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NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES

SYNTHESIS AND CHARACTERIZATION OF A SUBSTITUTED MANNICH BASE OF BENZIMIDAZOLE DERIVATIVE

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MS THESIS

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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 defense was online and all the jury members declared their approval as recorded.

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ABSTRACT

Benzimidazole is an important pharmacophore with reactivity that has led to the production of compounds with biological activities. In the search of manufacturing new drugs molecules with less toxicity and more potency, benzimidazole reaction with primary or secondary amines and formaldehyde via Mannich reaction has furnished new compounds with good pharmacological effects such as antimicrobial, anticancer, anti-inflammatory, antiparkinson, antiviral as shown in previous literature. It is in this light that we synthesized N1-substituted benzimidazole Mannich base derivative which based on previous studies, has antimicrobial effects. Our synthesized molecule was purified by using recrystallization and TLC methods and then the structure was confirmed by the ^{1} H-NMR and FT-IR spectra.

Keywords: Benzimidazole; Mannich base; antimicrobial activity; Mannich reaction.

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LIST OF ABBREVIATIONS

NMR……. Nuclear Magnetic Resonance

FT-IR……. Fourier Transform Spectroscopy

SAR……. Structural Activity Relationship

Rf……. Retention Factor

TLC……. Thin Layer Chromatography

ppm……. part per million

grps…… groups

1. Introduction

Benzimidazole which is a heterocyclic compound made up of benzene and imidazole ring fused together (Figure 2.1) has been an important explored pharmacophore in medicinal chemistry. Its structure is present in many clinical drugs which are already in the market. Among them, albendazole is used for worm treatment; omeprazole, for gastric ulcer; astemizole for allergy; ridinazole (PRESTON, 1974) used for bacterial infection; samastavir for viral infections; candesartan for high blood pressure; adibendan is to treat inflammation (Karakurt et al., 2017); just to name a few.

But because of the negative side effects that come with the use of these medications, for instance, toxicity (Salahuddin et al., 2017), constipation, gastrointestinal bleeding, nausea, apnea and so on and so forth, there is a call for the development of new drugs molecules devoid of these side effects.

Some synthesized compounds via Mannich reaction have been evaluated in vitro and found to exhibit anti-inflammatory, analgesic effects (Sagumaran & Silvadevi, 2011). Furthermore, other researchers have reported that heterocyclic Mannich bases also possess anticonvulsant and antioxidant. Moreover, Mannich bases have been studied recently and reported to be a multifunctional agent against Parkinson disease with good neuroprotective effects (Marinescu et al., 2020).

Benzimidazole reaction with substituted piperazine Mannich bases have been found to produce benzimidazoles derivatives with broad spectrum activity (Gul et al., 2019). Also, some studies have found that the pharmacological activity of a compound will depend on the position of the base on the benzimidazole nucleus as shown on the

structural activity relationship reports (Sahoo et al., 2019). Therefore, various compound with substitution at a particular position will have a specific activity.

With that said, benzimidazole skeleton is considered as a privileged substructure due to its high reactivity, especially with Mannich bases. It is in this light that we are going to explore it to produce a compound with good therapeutic effects and reduced sides effects and also less toxic than the preexisting ones (Ronald et al.,1968).

2. Literarature Review

2.1 Chemistry of Benzimidazoles

The benzimidazoles, also known as benzimidazoles or benzoglyoxalines have a phenyl ring fused to an imidazole ring, as shown in the figure2.1.

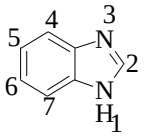


Figure 2.1 Chemical structure of benzimidazole

Hoebrecker prepared the first benzimidazole in 1872 by reducing 2-nitro-4-methylacetanilide to obtain 2, 5(or 2, 6) dimethyl benzimidazole.

Ladenburg obtained the same compound several years later by refluxing 3-,4-diaminotoluene with acetic acid. Because these compounds were formed by the loss of water, they were named “anhydrobases” in the early literature. It was later demonstrated that “anhydrobases” of this type could only be formed by compounds in which the nitrogen-containing groups were ortho to each other; that the ring formed was an imidazole ring as indicated by certain benzimidazole reactions, such as the fact that imidazole dicarboxylic acid can be obtained, though in low yield, by benzimidazole oxidation (Wright, 1951).

Benzimidazoles have also been identified as o-phenylenediamine derivatives, particularly in early literature. According to this nomenclature, benzimidazole is methenyl-o-phenylenediamine, and 2-methylbenzimidazole is ethenyl-o-

phenylenediamine. They have also been designated as derivatives of the grouping that makes up the imidazole portion of the ring. Thus, benzimidazole is also known as o-phenyleneformamidine), and 2(3H)-benzimidazolone and 2(3H)-benzimidazolethione are also known as o-phenyleneurea and o-phenylenethiourea, respectively.

The benzimidazole numbering scheme is as follows

2.2 Tautomerism in benzimidazoles

Tautomerization of benzimidazoles with hydrogen atom attached to nitrogen can be shown as follow: (Sahoo et al.,2019).

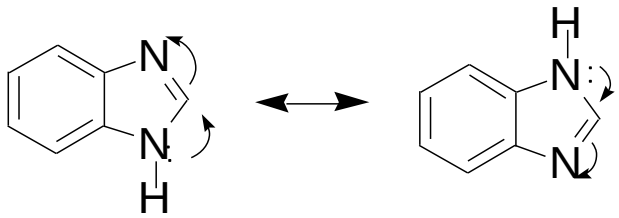


Figure 2.2 Tautomerism in benzimidazole

Tautomerization also happened in amidines and imidazoles structure. As a result, benzimidazoles and amidines are cyclic analogs.

Because of this tautomerism in benzimidazoles, certain derivatives that appear to be isomers at first glance are actually tautomers; although two non-equivalent structures can be written, only one compound is known. This can be demonstrated using 5(or 6)-methyl benzimidazole:

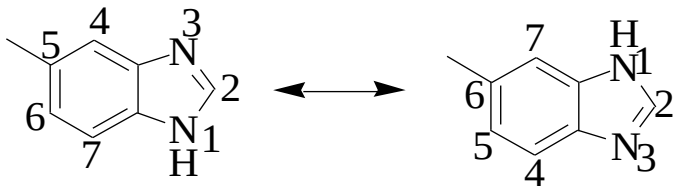


Figure 2.3: Tautomerism in 5 (or 6)-methylbezimidazole

As a result, 5-methylbenzimidazole is a tautomer of 6-methylbenzimidazole, and the two structures represent the same compound. When naming such tautomeric compounds, two numbers or sets of numbers are typically used to indicate the positions of the substituent group (or groups), with the second number in parentheses or set of numbers enclosed.

When the group attached to the nitrogen in the 1-position is larger than hydrogen, tautomerism does not exist, and isomeric forms exist. As a result, 1, 5-dimethylbenzimidazole and 1, 6-dimethylbenzimidazole are distinct compounds (Wright, 1951).

2.3 Solubility of Benzimidazoles

Because benzimidazoles are weak bases in nature, slightly less basic than imidazoles they are soluble in dilute acids. Benzimidazoles are also sufficiently NH-acidic to form N-metallic compounds in aqueous alkali. The acidic properties of benzimidazoles, like those of imidazoles appear to be the result of ion stabilization via resonance. Above 300°C, benzimidazoles can be distilled unchanged.

Benzimidazoles with hydrogen at position 1 position (i.e., imide nitrogen) are typically soluble in polar solvents but less so in organic solvents. Thus, while benzimidazole is

easily soluble in hot water, it is very poorly soluble in ether and insoluble in benzene and ligroin. However, incorporation of nonpolar substituents at various sites on the benzimidazole nucleus increases solubility in nonpolar solvents, as seen with 2-methylbenzimidazole, which is easily soluble in ether. In contrast, adding polar substituents to the benzimidazole nucleus increases its solubility in polar solvents; for example, 2-aminobenzimidazole is soluble in water. Overall, the solubility of various benzimidazole derivatives in alkaline solutions is dependent on the compound. There is also enough evidence to suggest molecular association via N-H-N bonds in benzimidazoles with an unsubstituted NH grouping (Singh & Silakari, 2018a).

2.4 Some Benzimidazole Reactions

Due to the high stability of benzimidazole ring, heating it with concentrated sulfuric acid to 270 ^{0}C under pressure nor treating it with hot hydrochloric acid or alkalis will not affect it. Although it is quite resistant to reduction; however, catalytic reduction of the benzene ring to tetrahydro- and hexahydrobenimidazoles is possible under certain conditions (PRESTON,1974). The benzene ring of the benzimidazole undergoes oxidation cleavage only under vigorous conditions. Benzimidazole do not react with sodium nitroprusside and alkali, that is, it gives a negative test. But 2(3H)-Benzimidazolethione gives a red color with these reagents (Singh & Silakari,2018)

2.4.1. Alkylation

In small amount of alkyl halides, benzimidazole give the first product and under vigorous conditions, the second product is obtained.

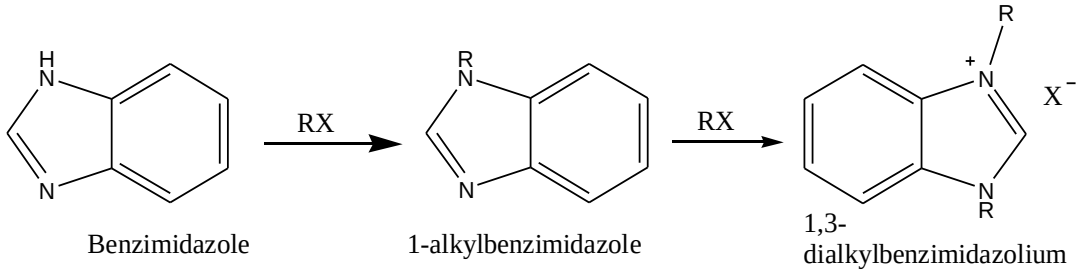
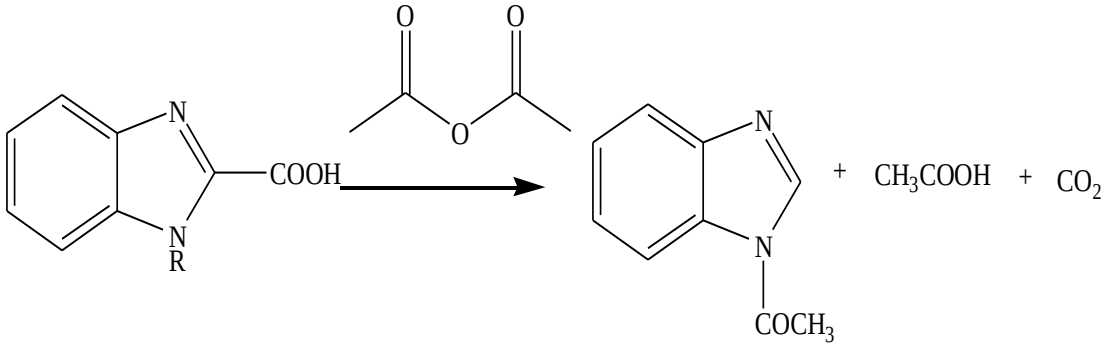


Figure 2.4: Reaction of benzimidazole with alkyl halides

2.4.2. Acylation

To prepare N-acyl benzimidazole, anhydrides or acid chlorides can be used with the corresponding benzimidazoles. The reaction should be carried out in the absence of water because in the presence of water and especially alkaline solution, cleavage of the imidazole ring can occur as shown by Schotten-Baumann procedure.



Acylation of 2-benzimidazole carboxylic acid with acetic anhydride to give 1-acetylbenzimidazole

Figure *2.5*: Acylation of 2-benzimidazole carboxylic acid with acetic anhydride to give 1-acetylbenzimidazole

2.4.3 Action of Grignard reagents on benzimidazoles

As shown in the following reaction, at position 1 Grignard reagents react with the active hydrogen.

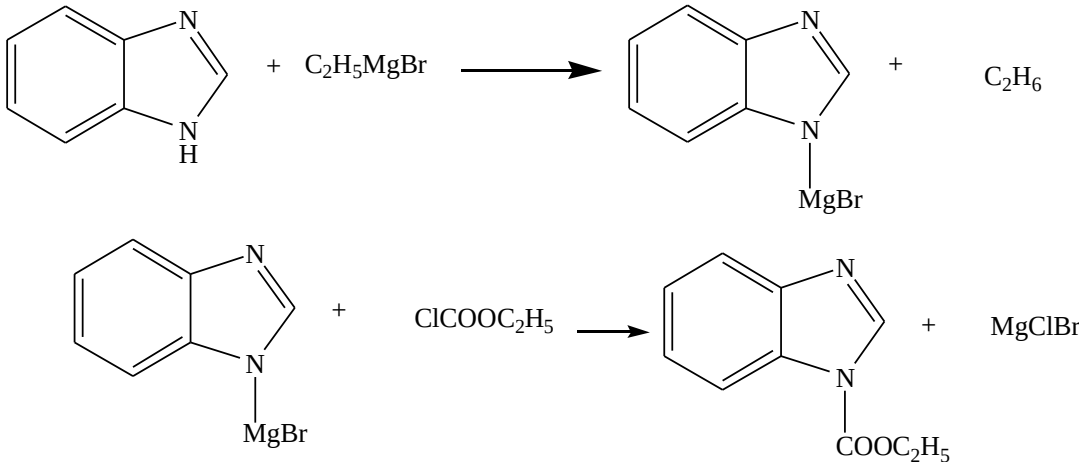


Figure 2.6: Action of Grignard reagent on benzimidazole

2.5 Benzimidazoles and Mannich bases

In the Mannich reaction with benzimidazoles shown by Bachman and Heisey, equal moles of benzimidazoles, formaldehyde and piperidine produce 97% of 1-(piperirinomethyl)benzimidazole.

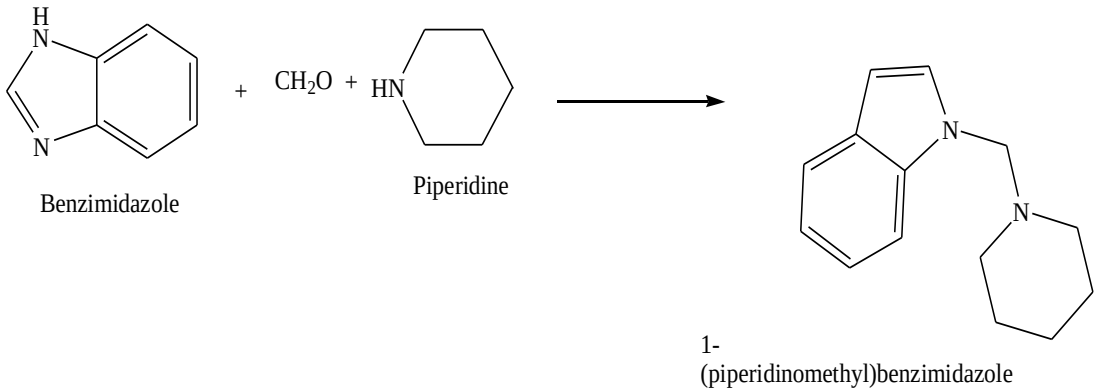


Figure 2.7: Synthesis of 1-(piperidinomethyl)benzimidazole

It is important to note that using primary amines or replacing formaldehyde with higher aldehydes will make this reaction impossible.

2.6 Reaction of Benzimidazole with Metals

Metals such as zinc, mercury, cadmium, cobalt, nickel, copper and silver form salt with benzimidazoles. Lorenzen and Bamberger were the first to show that the acidic hydrogen at position 1 could be replaced by metals to form salts of benzimidazoles. It was also shown that silver and mercury salts were formed with 2-methoxymethylbenzimidazole, 2-phenoxymethylbenzimidazole and 2-ethoxymethylbenzimidazole. However, 1-benzylbenzimidazole, 1,6-dimethylbenzimidazole and 1-benzylbenzimidazole do not form salts with silver, zinc, cobalt cadmium and copper because of no hydrogen at the first position. But, 1-1-phenylbenzimidazole and 1-tolylbenzimidazole salts were reported with mercurous chloride.

For example, the coordination of 2-aminomethylbenzimidazole gives the complex below:

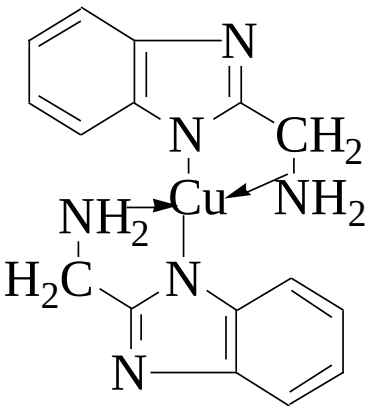
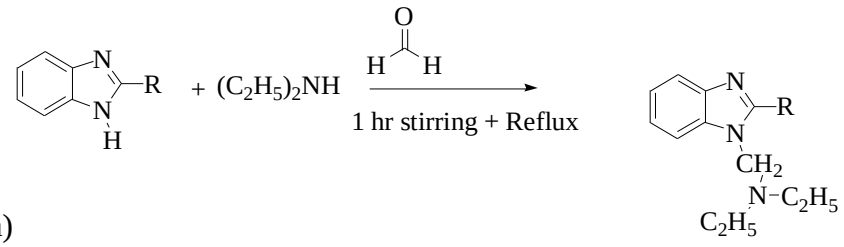


Figure 2.8: Reaction of copper with benzimidazole

2.7 Acid base reactions

Examples of such reaction is the reaction of benzimidazoles with bases such as primary or secondary amine, and formaldehyde as shown below (Rehman et al., 2013).



a)

Figure 2.9: Reaction of 2-substituted benzimidazole with secondary amine and formaldehyde

b)

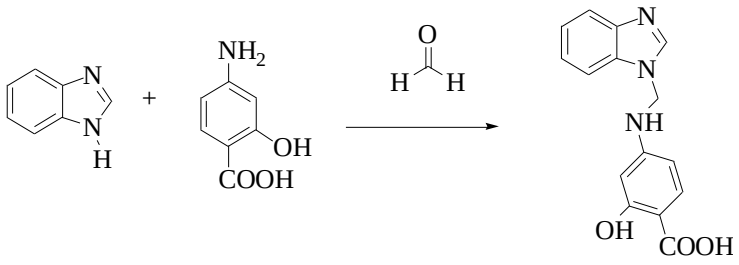


Figure 2.10: Reaction of 4-amino salicylic acid with benzimidazole and formaldehyde

c)

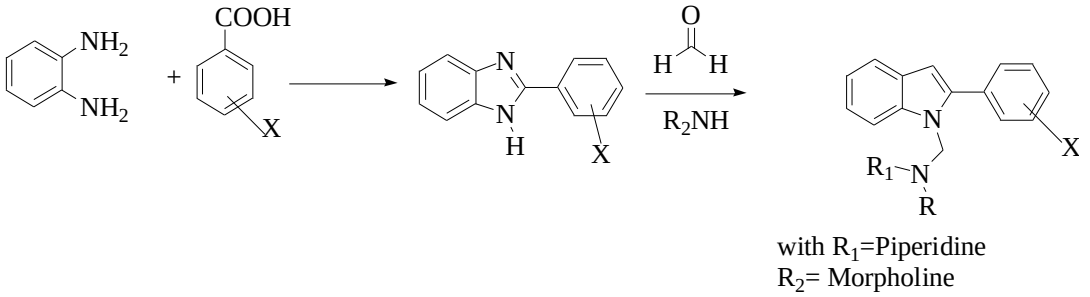


Figure 2.11: Reaction of 2-phenylbenzimidazole with formaldehyde and secondary amine

d)

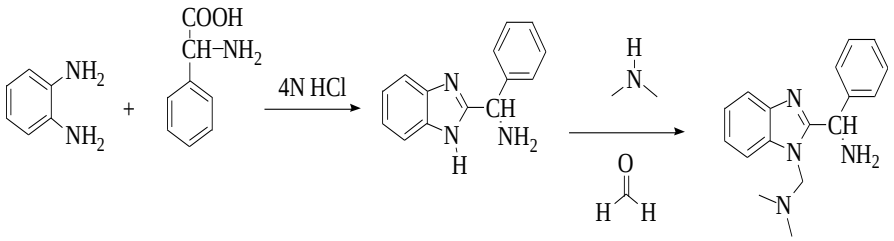


Figure 2.12: Synthesis of 1,2-Disubstituted Mannich base

Apart from this reaction, the benzimidazoles may undergo cleavage.

2.8 Cleavage of the Benzimidazoles

The imidazole ring of benzimidazoles may be cleaved by one of the following methods:

a) Aroyl halides in the presence of water

b) Reactions involving ‘’pseudo bases’’

c) By treatment of 1-benzimidazoylmagnesium with aroyl chlorides

d) By treatment with acid anhydrides.

For example, Wright (1951) showed in a cleavage reaction with ‘’Pseudo base’’ that, in the presence of sodium nitrite ($NaNO_2$) in diluted sulfuric acid, 2-Hydroxy-1,2,3,5-tetramethyl-2,3-dihydrobenzimidazolegave gave 3-acetylmethylamino-4-methylnitrosoaminotoluene as illustrated in figure (PRESTON, 1974).

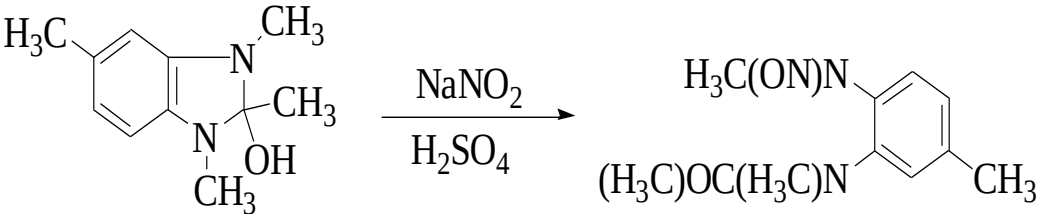
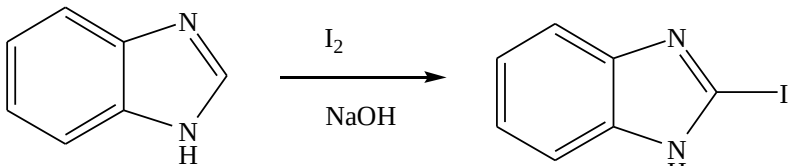


Figure 2.13: Cleavage of benzimidazole by ''pseudo base''

2.9 Halogenation of Benzimidazole

In the presence of sodium hydroxide, treatment of benzimidazole in aqueous solution with the theoretical amount of iodine(N/10) gives a quantitative yield of 2-iodobenzimidazole. (Wright, 1951).



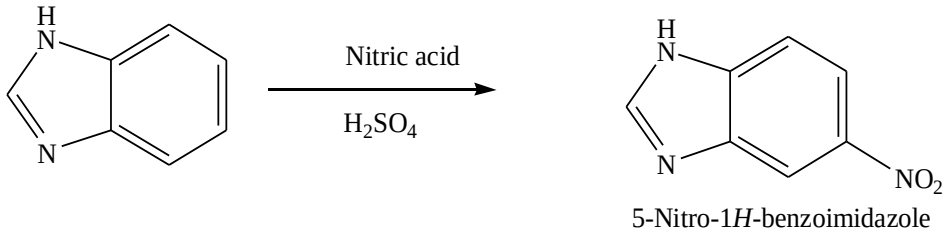
Benzimidazole

2-Iodo-1*H*-benzoimidazole

Figure 2.14: Halogenation reaction of benzimidazole

2.10 Nitration

The process of this reaction with benzimidazoles is not difficult. Nitration appears at the 5- or 6-position in most cases. However, if it is blocked, the nitro group may appear at position 4- or 7- of the benzimidazoles.



5-Nitro-1*H*-benzoimidazole

Figure 2.15: Nitration of benzimidazole

2.11. Mannich Reaction

According to Aditya, (2011), the hydrogen atom of the benzimidazole is quite active to participate in this reaction. It is a reaction which plays an important role in pharmaceutical chemistry because the beta-carbonyl compounds can be easily reduced to amino alcohols which has pharmacological effect.

In this reaction, three molecules, an amine, non-enolizable carbonyl compound like formaldehyde and an enolizable carbonyl also called alpha-CH acidic compound condense to furnish a beta-aminocarbonyl compound, also known as Mannich bases.

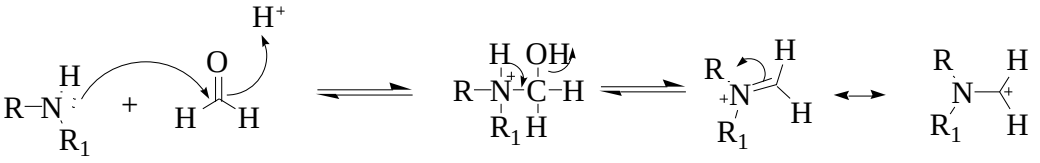
Since the reaction is carried out under acidic condition, the enolizable carbonyl compound is converted to enol form, which then attack the iminium ion at positively charged carbon adjacent to nitrogen to give the final beta-aminocarbonyl compound.

It is important to note that the aromatic amine do not undergo Mannich reaction.

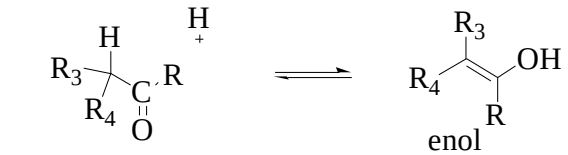
Examples of alpha-CH acidic compounds include indole, thiophene, pyrrole, furan, which are electron- rich heterocycles; activated phenyl group, alpha-alkylpyridines or imines, aliphatic nitro compounds, carbonyl compounds, nitriles.

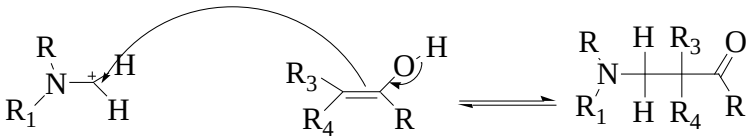
The mechanism is given as follow:

step1: Formation of iminium ion



Step 2: convertion of the enolizable cabonyl to enol



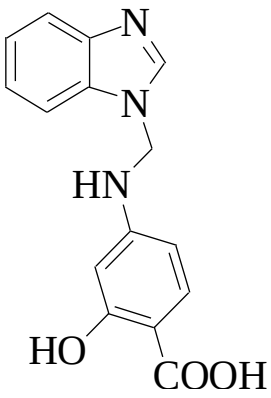
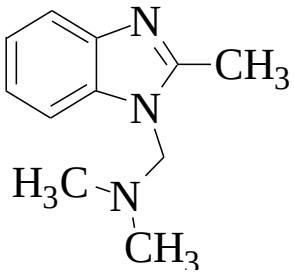


Step 3: Attack of the iminium ion by the enol to give b-aminocarbonyl compound

Figure 2.16: Mannich reaction mechanism

2.11.1 Some examples of Mannich bases

The following table shows some examples of Mannich bases that were synthesized and proven to possess biological activity (Vinoth et al., 2013)

Structures	IUPAC Name	Use
	4-[(Benzimidazol-1-ylmethyl)-amino]-2-hydroxy-benzoic acid	Antimicrobial
	N, N-Dimethyl-1-(2-methyl-1H-benzimidzol-1-yl) methanamine	Antimicrobial

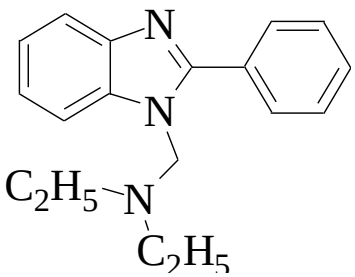
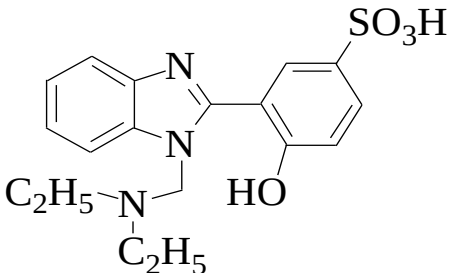
	N-Ethyl-N-[(2-phenyl-1H-benzimidazol-1-yl) methyl] ethanamine	Antimicrobial
	3-{1-[(Diethylamino) methyl] -1H-benzimidazol-2-yl}-4-hydroxy benzene sulfonic acid	Antifungal

Table 1: Mannich bases

2.12 Laboratory synthesis of benzimidazole

According to Wagner and Miller, (1939), the equation of benzimidazole synthesis is given as follow:

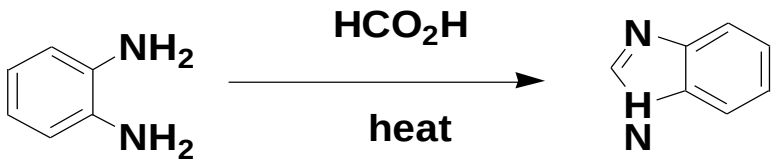


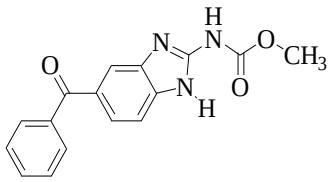
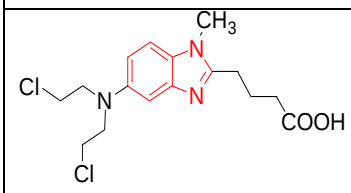
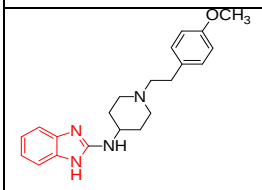
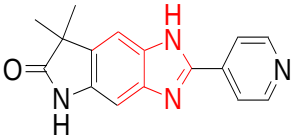
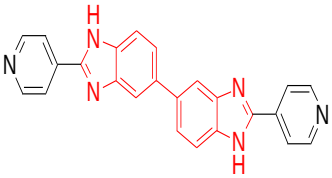
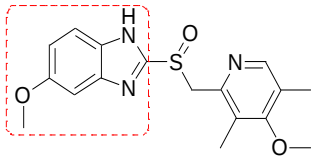
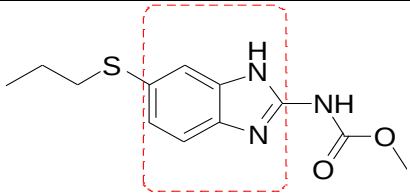
Figure 2.17: Synthesis of benzimidazole

2.13. Pharmacological Activities of Benzimidazoles

The prominent biological activity of benzimidazoles and its derivatives has made them to be an important building blocks in the pharmaceutical industry (Daw et al., 2017). The fact that we can place substituents on various position of benzimidazole nucleus has led to a variety of bioactivities as shown on table 2 below. Another important feature of the benzimidazole moiety that makes it to be favored is its structural similarity with naturally occurring nucleotides, which allows it to easily recognize human body biopolymers such as proteins, enzymes, and receptors (PRESTON, 1974). Also, many pharmaceutical chemists have caried out many research on benzimidazoles derivatives in order to investigate its biological activities. In medicinal chemistry, the pharmacophore has been thoroughly investigated and proven to have applications in a wide range of therapeutic and clinical areas (Rashid, 2011). Furthermore, the benzimidazole skeleton is an important pharmacophore in modern drug discovery and

its derivatives are important bioactive molecules. Drugs such as mebendazole and thiabendazole are being currently used as anthelmintic and antifungal (Li et al., 2019)

Table 2: Clinical used drugs with benzimidazole moiety

Drug structure	Name	Use
	**Mebendazole**	Worm infections
	**Bendamustine**	For the treatment of cancer
	**Astemizole**	For the treatment of allergic rhinitis
	**Adibendan**	For the treatment of patients with severe congestive heart failure (Voelker et al., n.d.)
	**Ridinazole**	For the treatment of bacterial infections
	**Omeprazole**	Gastric ulcer
	**Albendazole**	Anthelmintic

Source: (Salahuddin et al., 2017)

Their therapeutic action include: antitumor (Hameed et al., 2019)antimicrobial, anthelmintics, proton pump inhibitors, anti-inflammatory, and anti-hypertensive drugs. Benzimidazole derivatives have stimulated considerable interest in the medical domain. Among the various compounds developed as anti-inflammatory and analgesic agents, 2-substituted benzimidazoles and N-Mannich bases of various heterocyclic compounds have been shown to have anti-inflammatory and analgesic properties (Jesudason et al.,2009). Also, a large number of benzimidazole compounds have been employed as candidates for the treatment of various types of diseases or as clinical drugs. Such include anticancer agents (Pracinostat, Bendamustine), antihistamine agents (Astemizole)(Richards,1984), anthelmintic agents (Albendazole, Mebendazole) antibacterial agents (Ridinazole), antihypertensive agents (Candesartan), proton pump inhibitors (Pantoprazole, omeprazole), antiviral agents (Samatasvir) and phosphodiesterase inhibitors (Adibendan).

Other researchers discovered that molecules with low polarity have an advantage for antimicrobial activity. Additionally, the presence of functional groups, such as benzimidazole esters as antifungal agents and amino acids for antimicrobial activity, is associated with antimicrobial activity (Marinescu et al., 2020).

2.13.1. Antimicrobial Structural Activity Relationship of benzimidazoles

According to Sahoo et al., (2019), structure activity relationship (SAR) is the relationship between the chemical or 3D structure of a drug molecule and its biological activity. It is based on the alteration of the drug structure in order to modify its activity or increase its potency.

A drug is said to have antimicrobial activity when it is capable of inhibiting the growth of microorganisms such as bacteria, protozoa, Helminthes, fungi and viruses. It was after the year 2000 that researchers developed antimicrobials drugs from benzimidazole nucleus. In order to produce potent antifungal and antibacterial drugs. For instance, they coupled 2-alkylthiobenzimidazole with β-lactam ring.

In addition, from the compounds produced from the benzimidazole, they found that the most potent ones were the compounds with substitution on both 1 and 2-position.

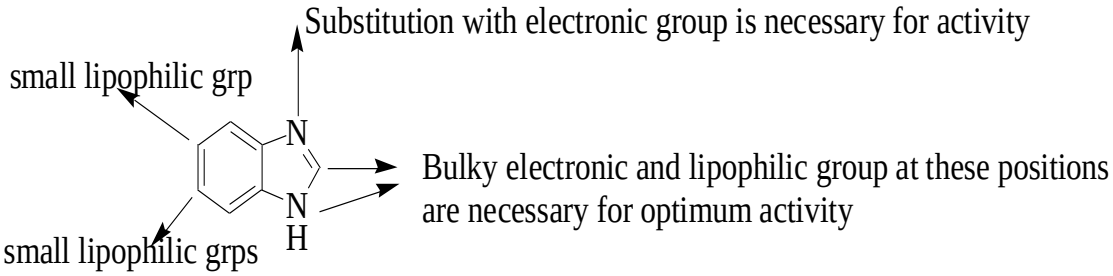


Figure 2.18: Antimicrobial SAR of benzimidazole

Some small electronic groups include halogens, nitro, amino, methyl, and arylalkyl groups (Singh & Silakari, 2018b)

3.Materials and Methods

3.1. Materials

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.

The attenuated reflection of the synthesized molecule was examined from Infrared spectroscopy using a spectrophotometer, Perkin Elmer Spectrum 100 shown in wave numbers (cm^{-1}). The proton nuclear magnetic resonance spectrum of each molecules was examined on NMR device of Mercury Varian 400 MHz where tetramethylsilane was used as a standard solution.

As a solvent, deuterated chloroform and dimethylsulfoxide ($CDCl_3$) were suitable solvents for analysis. Values of different types of protons on the structure was measured in parts per million (ppm) as chemical shifts (δ) . For the purification of compounds, thin layer chromatography method was used on silica gel GF 254 (DC-Alufplien-Kieselgel, Germany)

3.2. Synthesis of 1-(4-phenyl-piperazin-1-ylmethyl)-1H-benzimidazole

500 mg (0.004 mol) of 1H-benzimidazole and 690 mg (0.004 mol) of 1-phenylpiperazine were dissolved in 12 mL of ethanol. 0.3 mL of 35% (w/v) formalin solution was dissolved in 3 ml of ethanol before adding into the benzimidazole solution. The resulted mixture was then refluxed in a water bath for 3 hours. After completion, the mixture was poured into crushed ice upon which a precipitate was formed. The resulting solid was filtered using vacuum filtration method. The reaction

was monitored by TLC and the resulting white precipitate was purified by recrystallization with acetonitrile.

3.3. Spectroscopy

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 deuterated chloroform ($CDCl_3$) was used as solvent for analysis.

4.Results

4.1. Synthesis scheme

The new benzimidazole derivative was synthesized according to the scheme given below:

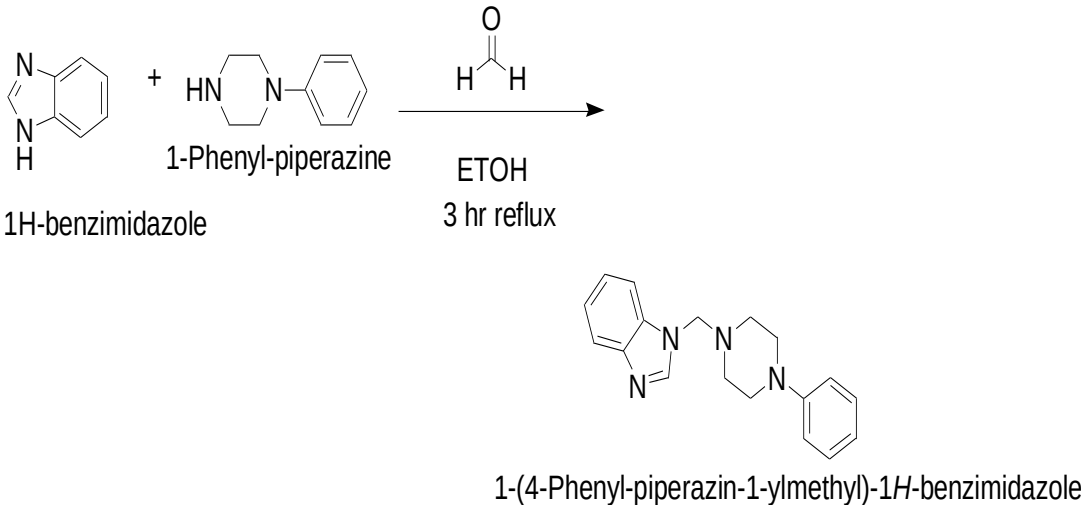


Figure 4.1: Synthesis scheme of 1-(4-phenyl-piperazin-1-ylmethyl)-1H-benzimidazole

4.2. Chemical information of the synthesized compound

IUPAC Name: 1-[(4-phenyl) piperazin-1-methyl]-1H-benzimidazole

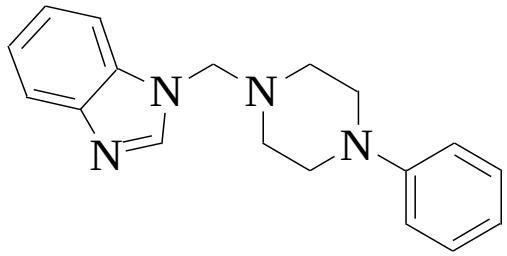


Figure 4.2.: Chemical structure of the synthesized compound

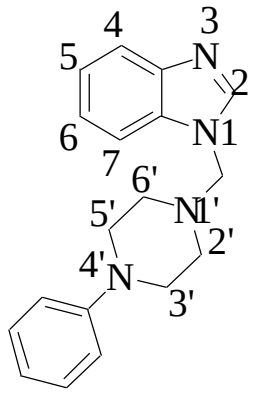


Figure 4.3.: Hydrogen numbering in the synthesized compound

Yield (%) : 65%

For TLC (Thin Layer Chromatography)

Stationary Phase: Silica gel GF 254

Mobile Phases: 2 were used

1): Hexane(3): Methanol (1) with Rf_1= 0.37

2): Hexane(3): Ethylacetate(5) with Rf_2= 0.1

Spot Detection: Under UV lamp at 254 nm

Retention Factors (Rf): Rf_1= 0.37

Rf_2= 0.1

Physical Appearance: white crystalline powder.

Solubility: Completely soluble in chloroform

Melting Point : 164°C

Molecular Formula: $C_{18}H_{20}N_4$

Molecular Weight (g/mol): 292

^{1}H NMR

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.8-7.8 (m, 9 H, Ar-CH), 4.9(s, 2 H, CH_2), 3.2 (t, 4 H , pip-CH_2 $H^{3'}$, $H^{5'}$), 2.7 (t, 4 H, pip-CH_2, $H^{2'}$, $H^{6'}$), 8 (s, H^2, Benzimidazole-H).

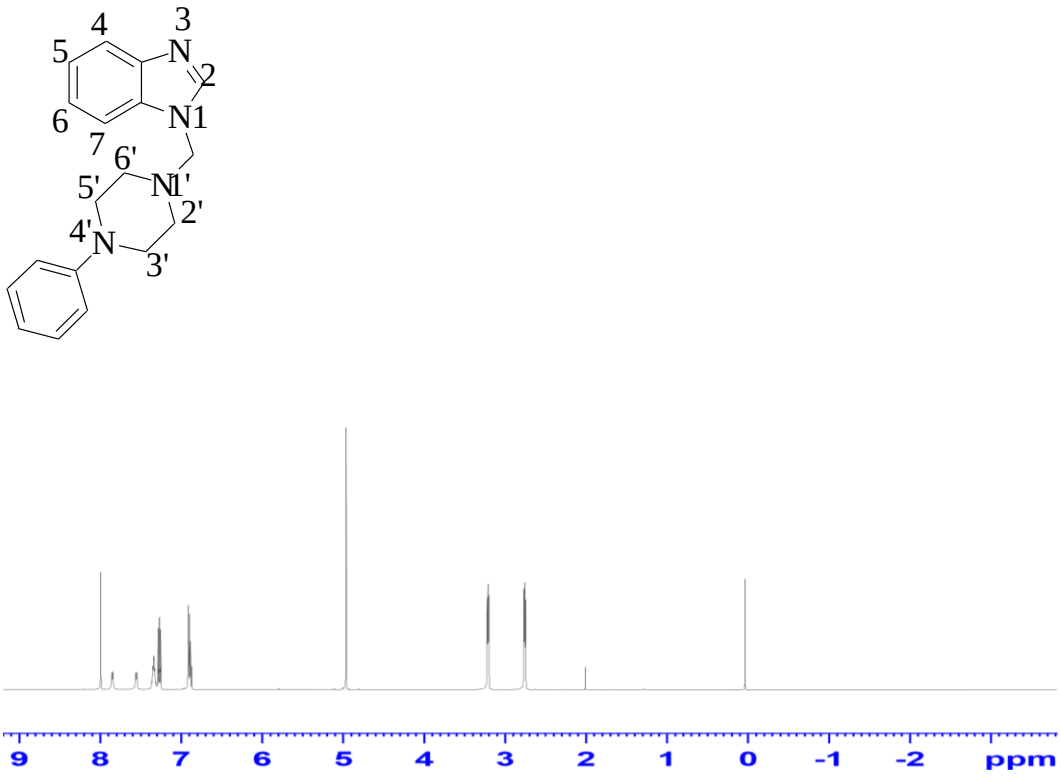
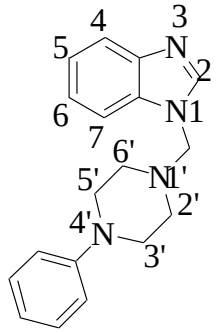


Figure 4.4.: H NMR of the synthesized compound

FT-IR Infrared Spectrum

ν_{max} (KBr, cm^{-1}) : 3056 (Aromatic C-H)**,** 2890-2946 (due to -CH_2- of the molecule), 1600(C=C)



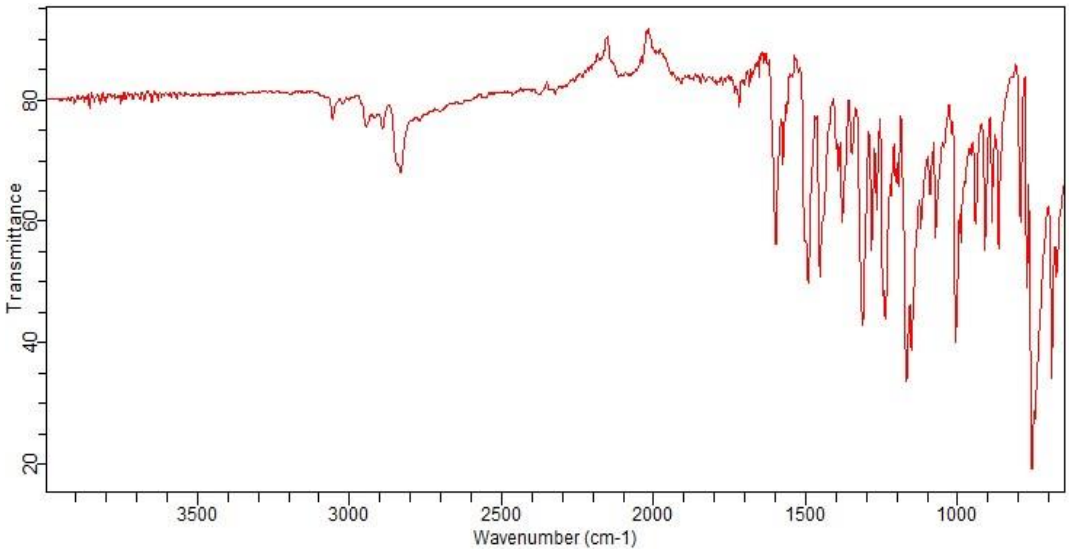


Figure 4.5.: FT-IR of the synthesized compound

5.Discussion

The 1-substituted benzimidazole derivative synthesis was via Mannich reaction as shown in the figure (5.1) bellow. In this reaction, 1H-benzimidazole, 1-phenylpiperazine in equal moles and formaldehyde were condensed to furnish the benzimidazole derivative. The reaction was done in 3 hours.

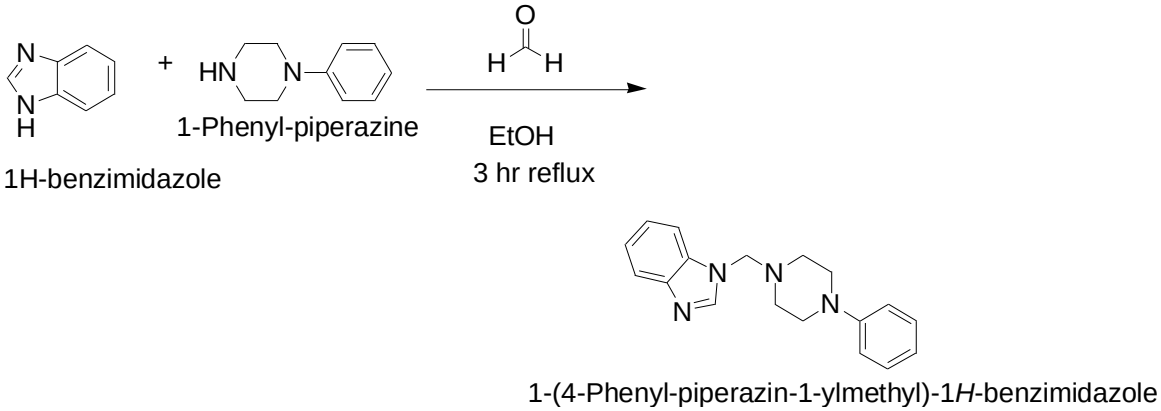


Figure 5.1.: Synthesis scheme of the compound

The yield was quite good as we can see in the table below with melting point as well. The chemical reaction carried out under reflux was completed after 3 hours of heating. The benzimidazole Mannich base derivative showed the IR absorption band in the region of 3056 cm-1, 2890-2946 cm-1 and 1600 corresponding to CH arenes, CH aliphatic due to the -CH_{2}- bridge and C=C respectively.

We could see that the N-H absorption band disappeared and that was as a result of reaction between the 1 Hydrogen of benzimidazole with 1-phenylpiperazine and formaldehyde. Therefore, the observed band at 2890-2946 attributed to aliphatic C-H.

For the proton Nuclear Magnetic Resonance Spectroscopy (^{1} H-NMR, $CDCl_3$; ppm), the signal observed at δ 8 ppm was for the hydrogen numbered H^2 and its high value was due to it being positioned between two heteroatoms with one carrying unsaturated bond.

Another signal at δ 4.9 ppm was due to the presence of methylene(-CH_2-), numbered $H^{1'}$ confirming the proposed structure.

Multiplicity at δ 6.8-7.8 ppm was due to the aromatic protons, whereas the triplets at 2.7 and 3.2 ppm was the result of the piperazine hydrogens.

Table 3: Melting point and yield of the synthesized compound

Yield (%)	**Melting point (^{0} C)**
65	164

6.Conclusion

Our 1-substituted benzimidazole Mannich base derivative was synthesized via the Mannich reaction using a method commonly called conventional method and the structure was confirmed by spectroscopy methods; H NMR and FT-IR.

The biological activity was not done because of time constraint. Moreover, the molecule is not soluble in DMSO and ethanol, and because of that, the cytotoxicity could not be studied too.

In the future, we are going to explore it in order to improve on the solubility and also study the cytotoxicity.

This type of molecule has been proven in previous literature to have antimicrobial activity which implies that this property would be studied by making its derivatives.

References

Daw, P., Ben-David, Y., & Milstein, D. (2017). Direct Synthesis of Benzimidazoles by Dehydrogenative Coupling of Aromatic Diamines and Alcohols Catalyzed by Cobalt. *ACS Catalysis*, *7*(11), 7456–7460. https://doi.org/10.1021/acscatal.7b02777

Gul, H. I., Tugrak, M., Gul, M., Mazlumoglu, S., Sakagami, H., Gulcin, I., & Supuran, C. T. (2019). New phenolic Mannich bases with piperazines and their bioactivities. *Bioorganic Chemistry*, *90*. https://doi.org/10.1016/j.bioorg.2019.103057

Hameed, A., Hameed, A., Farooq, T., Noreen, R., Javed, S., Batool, S., Ahmad, A., Gulzar, T., & Ahmad, M. (2019). Evaluation of structurally different benzimidazoles as priming agents, plant defense activators and growth enhancers in wheat. *BMC Chemistry*, *13*(3). https://doi.org/10.1186/s13065-019-0546-2

Li, G., He, R., Liu, Q., Wang, Z., Liu, Y., & Wang, Q. (2019). Formation of Amidinyl Radicals via Visible-Light-Promoted Reduction of N-Phenyl Amidoxime Esters and Application to the Synthesis of 2-Substituted Benzimidazoles. *Journal of Organic Chemistry*, *84*(13), 8646–8660. https://doi.org/10.1021/acs.joc.9b01158

Marinescu, M., Cinteză, L. O., Marton, G. I., Chifiriuc, M. C., Popa, M., Stănculescu, I., Zălaru, C. M., & Stavarache, C. E. (2020). Synthesis, density functional theory study and in vitro antimicrobial evaluation of new benzimidazole Mannich bases. *BMC Chemistry*, *14*(1). https://doi.org/10.1186/s13065-020-00697-z

Rashid, M. (2011). Benzimidazole: A Valuable Insight into The Recent Advances and Biological Activities. *Journal of Pharmacy Research*, *4*(2), 413–419. http://jprsolutions.info

Sahoo, B. M., Banik, B. K., Mazaharunnisa, Rao, N. S., & Raju, B. (2019). Microwave Assisted Green Synthesis of Benzimidazole Derivatives and Evaluation of Their Anticonvulsant Activity. *Current Microwave Chemistry*, *6*(1), 23–29. https://doi.org/10.2174/2213335606666190429124745

Salahuddin, Shaharyar, M., & Mazumder, A. (2017). Benzimidazoles: A biologically active compounds. In *Arabian Journal of Chemistry* (Vol. 10, pp. S157–S173). Elsevier B.V. https://doi.org/10.1016/j.arabjc.2012.07.017

Singh, P. K., & Silakari, O. (2018a). Benzimidazole. In *Key Heterocycle Cores for Designing Multitargeting Molecules* (pp. 31–52). Elsevier. https://doi.org/10.1016/b978-0-08-102083-8.00002-9

Singh, P. K., & Silakari, O. (2018b). Benzimidazole. In *Key Heterocycle Cores for Designing Multitargeting Molecules* (pp. 31–52). Elsevier. https://doi.org/10.1016/b978-0-08-102083-8.00002-9

Vinoth Kumar, S., Subramanian, M. R., & Chinnaiyan, S. K. (2013). Synthesis, characterization and evaluation of N-mannich bases of 2-substituted Benzimidazole derivatives. *Journal of Young Pharmacists*, *5*(4), 154–159. https://doi.org/10.1016/j.jyp.2013.11.004

Voelker, W., Mauser, M., Preisack, M., Med, C., & Karsch, K. R. (n.d.). *Acute Hemodynamic Effects of Adibendan, a New Phosphodiesterase Inhibitor, for Severe Congestive Heart Failure*.

Aditya. (2011). *Mannich reaction*. https://www.adichemistry.com/organic/namedreactions/mannich/mannich-reaction-1.html. https://www.adichemistry.com/organic/namedreactions/mannich/mannich-reaction-1.html

Cennett Arslaner, Sedar Karakurt, Ziya Erdem Koc. (2017). Synthesis of benzimidazole Schiff base derivatives and cytotoxic effects on colon and cervix cancer cell lines. *Synthesis of benzimidazole Schiff base derivatives and cytotoxic effects on colon and cervix cancer cell lines*, *7*(4). https://www.researchgate.net/publication/320614151

Jesudason, E. P., Sridhar, S. K., Malar, E. J., Shanmugapandiyan, P., Inayathullah, M., Arul, V., Selvaraj, D., & Jayakumar, R. (2009). ChemInform abstract: Synthesis, pharmacological screening, quantum chemical and in vitro permeability studies of N-Mannich bases of Benzimidazoles through bovine cornea. *ChemInform*, *40*(33). https://doi.org/10.1002/chin.200933132

P. N. PRESTON. (1974). *Synthesis, Reactions, and Properties of Benzimidazoles*.

REDDY ANIL B. (2010). Synthesis, Characterization and Biological Evaluation of 1,2-Disubstituted Benzimidazole Derivatives using Mannich Bases. *E-Journal of Chemistry*, *7*(1), 222-226.

Reham, et al. (2013). Mannich Base Derivatives of Benzimidazole: Synthesis and Antimicrobial Properties- A Short Review. *@2013 WAP journal*, *3*(12).

T. M. Richards, R. N. Brogden, R. C. Heel, T. M. Speight, G.S. Avery. (1984). *Astemizole A Review of its Pharmacodynamic Properties and Therapeutic Efficacy* (pp. 38-61). ADIS press Limited.

Murugesan Sugumaran, Sathiyamoorthy Sivaderi. (2011). Synthesis, Spectral analysis and Biological activity of Mannich Bases of 2-Substituted benzimidazole. *Synthesis, Spectral analysis and Biological activity of Mannich Bases of 2-Substituted benzimidazole*, *4*(8).

Singh, Silakari. (2018). *Benzimidazole: Journey from Single Targeting to Multitargeting Molecule*. Elsevier. https://doi.org/10.1016/B978-0-08-102083-8.00002-9

Wagner and Millett. (1939). *Organic Syntheses*. Coll. vol 2,501. https://doi.org/10.15227/orgsyn.019.0012

Wright B. John. (1951). *THE CHEMISTRY OF BENZIMIDAZOLES*. The upjohn company.