corona pandemic, which greatly affected humans with high ratio. Medicinal chemistry helped to invent many medicines such as antibiotics to face these infectious microorganisms that cause infectious diseases, so the scale tilted in favor of the human race, especially in the early forties, after penicillin became available for use, and because of the misuse of these antibiotics by the human race, bacteria were able to show resistance, so in order to resist and control infectious diseases serving humanity in the world, scientists' attention has been directed to providing alternatives to these antibiotics [7].

2.1.3. Antibacterial Agents and Their Mechanisms of Action?

The antibacterial treating infections caused by bacteria are divided into several mechanisms of action [8,9], shown in Figure 1.

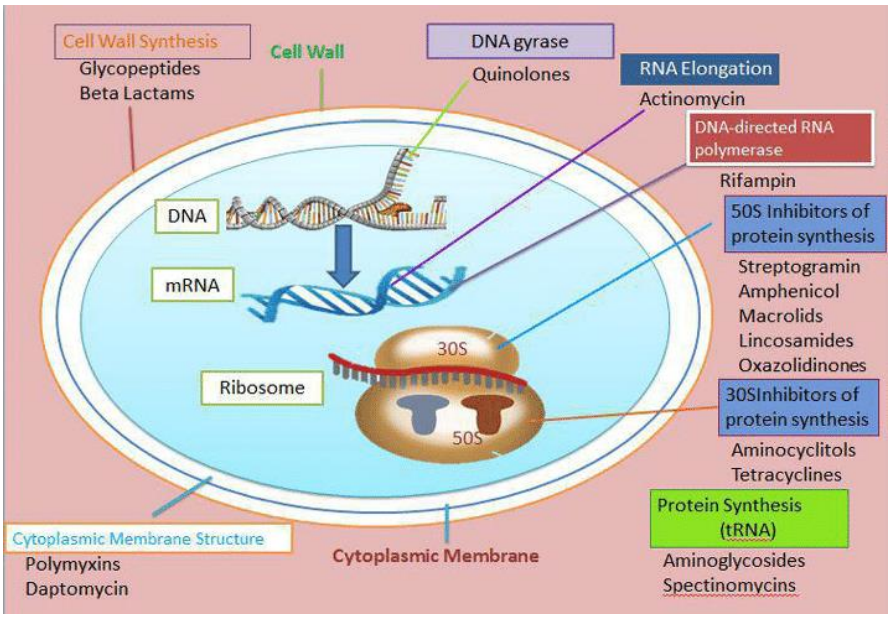


Figure 1: Antibiotics' mechanisms

2.1.4. Disk Diffusion Technique

The Broth dilution technique is considered as the best technique for AST test, but because of the disadvantages of this technique, microbiologists 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 is disk diffusion technique. In 1956, Kirby, M., and his Washington School of Medicine colleagues proposed a single unified disk technique for AST. It is now referred to as (Kirby-Baurer disk diffusion technique). WHO created a committee in 1961 to establish guiding and the fundamentals of 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 need any additional equipment. It is the most practical and accessible test [10]

The antimicrobial agents are injected into small discs with standard and known concentrations and placed on a Mueller-Hinton agar surface that is injected with (0.5 McFarland) of the isolated strain such as *Staphylococcus aureus* and *E. coli* then incubated for 24 hours under 37 °C, injected discs diffused to the agar and form a zone called “inhibition zone”. and inhibition zone diameter was measured with millimetric scale for each disk. In term of resistance/susceptibility this technique is called disk diffusion technique Figure 2 [11]

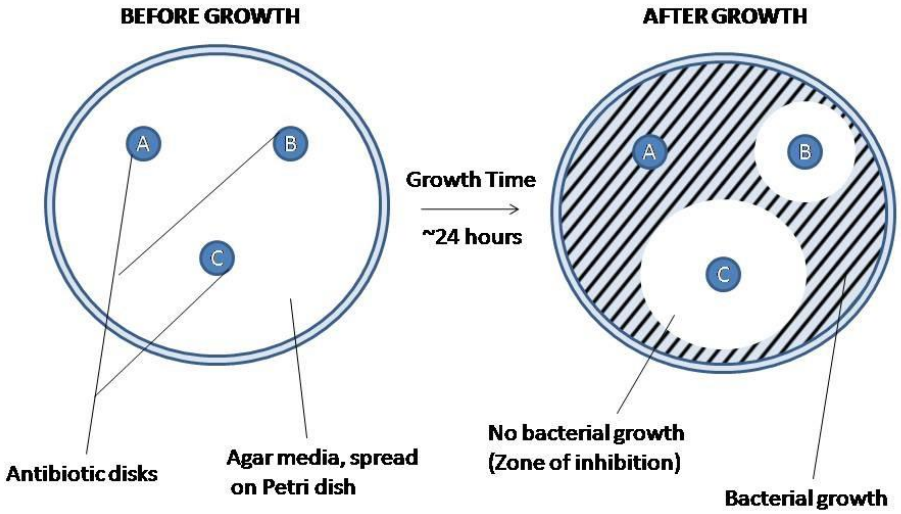


Figure 2: Disk diffusion technique before and after bacterial growth

2.3. Imidazole

Imidazole is a heterocyclic molecule that is used in many pharmaceutical agents and has essential properties. This molecule has a five-membered planar aromatic ring which is mostly hydrophilic and polar solvent soluble. A hydrogen atom can be present on either N-1 or N3 atoms. That's why imidazole has two identical tautomeric forms. The measured dipole of 3.61D indicates that it is a strongly polar substance that is fully soluble in water. The inclusion of a protonated nitrogen atom and an electron-electron hex term composed of electron pairs from each of the other four atoms in the ring classifies this compound as aromatic. Imidazole is an amphoteric compound. That has the properties of both an acid and a base. [12]

The hydrogen atom bound to imidazole nitrogen has a comparatively low acidity [pKa1= 14.4], This is because in aqueous solutions, imidazole acts like a base and forms cations [Imidazole H^+, see Figure 3b], with N3 protonated [pKa2= 6.99]. while is a strong base solution where imidazole deprotonated and forms [Imidazole$^-$ anion Figure 3c. The aromatic [imidazolium ylide] is a different kind of imidazole. It's a neutral, dipolar tautomer of imidazole, with an unshared pair of ó electrons on C2 that's produced by intramolecule hydrogen rearrangement. Figure 3d. As a result, the presence of the above four imidazole structures make imidazole have an amphoteric property. [13]

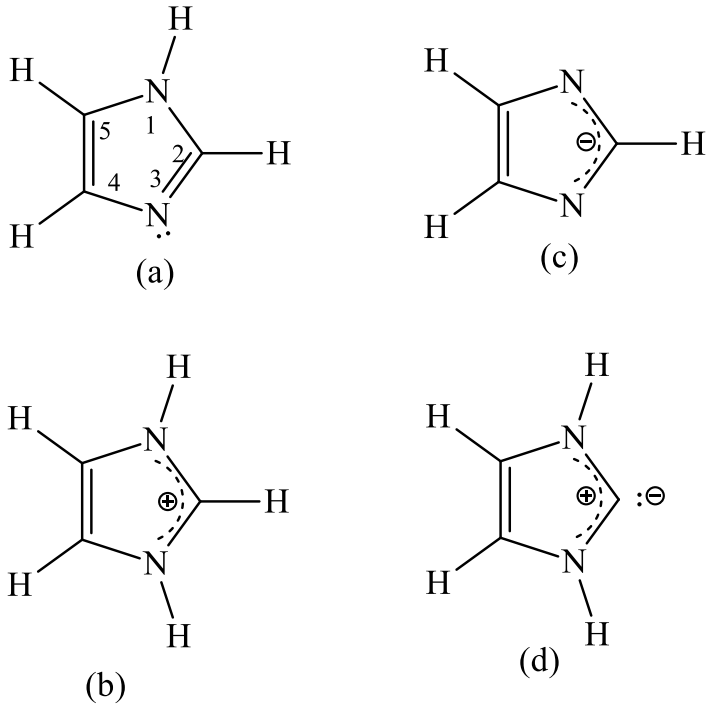


Figure 3: Different tautomerization of imidazole molecule

2.3.1. Imidazole Synthesis

There are several classical techniques for synthesizing the imidazole ring, including synthesis of imidazole via Wallach [14], synthesis of imidazole via Debus-Radziszewski [15], synthesis of imidazole via van Leusen [16], and others. van Leusen imidazole technique depending on Toluenesulfonylmethylisocyanide (TosMICs) is considered the famous technique among all other techniques for imidazole-based preparation which has rapidly developed in recent decades due to its good advantages such as ease of availability of raw materials, simple manipulation of the structure, diverse variety of substrates, highly bioactive and less toxic [17]. Figure 4.

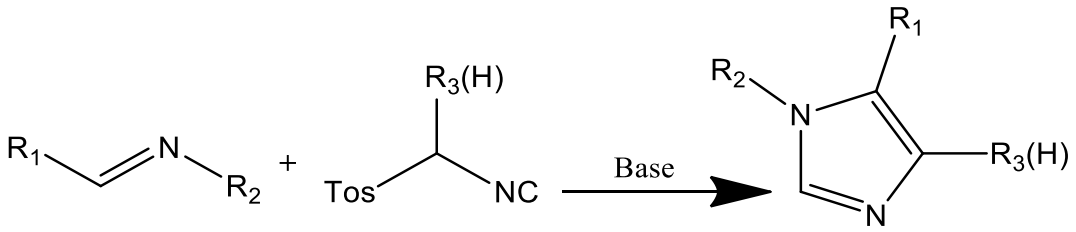


Figure 4: General van Leusen imidazole synthesis

Imidazole and its derivatives have a variety of pharmacological functions, according to the literature [12], including: anti-bacterial, anti-fungal, anti-cancer, anti-viral, analgesic, anti-inflammatory, anti-tuberculosis, anti-depressant and antileishmanial activities

2.3.2. Anti-Bacterial and Anti- Fungal Activity

A new molecule of five-[bromo/nitro] 1H-benzo[d]imidazole derivatives has been developed and examined by Ramya v et al for investigation of both anti-fungal activity against *Candida spp and Aspergillus spp*, and anti-bacterial activity against *Staphylococcus spp, Enterococcus spp, Klebsiella spp*, and *E. coli*, Figure 5. This could be similar compared to ciprofloxacin. [18]

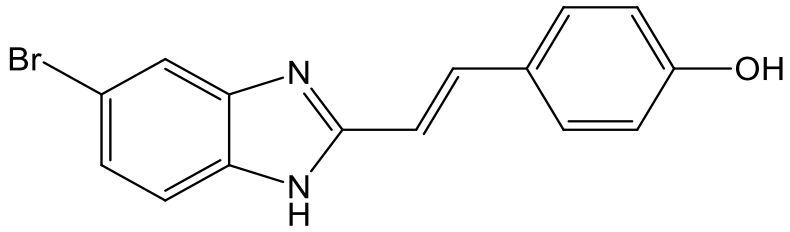


Figure 5: (E)-2-(2-(4-hydroxyphenyl)vinyl) (5-bromo-1H-benzo[d]imidazol-2-yl) showing anti-fungal and anti-bacterial activity

2-phenyl-1-(phenyloxomethyl)imidazole substituted has been synthesized by Deepika Sharma et al against fungal species also it has shown gram [G+ve & G- ve] anti-microbial activity. while Norfloxacin is utilized as standard Figure 6 [19]. The other substitution below shows fantastic potency.

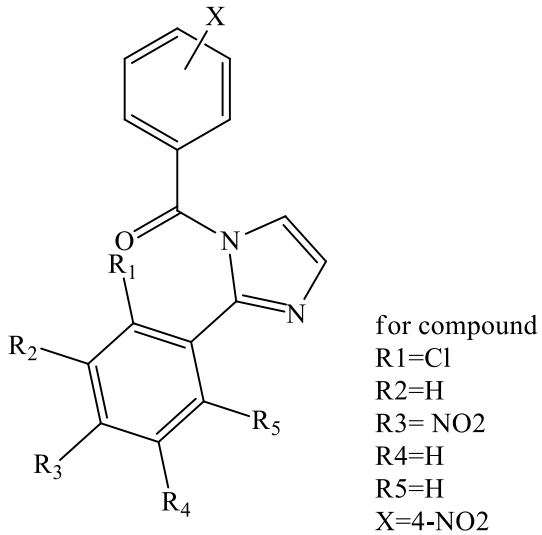


Figure 6: Basic structure of 2-phenyl-1-(phenyloxomethyl)imidazole substituted showing anti-bacterial activity

2.3.3. Anti-Cancer Activity

A new molecule of phenyl substituted imidazole-and azole derivatives have been developed and synthesized by Yusuf Ozkay et al for investigation of anti-cancer activity. These were the most active compounds in the sequence, according to anti-cancer activity screening findings. Cisplatin was utilized as a standard. Figure 7 [20].

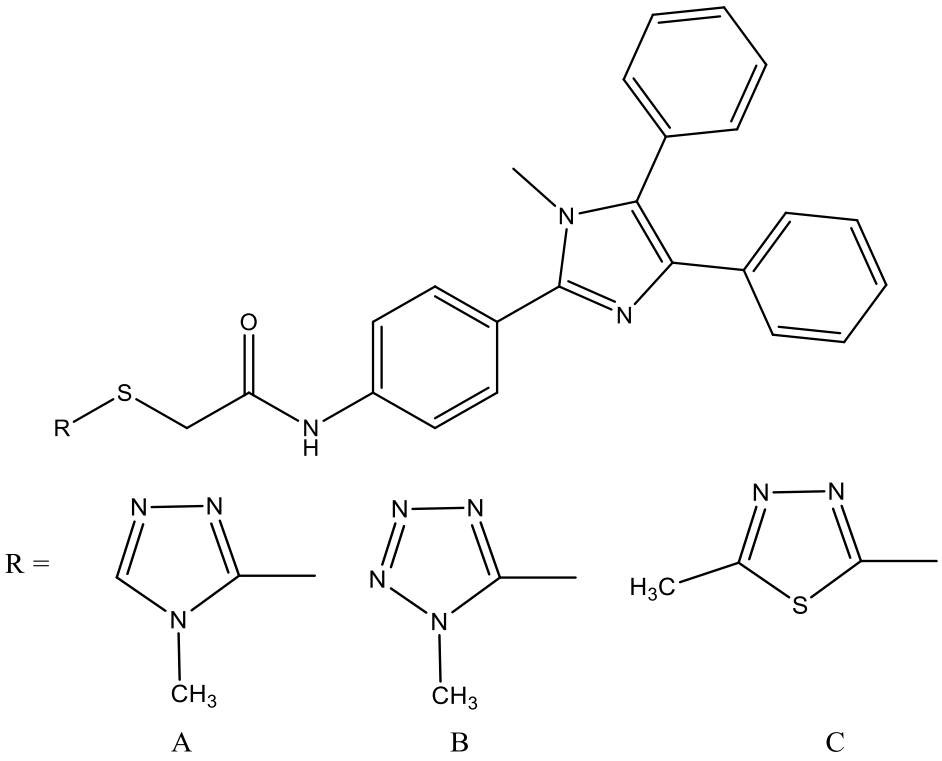


Figure 7: Basic structure of phenyl substituted imidazole-and azole showing anti-cancer activity

Also, a various novel of 2-substituted benzimidazole synthesized by Hanan M. Refaat for investigation of anti-cancer activity. All of the compounds examined had antitumor efficacy against breast cancer, colon carcinoma, adenocarcinoma and human hepatocellular carcinoma, according to the findings. The most potent against human hepatocellular carcinoma are 3a and 4a. Figure 8-9 [21]

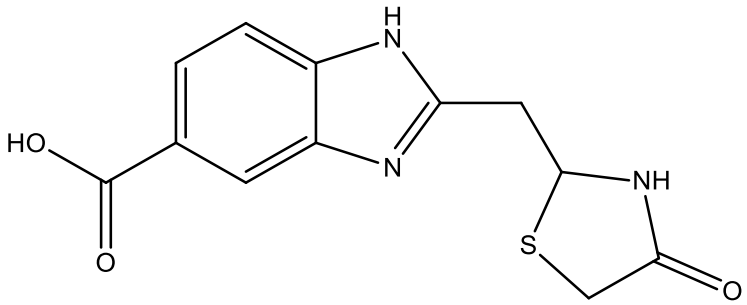


Figure 8: Compound [3a] 2-((4-oxo-thiazolidin-2-yl)methyl)-5-carboxylic acid-1H-benzo[d]imidazole showing human hepatocellular carcinoma activity

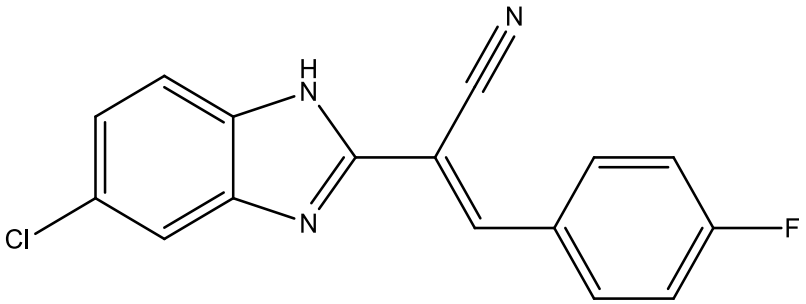


Figure 9: Compound [4a] (E)-2-(5-chloro-1H-benzo[d]imidazol-2-yl)-3-(4-fluorophenyl) acrylonitrile showing human hepatocellular carcinoma activity

However, the results showed that compounds 5a and 6a were more successful and biologically active in the treatment of human breast adenocarcinoma than human colon cancer. Figure 10,11

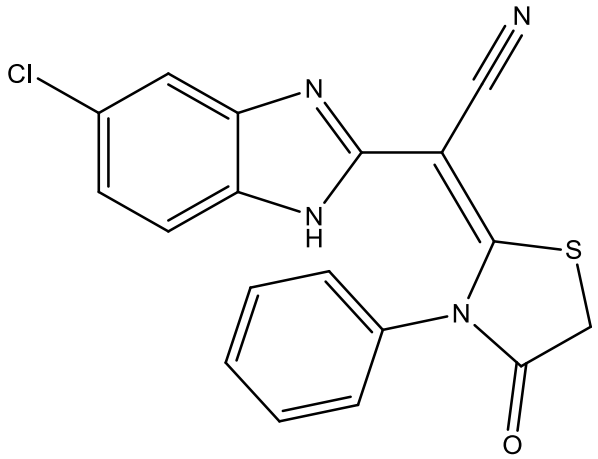


Figure 10: Compound [5a] [E]-2-(5-chloro-1H-benzo[d]imidazol-2-yl)-2-(4-oxo-3-phenylthiazolidin-2-ylidene)acetonitrile showing anti- human colon cancer activity

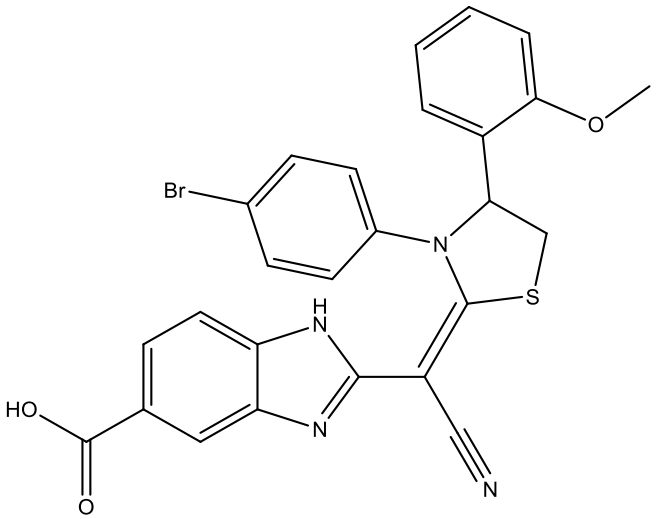


Figure 11: Compound [6a] (E)-2-((3-(4-bromophenyl)-4-(2-methoxyphenyl)thiazolidin-2-ylidene)(cyano)methyl)-1H-benzo[d]imidazole-5-carboxylic acid showing anti- human colon cancer activity

A 1, 4-diarylimidazole-2[3H]-one derivatives and their 2-thione were synthesized by Cenzo congiu et al for anti-cancer activity analogues. This compound has anti-cancer properties Figure 12 [22].

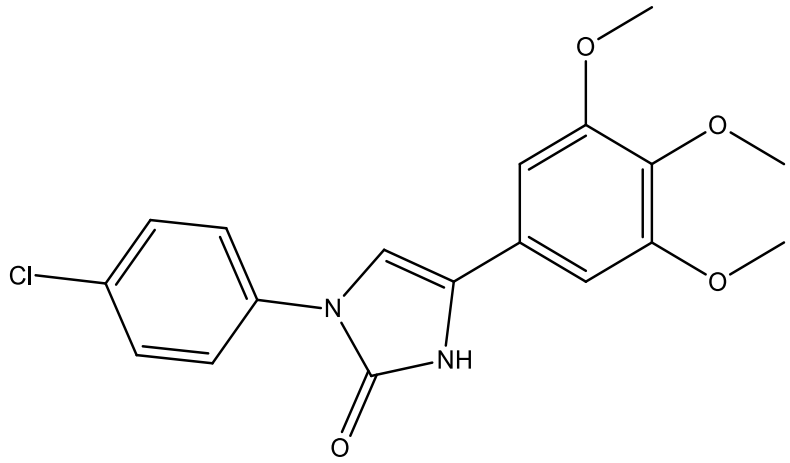
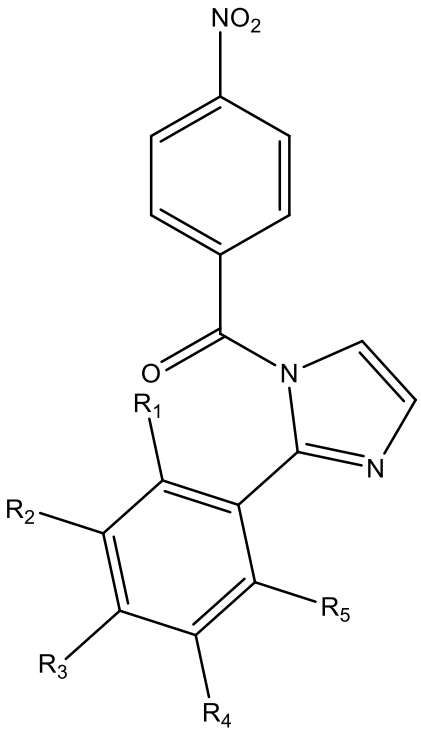


Figure 12: Structure of 1-(4-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)-1H-imidazol-2(3H)-one showing anti-cancer activity analogues

2.3.4. Anti-Viral Activity

4-nitrophenyl [2-phenyl substituted-1H-imidazol-1-yl] methanone has been synthesized by Deepika Sharma et al. Compounds A and B were shown to be the most effective anti-viral agents against viral strains. Ribavirin was utilized as a standard. Figure 13 [23]



For compound
R1 =H
R2 =H
R3 =NO2
R4 =H
R5 =H

Figure 13: Structure of 4 nitophenyl (2-phenyl substituted-1H-imidazol-1-yl) methanone showing anti-viral activity

2.3.5. Analgesic Activity Anti-Inflammatory Activity

2-methylaminibenzimidazole derivatives have been synthesized and tested by Kavitha C.S.et al for analgesic and anti-inflammatory activities. The new compound shows potent analgesic activity were Nimesulide was utilized as standard. Figure 14 [24]

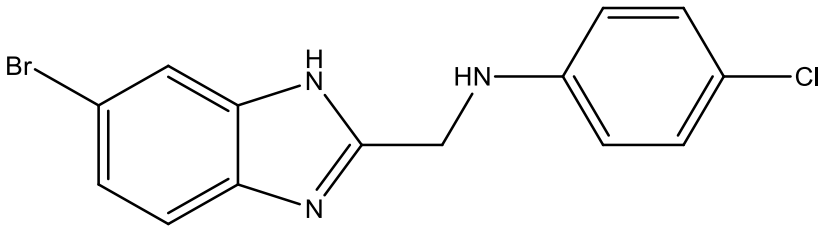


Figure 14: Structure of N-((6-bromo-1H-benzo[d]imidazol-2-yl)methyl)-4-chlorobenzenamine showing analgesic activity

investigation of Puratchikody A.et al for anti-inflammatory activity of 2-substituted-4, 5-diphenyl-1H-imidazoles according to the Carrageenan-induced paw edema technique. This compound has shown the highest anti-inflammatory activity, and indomethacin was utilized as a standard. Figure 15 [25]

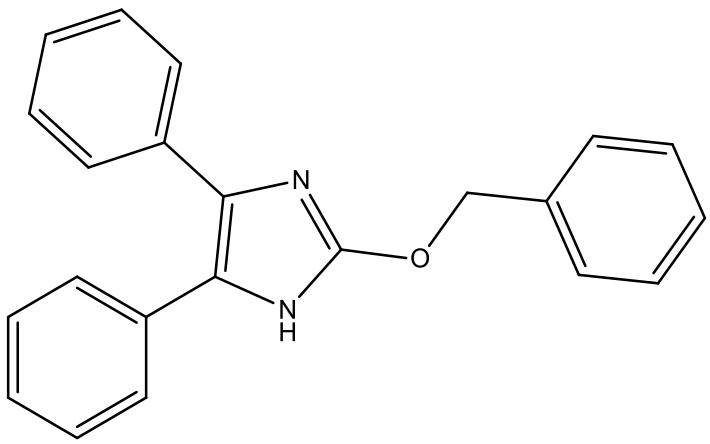
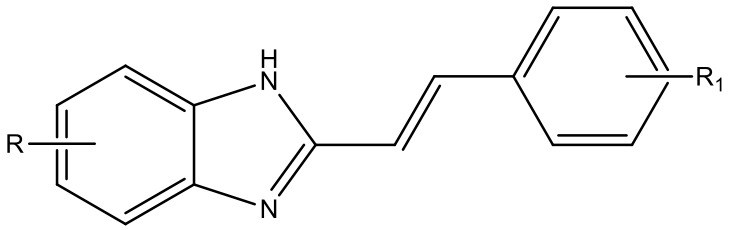


Figure 15: Structure of 2-(benzyloxy)-4,5-diphenyl-1H-imidazole showing anti-inflammatory activity

2.3.6. Anti-Tuberculosis Activity

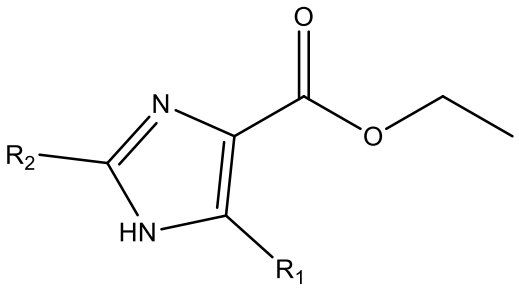
A novel 5-[nitro/bromo]-styryl-2 benzimidazole (1–12) derivative has been synthesized and tested by Ramya v et al for anti-tubercular activity against Mycobacterium tuberculosis. and these compounds showed good anti-tubercular activities. Streptomycin was utilized as standard. Figure 16 [18].



For This compound
A -R=Br, R1=H
B -R=Br, R1=3,4-OCH3
C -R=Br, R1=4-CH3
D -R=Br, R1=2,4-Cl

Figure 16: Structure of 5-(nitro/bromo)-styryl-2 benzimidazole derivatives showing anti-tubercular activities

Ring substituted-1Himidazole-4-carboxylic acid derivatives and 3-(2-alkyl-1H-imidazole-4-yl)-propionic acid derivatives were studied by Preeti Gupta et al to investigate anti-mycobacterium tuberculosis activities against resistant and sensitive drug of M. tuberculosis strains. The most potent compounds were 1b and 2b. Figure 17 [26]

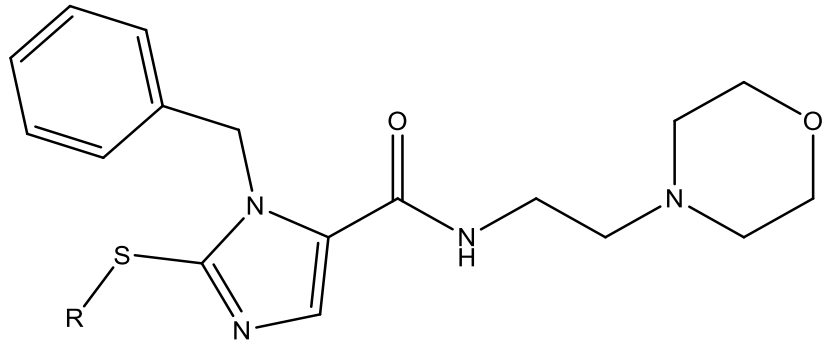


For compound
1b -R1=R2=c-C5H9
2b -R1=R2=C6H11

Figure 17: Structure of ethyl 2,5-di-substituted-1H-imidazole-4-carboxylate showing anti-tubercular activities

2.3.7. Anti-Depressant Activity

Farzin Hadizadeh et al synthesized moclobemide analogues of substituted imidazole with moclobemide phenyl ring for investigation of antidepressant activity. as a result, the analogues 3c compound were discovered to be more effective than moclobemide as an anti-depressant. Figure 18 [27]

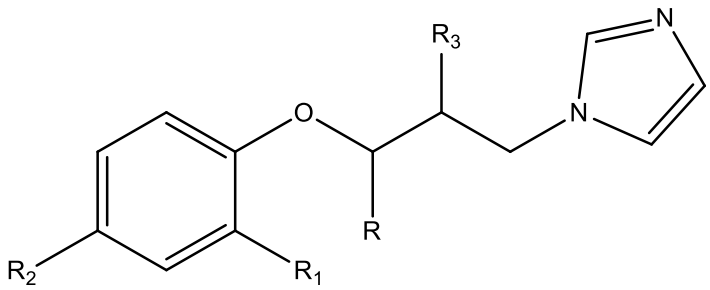


For this compound
1c -R= CH3
2c -R= C2H5
3c - R= CH2C6H5

Figure 18: Structure of 1-benzyl-2-mercapto-N-(2-morpholinoethyl)-1H-imidazole-5-carboxamide showing anti-depressant activity

2.3.8. Antileishmanial Activity

Deepika Sharma et al have synthesized an aryloxy aryl alkyl imidazole for investigation of anti-leishmanial activity aginist Leshmania donovani. As a result, 94-100% were inhibited for all compounds. Figure 19 [28]

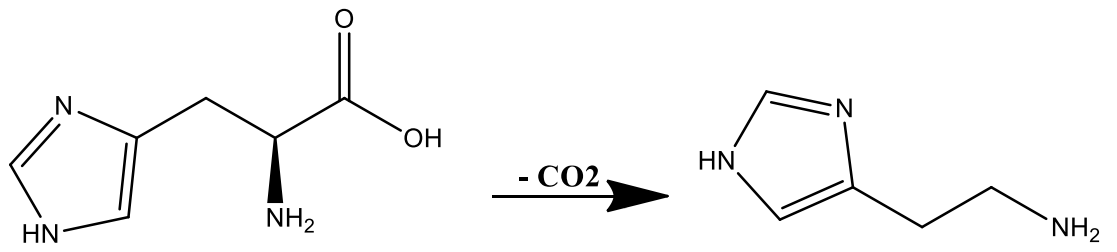


General Compounds R= Ph R_1=H R_2=CF_3 R^3=H

Figure 19: General structure of 1-(3-phenoxypropyl)-1H-imidazole showing anti-leishmanial activity

2.3.9. Biochemical Importance of Imidazole

Imidazole is utilized in a variety of biomolecules. The molecule which contains imidazole side chain like histidine amino acid is the most important. Histidine can be found in many enzymes and proteins and is important for the structure and function of hemoglobin binding. Decarboxylation of histidine to histamine is considered as common molecule biologically which causes urticaria due to the toxin that could be present because of allergic reaction. Figure 20 shows histidine and histamine relationship [12]



(S)-2-amino-3-(1H-imidazol-4-yl)propanoic acid
Histadine

2-(1H-imidazol-4-yl)ethanamine
Histamine

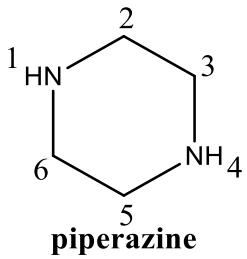
Figure 20: Decarboxylation of histidine to histamine

2.3.10. Some Imidazole's Applications

Standard solutions with a pH range of 6.2 to 7.8 can be prepared at room temperature utilizing imidazole. It is suggested that it be used as part of the horseradish peroxide screening buffer. It can be used as a divalent cation chelate. as a binder [29]. In the field of drug development, the nucleus of imidazole is a useful synthetic technique. Miconazole, Clotrimazole Azomycine, Moxonidine, Clonidine, and Ergothionine are examples of imidazole which are used as pharmaceutical drugs. Imidazole derivatives are also used as a material to treat denture stomatitis, which is considered as one of their most important applications. [30]. Industrial imidazoles are involved in many medical fields, especially antibiotics, antihypertensives, antifungals, as well as fungicides. Imidazole is also included in the composition of theophylline, as it is found in coffee beans and tea leaves, which stimulate the central nervous system. Amidazole is present in the anti-cancer drug mercapturin used by interfering with the DNA activities in leukemia. This is why amidazole is one of the most important parts of many medications now a days. [31]

2.4. Piperazine

1,4-Diazacyclohexane is a heterocyclic organic compound. The piperazine cyclic molecule consists of a six-member ring, and this ring consists of two nitrogen atoms at opposite sides and four carbon atoms, with the formula C4H10N2. It is a white crystalline solid, freely soluble in water [4], According to [Haroz and Greenberg, 2006] study, piperazine was first used in the 1950s as anthelmintic agents, and is still used as pharmacotherapy for both human and veterinary [32 – 33]. Figure 21.



piperazine

Figure 21: The chemical structure of piperazine

2.5. Mannich Reaction

Better medicines and improved effectiveness are required to combat Multi-Drug Resistant (MDR) cancers and bacteria strains [34-35]. It is possible to reduce costs and time-consuming modifications to producing and achieving a new novel bioactive technique. The Mannich reaction is an appropriate way to demonstrate an aminoalkyl substitution to a compound [36]. The Mannich derivatives show greater potency in many cases than the original compounds. because it enhances and develops the drug bioavailability via increasing its solubility. The results of this analysis review assess the advantages and limitations of using multiple Mannich reactions to develop many compounds that are biologically active, including anti-microbial, anti-fungal, antimalarial, anti-inflammatory, anti-cancer, and anticonvulsant etc.

2.5.1. The New Variants of Mannich Reaction

An essential and basic carbon - carbon bond formation process in organic synthesis is referred to as the Mannich reaction [37]. This reaction works well with a diverse range of functional groups, which supports its use in the development of medicinal chemistry. Many literature is available on the Mannich reaction, showing that the reaction has significant potential for application and diversification. [38,39] This technique has many derivatization possibilities which offer a reliable mechanism for the production of aminocarbonyl and other derivatives. [40,41] Figure 22 shows the synthesis.

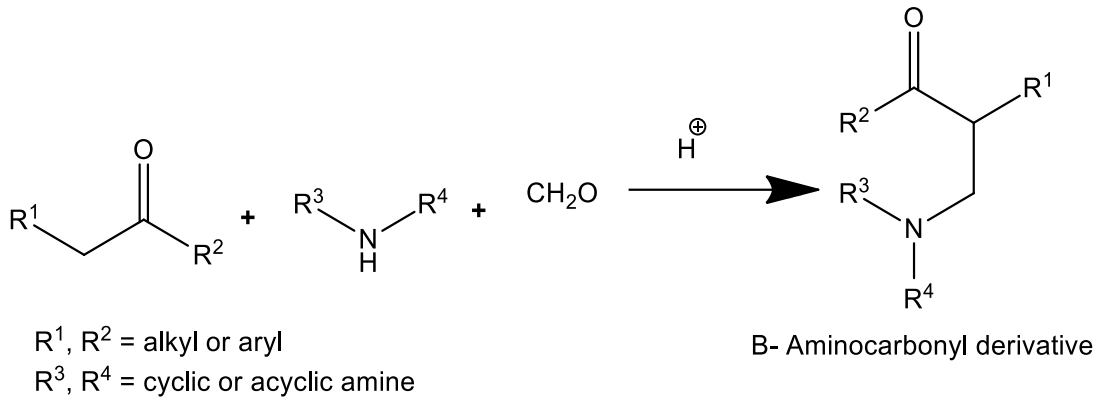


Figure 22. Scheme of Mannich reaction

2.5.2. Mannich Reaction Uses in Synthesis of Bioactive Molecules

Many different compounds, which have a variety of uses, are accessed via the Mannich reaction and its variations. The therapeutic/bioactive compounds produced by the Mannich reaction are listed in Figure 23, β-peptides and β-lactams, taxol (antitumour drug), among other bioactive compounds, are constructed from the aminocarbonyl Mannich products.

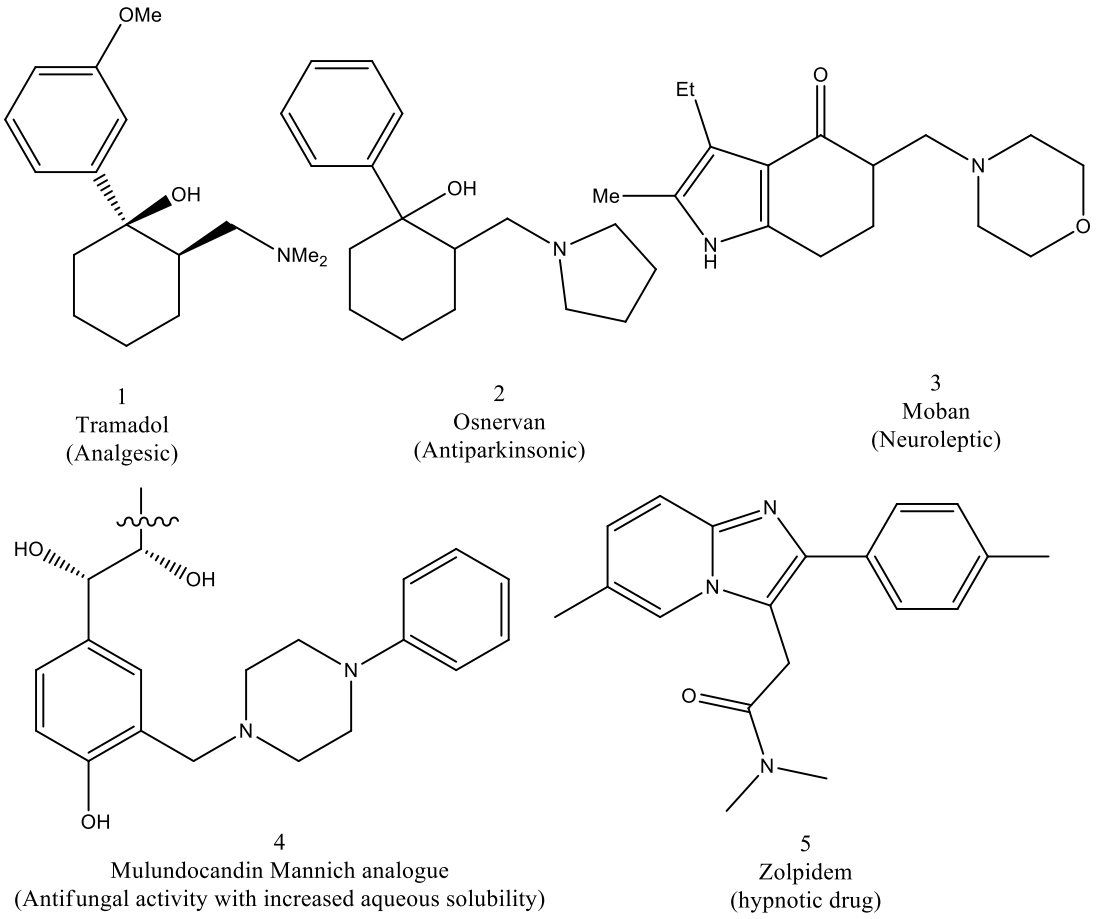


Figure 23. Synthesis of Bioactive Molecules by Mannich reaction

bestatine [immunological response modifier] and SCH48461 [anti-cholesterol agent].[42] Tramadol **1**, osnervan **2** and moban **3** are bioactive *β*-aminocarbonyl derivatives with analgesic, antiparkinson and neuroleptic properties Figure 23.[43] The theory is that in water the hydrogens of the basic amine nitrogen atom enhances the solubility of the Mannich derivatives.[44] In vitro activity against Candida species: Lipopeptides Mulundocandin has shown good activity. Nevertheless, the low

solubility prevents it from being widely used. In other words, lal et al. carried out a Mannich reaction-derived semi-synthetic modification of mulundocandin. [45] Improvement in solubility while maintaining activity was shown using the Mannich derivatives of mulundocandin 4, and this result was confirmed in the experiment shown in Figure 23. Zolpidem 5, a hypnotic medication used for the treatment of insomnia, was synthesized using the Mannich reaction. [46] C–H acidic substrates aldehyde, amine (cyclic or acyclic), and ketones, phenols, etc., are all components of the Mannich bases. Mannich bases are a major class of biologically active compounds that serve as a foundation. Cationic surfactant molecules that were obtained from Mannich bases are effective against both Gram-positive and Gram-negative bacteria while possessing a strong fungicidal effect. [47]

2.5.3. Mannich Bases of Pharmaceutical Chemistry and Drug Design

Many practical uses have been discovered for Mannich's bases in the manufacture of a variety of organic substances such as the field of medicinal chemistry, cosmetics, textiles, paper, skin, polymers synthesis and used in the petroleum sector, analytical reagents. [48]

Antibacterial activity

More recently, several publications have provided examples of Mannich's antimicrobial bases. A prominent feature of the Mannich reactions is their dependence on almost all types of significant aminomethylation substrates. the Mannich rules. Two distinct families of bacteria were selected for comparison: One gram-positive, one gram-negative. The findings from various tests cannot be compared due to variability in test techniques. studies on evaluation of Mannich's rules against mycobacterium species for tuberculosis (TB) are very important. [49]

Antimalarial activity

Elimination of malaria is proceeding well in many areas of the globe, although the overall number of reported cases stays high. Due to the expansion of malaria parasites, there is a hazardous scenario brewing in the international community. It is worth considering if antimalarial medicines, particularly the most frequently used ones, such as chloroquine, need to be utilized. Recently, new antimalarial medications have been created. due to the absence of development opportunities for developers in countries for this subject since the 1970s. Scientists started to implement numerous well-publicized public initiatives in light of this, a rise in interest in neglected illness medications has brought about an increase in funding for antimalarial treatments. Mannich reactions have been used for the preparation of antimalarial drugs for a long time. [50]

3. MATERIALS AND METHODS

3.1. Chemical Methods

The chemicals used for organic synthesis were obtained from Sigma Aldrich Chemical Co and the melting points of molecules were measured using Mettler Toledo FP 900 Thermo System device in the laboratory.

All spectrometric analysis were done at Central Laboratory, Ankara University, Turkey. The attenuated reflection of each synthesized molecule was examined from Infrared spectroscopy using a spectrophotometer, Perkin Elmer Spectrum 100 shown in wave numbers (cm^{-1}). The proton and carbon nuclear magnetic resonance spectrum of each molecule were examined on NMR device of Mercury Varian 400 MHz where tetramethylsilane was 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

3.2. Procedures for preparing 1-((4'-(4-fluorophenyl)piperazine-1-yl)methyl)-imidazole

300 mg of imidazole were weighed in a 50 ml round bottom flask then 8 ml of ethanol was added into it. 795 mg of 1- (4-fluorophenyl)-piperazine was then added into the flask. In another beaker 0.2 ml formaldehyde and 2.0 ml of ethanol were mixed, introduced into the other reaction mixture and refluxed for one hour. The resulting reaction mixture was extracted 3 times with chloroform-water and then the organic phase was evaporated to dryness under vacuum. The solid was recrystallized in ethanol to give a white crystalline solid.

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3.3. Microbiology

10mg/ml of the synthesized molecule injected to small discs and placed on Mueller-Hinton agar surface which is injected with isolated strain such as, *Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa*. After 24 hours of incubation under 37 °C, the injected discs diffused to the agar and form a zone called “inhibition zone”. and inhibition zone diameter was measured with millimetric scale for each disk. In term of resistance/susceptibility this technique is called disk diffusion technique

4. RESULTS AND DISCUSSION

4.1. Results

IUPAC Name. 1-((4'-(para-fluorophenyl)piperazine-1-yl)methyl)-imidazole

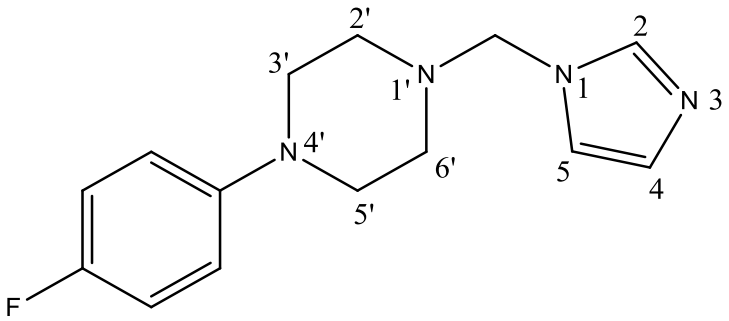


Figure 24. Structure of 1-((4'-(para-fluorophenyl)piperazine-1-yl)methyl)-imidazole

Table 1:Chemical and physical properties of 1-((4'-(para-fluorophenyl)piperazine-1-yl)methyl)-imidazole

Percentage yield	47 %
Mobile Phase	Ethanol. n-hexane (9:3)
Stationary Phase	Silica gel GF 254
Retention Factor (Rf)	0.19
Spot Detected	Under UV light at 254 nm
Physical Appearance	White powder solid
Solubility	Completely soluble in chloroform, Methanol, Ethanol, Propanol, Acetone, Ethyl acetate, Benzene and Acetonitrile Not soluble in n-hexane
Melting Point	141.7 °C
Molecular Formula	$C_{14}H_{17}FN_4$
Molecular Weight	260g/mol
m/z	260 (100.0%), 261 (16.8%), 262 (1.3%)

[FT-IR] [IR vmax]. The table 2 shows IR spectra results of target molecule

Table 2. IR spectra results of 1-((4'-(para-fluorophenyl)piperazine-1-yl)methyl)-imidazole

	Absorption (cm^{-1})	**Group**	**Compound Class**
1	3021 - 3054	C-H stretching	alkene
2	2816	C-H stretching	alkane
3	1638	C=C stretching	conjugated alkene (benzene and imidazole)
4	1148	C-F stretching	fluoro compound
5	1293	C-N stretching	Aromatic amine
6	1020 - 1250	C-N stretching	Amine
7	1448	C-H bending	methyl group
8	1705 - 1917	C-H bending	Aromatic

Absence of N-H stretch, which indicates that the reaction has actually taken place at position 1 of imidazole

[^{1}H NMR, $CDCl_3$; ppm]. 6.93 – 7.3 [m; 4H; Aromatic phenyl – H], 6.87 [m; 2H; imidazole H – 4, H – 5], 2.78 [s; CH_2, 2H; methyl], 3.17 – 3.25 [t; 4H; pip H3`-H5`]; 2.8 – 2.9 [t; 4H; pip H2` -H6`] ppm

Mass spectroscopy (ESI-MS). base peak is at m/z 164 amu.

[Disk diffusion technique]. shows activity to certain bacteria such as. *Pseudomonas aeruginosa*, *Staphylococcus aureus and Escherichia coli*

4.2. Discussion

In this study, the Mannich reaction was used in the synthesis of an imidazole derivative and this was carried out under reflux condition in ethanol, pFFP was attached on position 1 of imidazole to give the desired Mannich base. The general Mannich reaction for the target molecule is given below Figure 25.

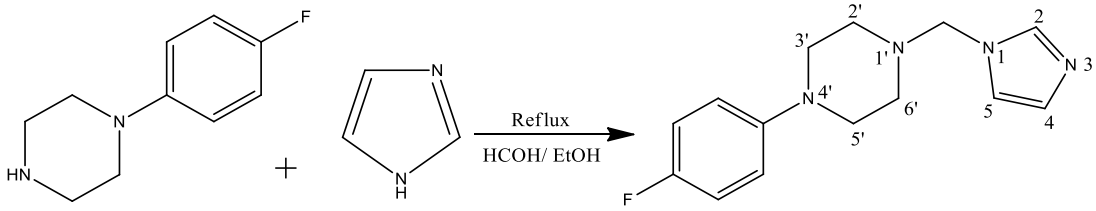


Figure 25. The general Mannich reaction for the target molecule

Mannich reaction mechanism of this study mainly undergoing with two steps, 1st step an iminium ion formation, 2nd step attacking of iminium ion by imidazole nucleus as shown in Figure 26.

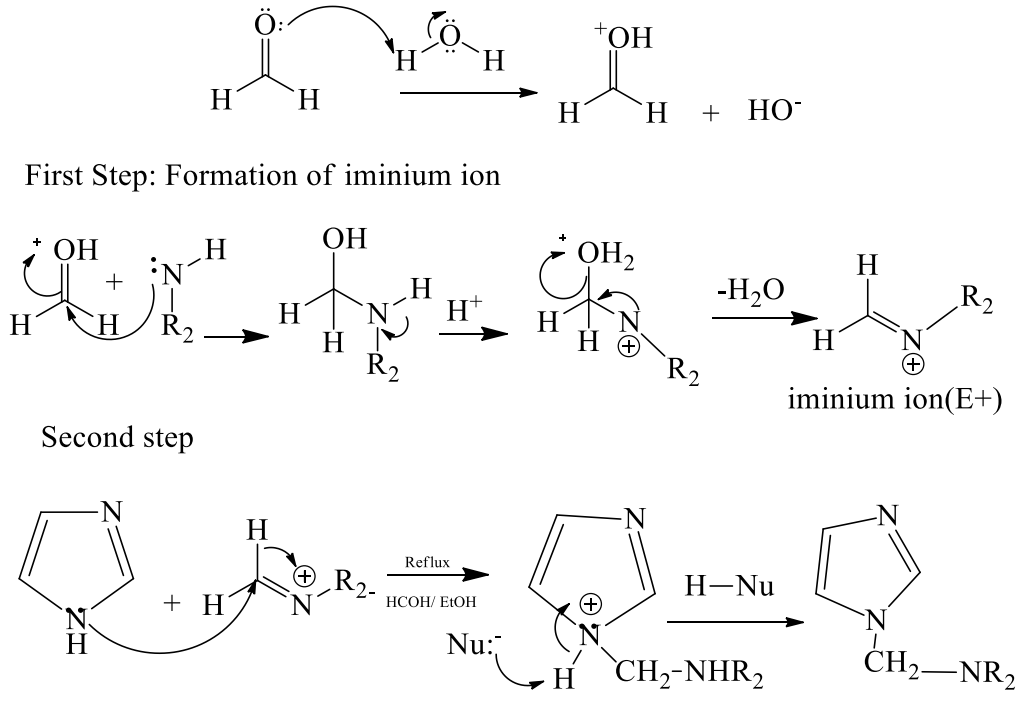
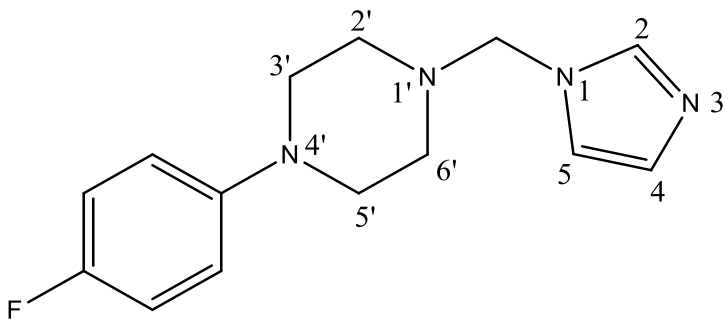


Figure 26. Mannich reaction mechanism of this study

Table 3. Summary of the target molecule

Target molecule	
IUPAC naming	1-((4'-(para-fluorophenyl)piperazine-1-yl)methyl)-imidazole
% Yield	47%
Condition/time	Reflux / 2hr
M.P.	141.7
Rf value	0.19

The synthesized molecule was characterized by Proton Nuclear Magnetic Resonance Spectroscopy [^{1}H-NMR] and Fourier Transform Infra-Red [FT-IR]. Thin layer chromatography and melting point were done for checking the purity and also to cross reference to the previously synthesized compound. The FT-IR spectra of the compound show the absence of N-H stretch, which indicates that the reaction has actually taken place at position 1 of imidazole

The HNMR and FT-IR spectra of the compound synthesized are shown in Figure. 27 and Figure. 28 below

Generally, 1H NMR spectra results show hydeogen atoms of the target molecule at different ppm. The multiplet peak appeared at about 6.8-73 ppm for aromatic hydrogens, the triplet peak appeared between 2.7-3.2 ppm for piperazine hydogens (H3` and H5`) and (H6` and H2`), and the most important peak that appeared was the methylene bridge as a singlet peak at 3.2 ppm that indicated the formation of the Mannich reaction. The HNMR spectra of the compound synthesized are shown in Figure. 27.

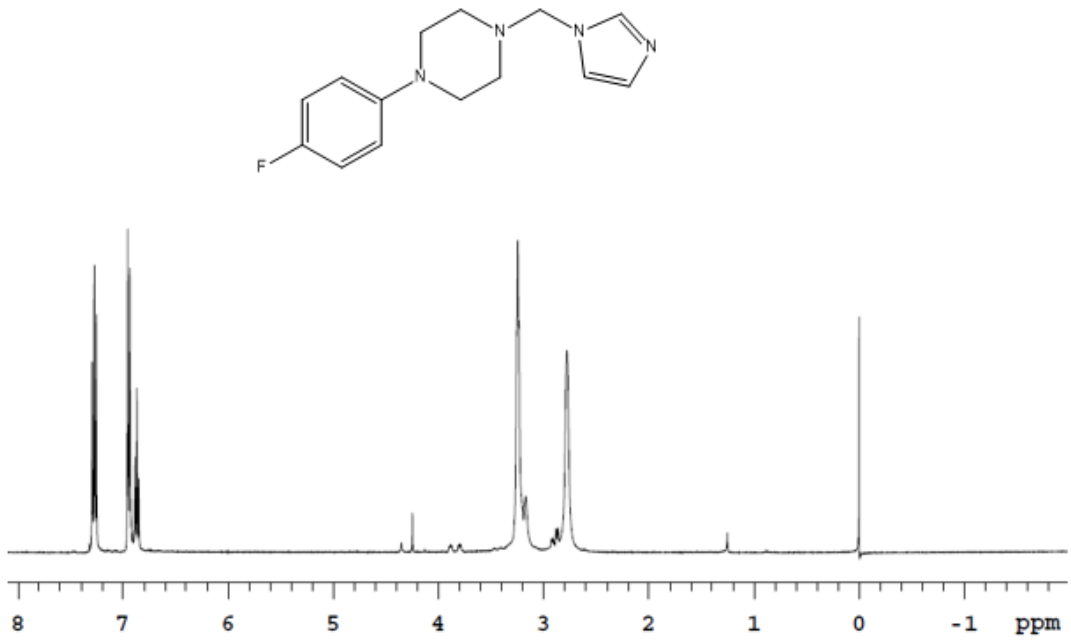


Figure 27. ^{1}H NMR Spectrum of 1-[4'-[para-fluorophenyl]-piperazin-1-yl]imidazole

The FT-IR spectra of the compound synthesized are shown in Figure. 28 below

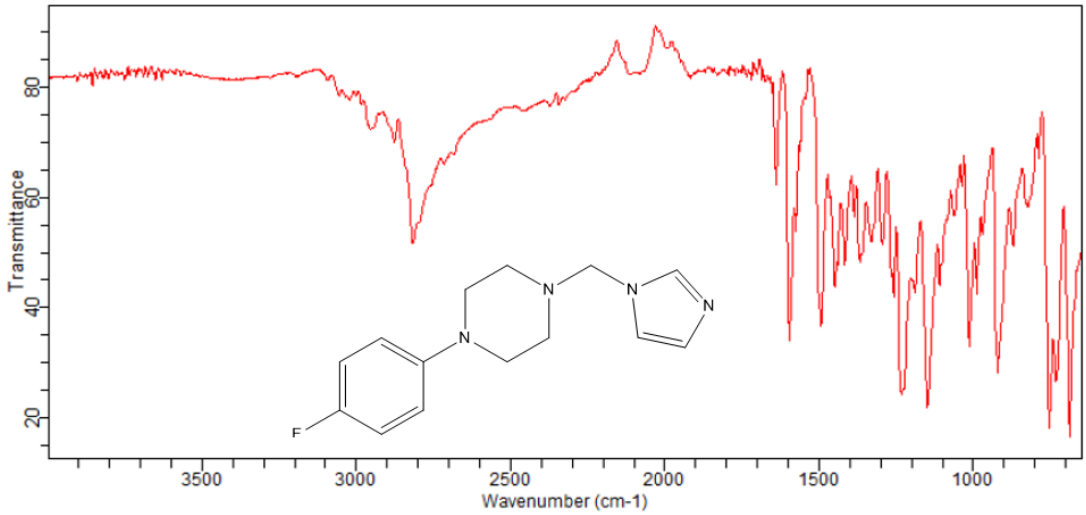


Figure 28. FT-IR Spectrum of 1-[4'-[para-fluorophenyl]-piperazin-1-yl]imidazole

In the mass spectroscopy (ESI-MS), the molecule ion is missing. As shown below, the base peak was found at m/z 164.amu Figure 29.

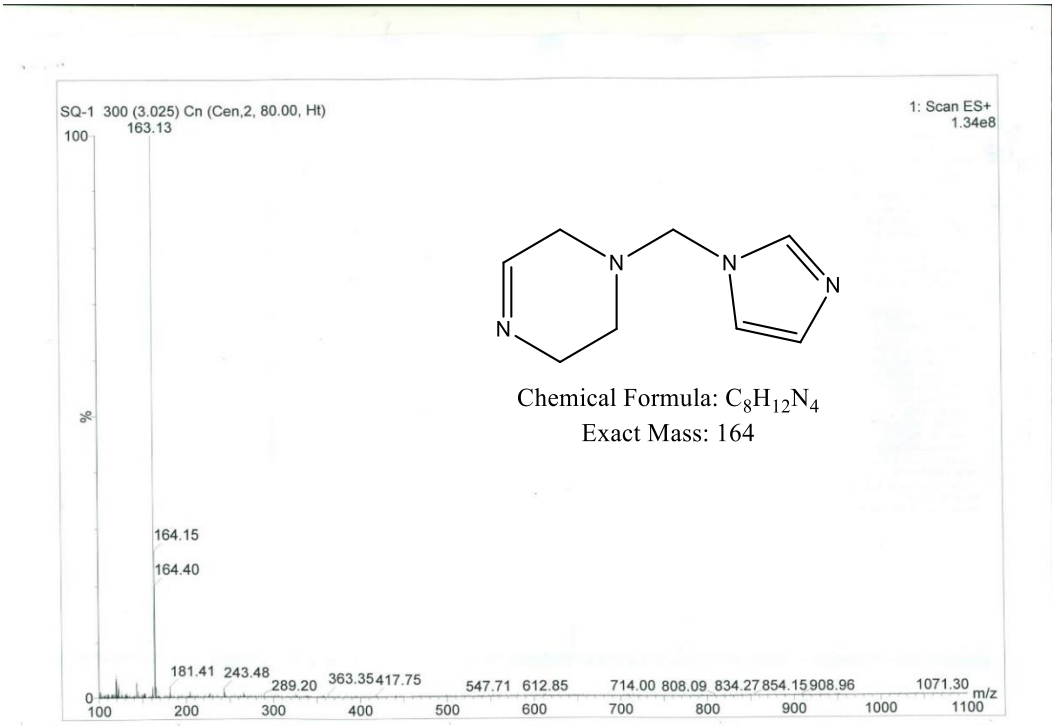


Figure 29. Mass spectra of 1-[4'-[para-fluorophenyl]-piperazin-1-yl]imidazole

Antimicrobial results

The target compound that was synthesized via Mannich reaction was tested at the clinical microbiology laboratory of Near East University; disk diffusion technique was done for investigation of biological activity. Table 3 shows antimicrobial activity against some bacterial spp.

Table 4. Antimicrobial results

Compound	**Zone of Inhibition (mm)**			
	S.aureus [1]	**P. aeruginosa**[2]	***E.coli*** [2]	***Candida albicans***[3]
	10 mm	5 mm	15 mm	0 mm
Positive Controls[1,2,3]	40 mm	40 mm	40 mm	15 mm

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

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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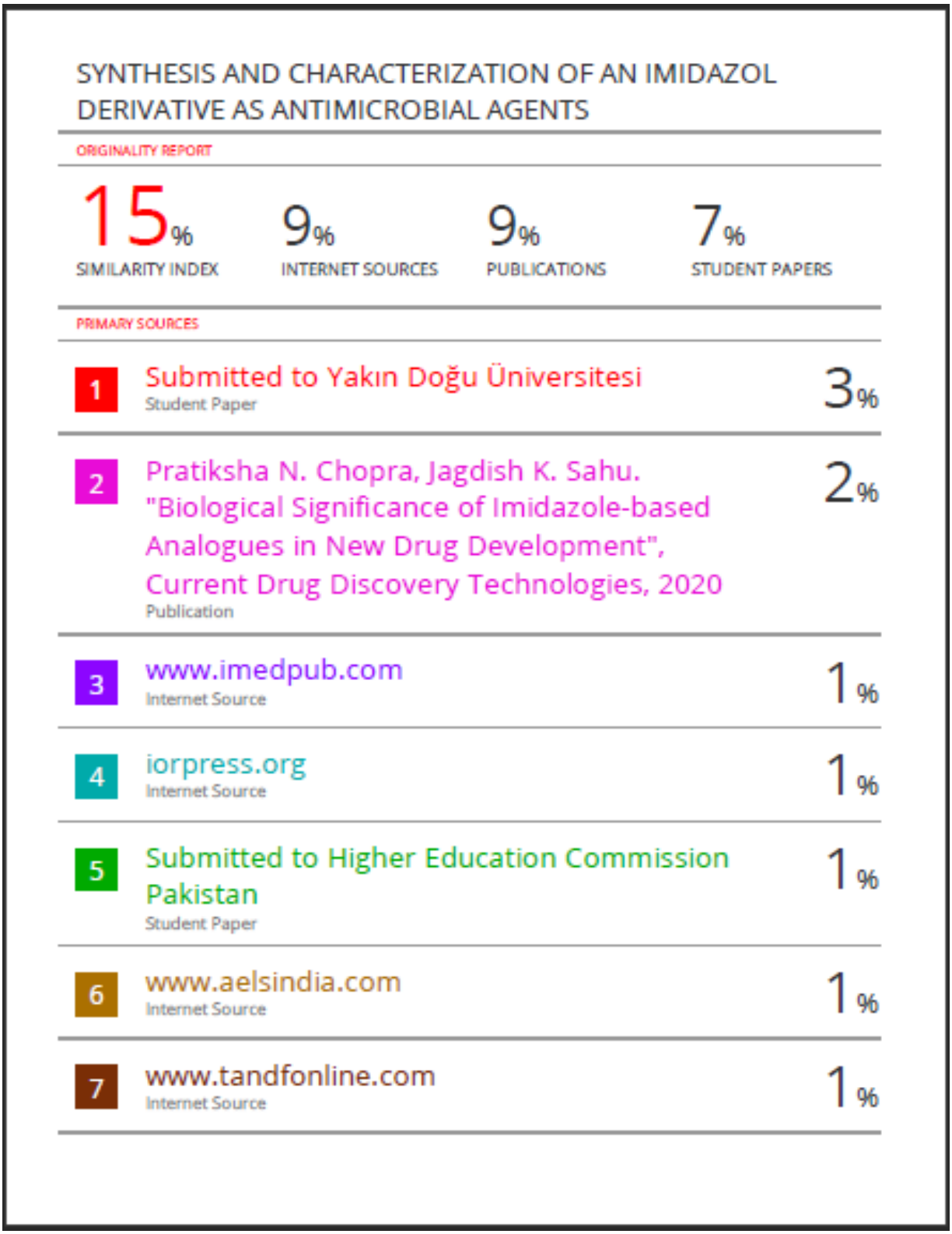
PLAGIARISM REPORT



SYNTHESIS AND CHARACTERIZATION OF AN IMIDAZOL DERIVATIVE AS ANTIMICROBIAL AGENTS

ORIGINALITY REPORT

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