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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF BENZIMIDAZOLE DERIVATIVES AND ITS NITRATED DERIVATIVES

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ABSTRCT:

Benzimidazoles are a significant class of chemicals that exhibit a variety of biological activities, including anthelmintic, antiviral, antifungal, antihypertensive, and antitumor properties. The most significant nitrogen-containing heterocycles that are extensively researched and used by the pharmaceutical industry for drug discovery are benzimidazole rings. Owing to their unique structural characteristics and electron-rich surroundings, medications containing benzimidazoles bind to a range of therapeutic targets and display a wide range of biological activities. Many medications with benzimidazole bases have been widely employed in clinical settings to treat a wide range of illnesses with promising treatment outcomes. o-phenylenediamine and derivatives of benzoic acid were reacted to create a series of benzimidazole derivatives in a single step. IR were used to verify the synthesized compounds purity and structural integrity. The compounds antifungal activity was assessed.

KEYWORDS: Benzimidazole, Antifungal activity, IR – Spectroscopy.

INTRODUCTION:

One type of heterocyclic aromatic organic compound is benzomidazole. In medicinal chemistry, it is a privileged structure and a significant pharmacophore. This compound is bicyclic in nature, arising from the combination of imidazole and benzene. There is a popular variety these days that has numerous pharmacological qualities. N-ribosyl-dimethylbenzimidazole is the most prominent benzimidazole compound found in nature. It functions as an axial ligand for cobalt in vitamin B12[1]. Hobrecker created the first derivative of benzimidazole in 1872[2]. The 1943 study by Goodman and Nancy Hart on the pharmacological characteristics of benzimidazole. Then, in 1944, Woolley reported on the antibacterial properties of certain benzimidazole derivatives. In 1949, Norman GB and Karl Folker reported 5, 6-dimethyl benzimidazole as a degradation product following the acid hydrolysis of vitamin B-12[3].

A heterocyclic ring with five members that contains nitrogen can be found in the structures of many synthetic compounds that are biologically active [4]. It has been stated that structural frameworks are privileged structures, and that N-containing polycyclic structures in particular are linked to a variety of biological activities. The great therapeutic potential of medications related to benzimidazoles has inspired medicinal chemists to create a wide range of innovative chemotherapeutic agents [5-7].

Due to its widespread presence in a variety of bioactive substances, including antiparasitics, anticonvulsants, analgesics, antihistaminics, antiulcers, antihypertensives, antiviral, anticancer, antifungals, anti-inflammatory agents, proton pump inhibitors, and anticoagulants, benzomidazole has developed into an important heterocyclic system over the course of many years of research[8-14]

Benzimidazole medications are frequently used to treat and prevent parasitic infections. Well-known benzimidazole medications include omeprazole,rabeprazole, lansoprazole, pantoprazole, and esomeprazole. More than 40 years ago, thiabendazole (TBZ) was the first benzimidazole to be marked. Following its launch, a number of benzimidazoles with comparable activity—such as parbendazole (PAR), cambendazole (CAM), mebendazole (MBZ) , and oxibendazole (OXI)—entered the market[15].

Benzimidazole nucleus has been used for therapeutic purposes since 1944. It functions similarly to purines in evoking certain optimal biological responses. Brink et al.[16].

EXPERIMENTAL:

MATERIALS AND CHEMICALS:

In round-bottom flasks, all of the reactions were conducted in an airtight environment. Reagents, substrate, and solvents have been purchased from Hi Pure Fine Chem Industry, HI Media Laboratories Pvt. Ltd., and Sri Kailash Chemicals for use in reactions, recrystallizations, and IR spectroscopy. Spectroscopy details are provided in the Supplementary Materials.

CHEMISTRY:

GENERAL PROCEDURE FOR 2- PHENYL SUBSTITUTED BENZIMIDAZOLES:

Place 2.7 gm of ortho phenyl diamine in 250 ml of round base flagon and add 5 ml of Benzoic Acid Derivatives and reflux. After condensing the round bottom flask for two hours in a 100-degree water bath, cool the contents and gradually add 10% sodium hydroxide solution while stirring constantly until the mixture becomes alkaline.

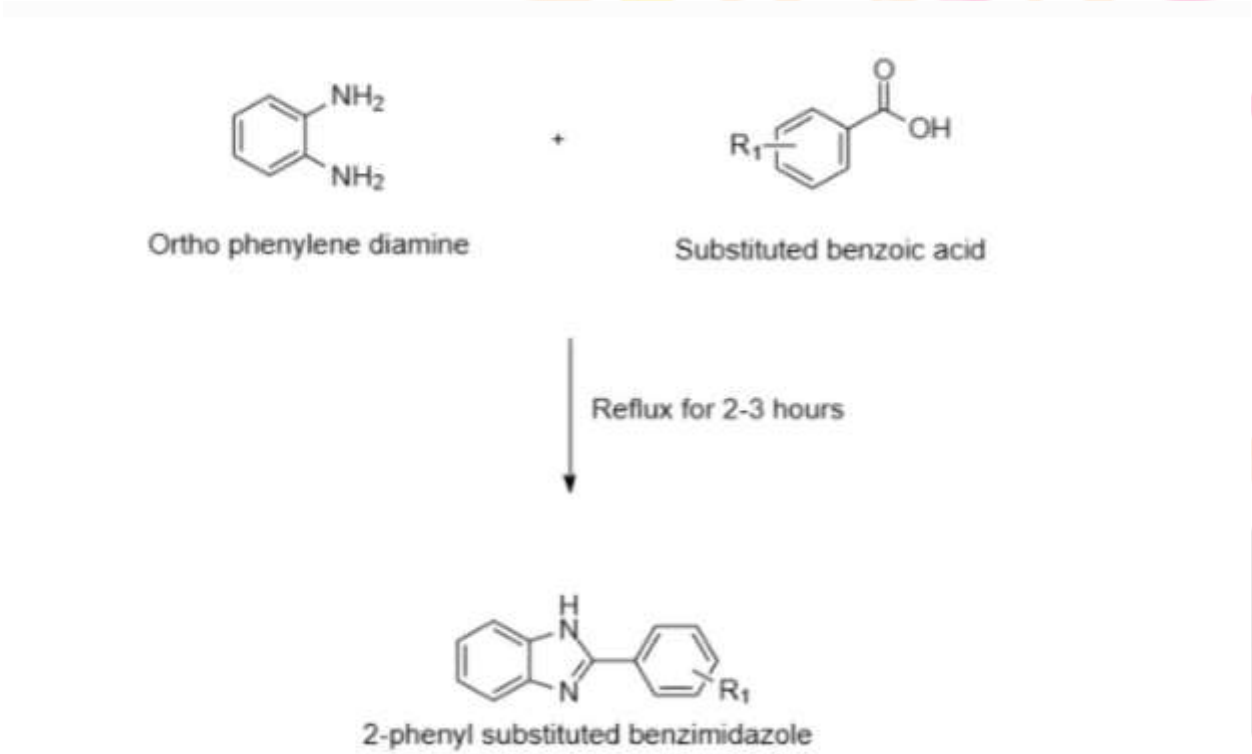
Channel the get item and wash with super cold water and channel nill break up the unrefined item in 50 ml of bubbling water and add a spot of decolourised carbon and overview it for 15 minutes . At the pump, connect the condenser filter to the heated bun funnel. cool the filtrate to over 10 degree centrigate channel off the got. Benzimidazole wash it with 25 ml of water and dry at 100 degree centrigate.

GENERAL PROCEDURE FOR NITRATION OF 2- PHENYL SUBSTITUTED BENZIMIDAZOLES:

7 ml of conc. HNO3 was set in a three necked round lined jar fitted with a mechanical stirrer. The cup was drenched in super cold water and 7.5 ml of conc. With slow stirring, H2SO4 was slowly added through the condenser. Measure of 2-subbed benzimidazole included segments during one hour at a rate, so the temperature don't surpass 35°C. After persistent blending for 30 mins at room temperature 35°C, the response combination was poured gradually over squashed ice with fiery mixing. The accelerated item was sifted at siphon, washed with cold water, depleted and gauged.

SCHEME:

GENERAL SCHEME FOR 2- PHENYL SUBSTITUTED BENZIMIDAZOLES:



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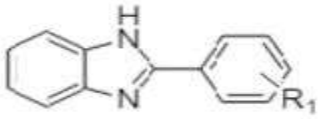
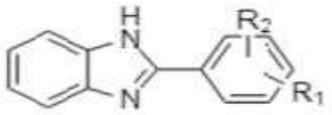
GENERAL SCHEMENITRATION OF 2- PHENYL SUBSTITUTED BENZIMIDAZOLES:



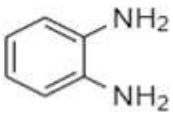
2-phenyl substituted benzimidazole

Nitration HNO3/H2SO4



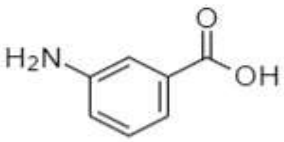
2-[nitro phenyl] substituted benzimidazole

COMPOUND I



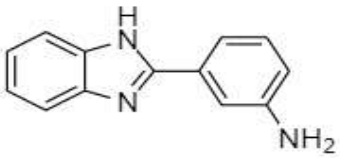
O-phenylene diamine

+



M-amino benzoic acid

Reflux for 2-3 hours



2-[5-amino phenyl] benzimidazole

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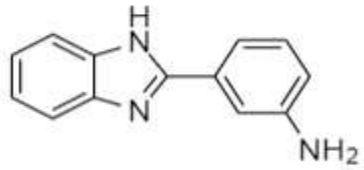
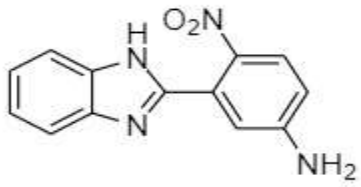
COMPOUND II



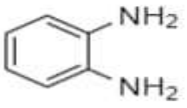
2-[5-amino phenyl] benzimidazole

Nitration | HNO_3/H_2SO_4



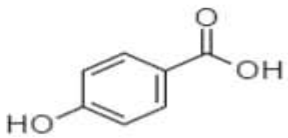
2-[5-amino-2-nitro phenyl] benzimidazole

COMPOUND III



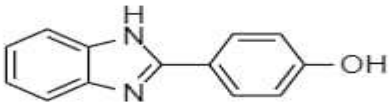
O-phenylene diamine

+



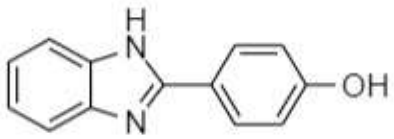
P-hydroxy benzoic acid

Reflux for 2-3 hours



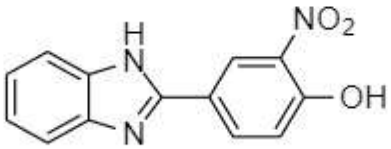
2-[4-hydroxy phenyl] benzimidazole

COMPOUND IV



2-[4-hydroxy phenyl] benzimidazole

Nitration | HNO_3/H_2SO_4

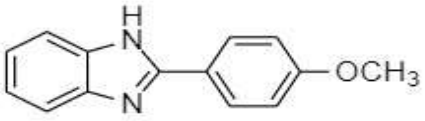


2-[4-hydroxy-3-nitro phenyl] benzimidazole

COMPOUND V

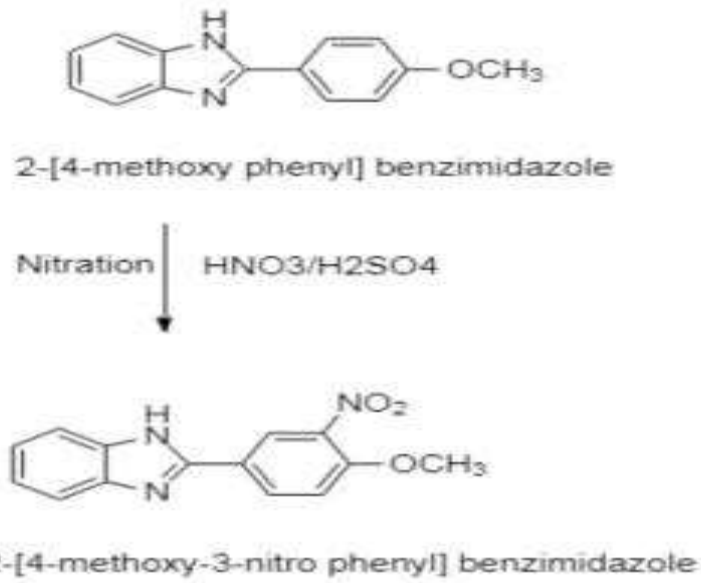
O-phenylene diamine + P-methoxy benzoic acid

Reflux for 2-3 hours



2-[4-methoxy phenyl] benzimidazole

COMPOUND VI



2-(5- Amino phenyl) Benzimidazole:

A grape color crystals yield 72%, BP 169 ºC; IR (KBr) υmax cm_1: C-Br (phenyl ring) 754; C-F (phenyl ring) 1479; N-H 3084; -CO-O- 1714; C-NH- 3310,3350; C-H 860; C=C(Arom) 1618; -CONH 1574.

2-(4- Amino -2-nitrophenyl) Benzimidazole:

A rasin color crystals, Yield 52%, BP 170 ^{O}C; IR (KBr) υmax cm_1: C-Br (phenyl ring) 771; C-F (phenyl ring) 1413; N-H 3182; -CO-O-1718; -OH-(stretching) 3300, 2500; C-H 891; C=C(Arom) 1595; -CONH 1718.

2-(4- Hydroxy phenyl) Benzimidazole:

A pink purple color crystals, Yield75%, BP 168 ºC; IR (KBr) υmax cm_1: C-Br (phenyl ring) 740; C-F (phenyl ring) 1408; N-H 3471; -CO-O- 1718; C-CH-(Stretching) 2830, 2815; C-H 864; C=C (Arom) 1616; -CONH 1654.

2-(4- Hydroxy -3-nitrophenyl) Benzimidazole:

A mulberry color crystals, yield 72%, BP 172 ^{O}C; IR (KBr) υmax cm_1: C-Br (phenyl ring) 754; C-F (phenyl ring) 1479; N-H 3084; -CO-O- 1716; C-NH- 3310,3350; C-H 863; C=C(Arom) 1618; -CONH 1576.

2-(4- Methoxy phenyl) Benzimidazole:

A lavender color crystals yield 68%, BP169ºC; IR (KBr) υmax cm_1: C-Br (phenyl ring) 742; C-F (phenyl ring) 1452; N-H 3383; -CO-O- 1722; -CH-(Stretching) 1450, 1375; C-H 964; C=C (Arom) 1616; -CONH 1543.

2-(4- Methoxy -3-nitrophenyl) Benzimidazole:

A purple color crystals, Yield75%, BP 170 ^{O}C; IR (KBr) υmax cm_1: C-Br (phenyl ring) 740; C-F (phenyl ring) 1408; N-H 3472; -CO-O- 1718; C-CH-(Stretching) 2831, 2817; C-H 864; C=C (Arom) 1618; -CONH 1654

RESULTS AND DISCUSSION:

The pharmacological uses for benzimidazole and halogen substituted benzimidazole are numerous.Every compound was synthesized in the manner shown in Scheme I. Using ethanol and methanol for recrystallization, the resultant compounds were purified.

The synthesized compounds were evaluated using FT-IR and other physical and spectral data. A review of recently created compounds' antifungal properties was conducted.

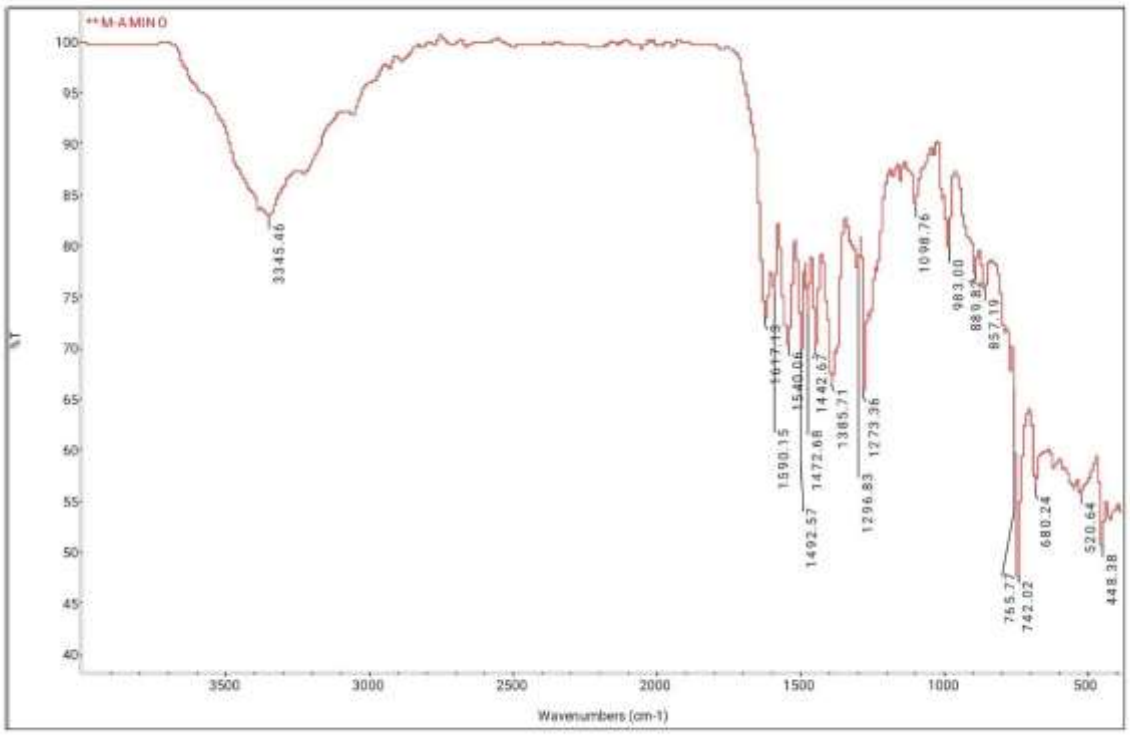
When the synthesized novel benzomidazole was characterized using spectral analysis, it was discovered that all of the benzomidazole had an IR presence of the C=N peak at about 1600 cm^{-1} determine whether the aforementioned compounds contained an additional element, IR qualitative approaches were applied.

SPECTRAL ANALYSIS:

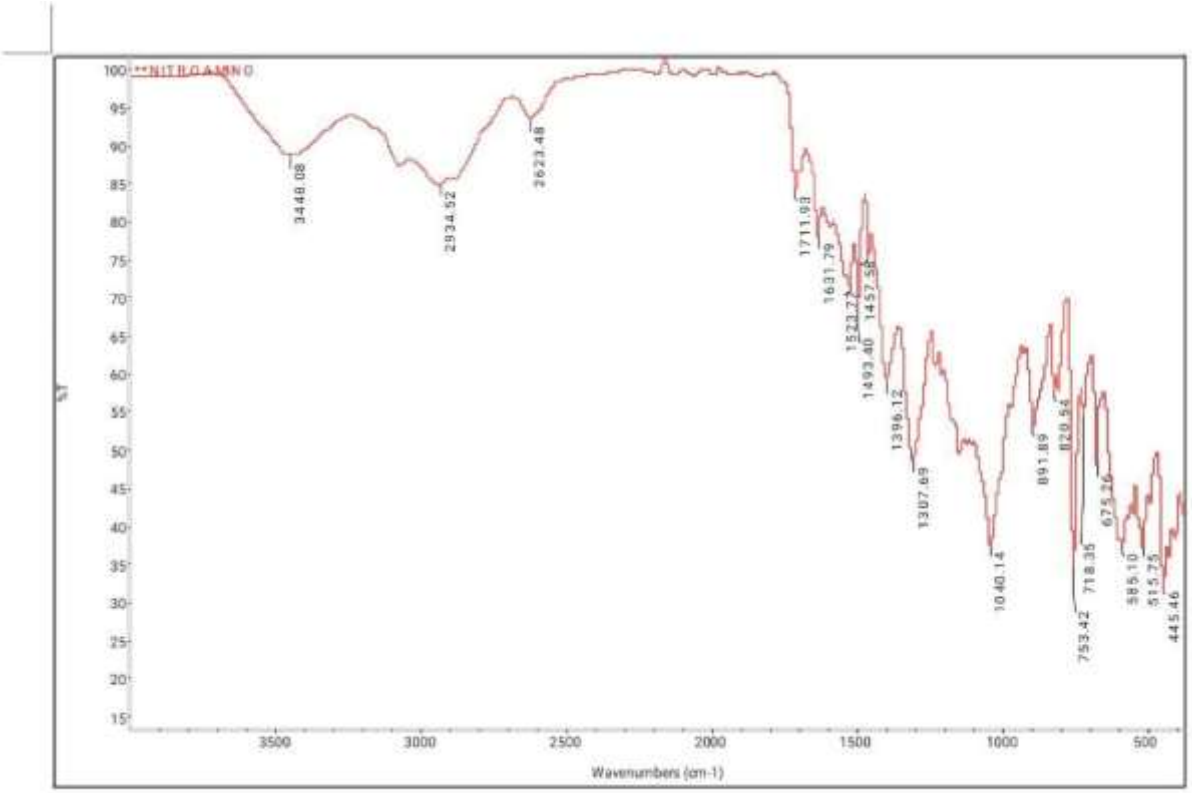
IR spectroscopy techniques were used to characterize the synthesized compounds.

INFRARED SPECTRAL ANALYSIS:

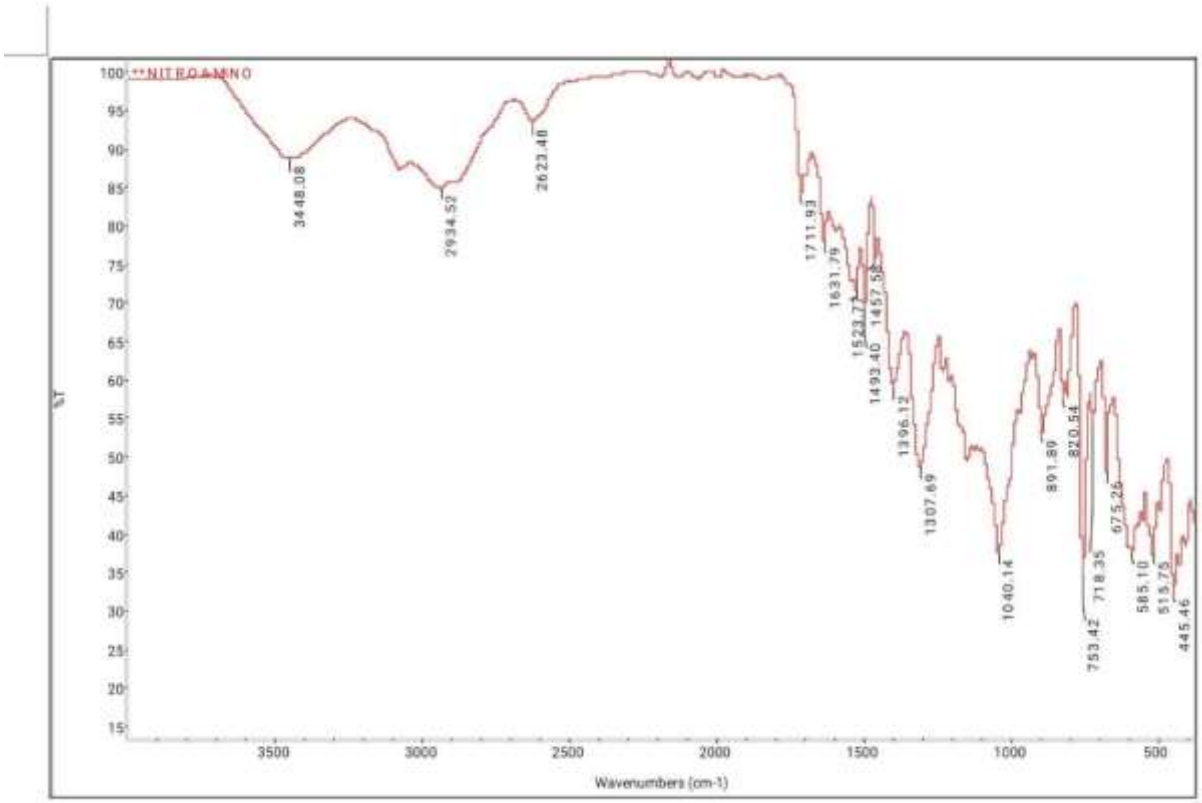
The JASCO FT-IR spectrophotometer was used to record the infrared spectrum. The table displays the results and the significant IR values expressed in cm-1.



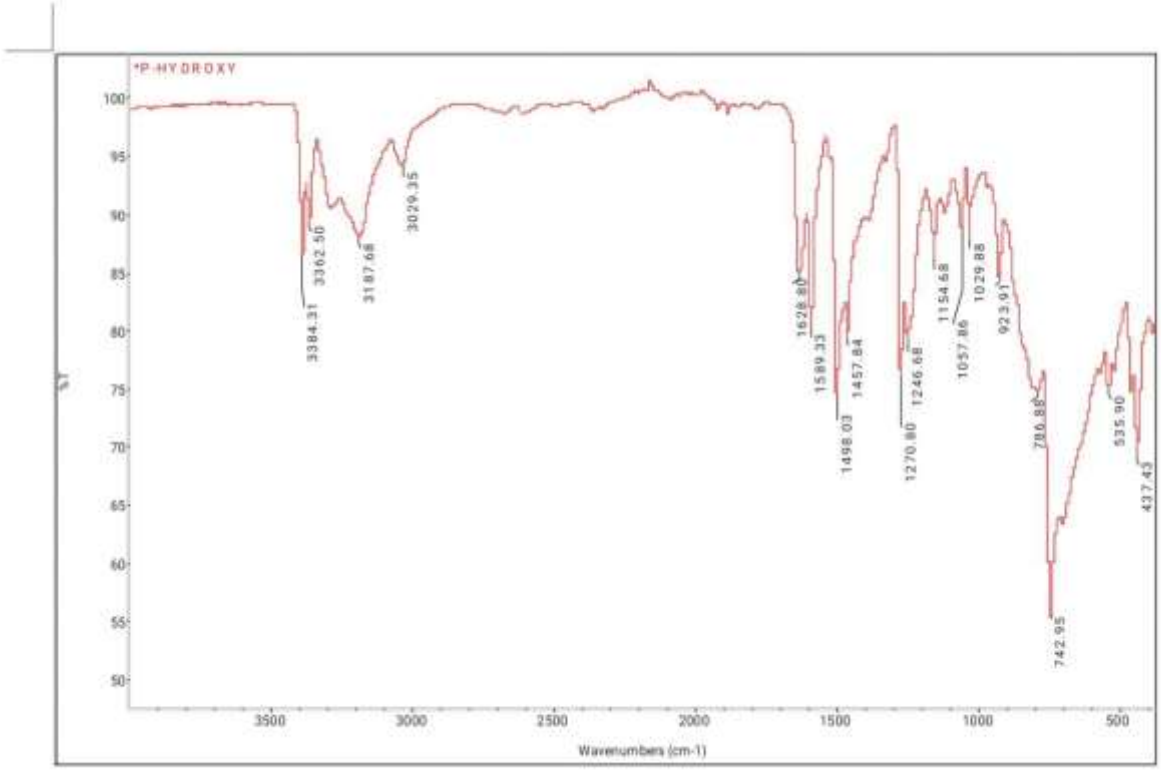
IR Spectrum of COM-1



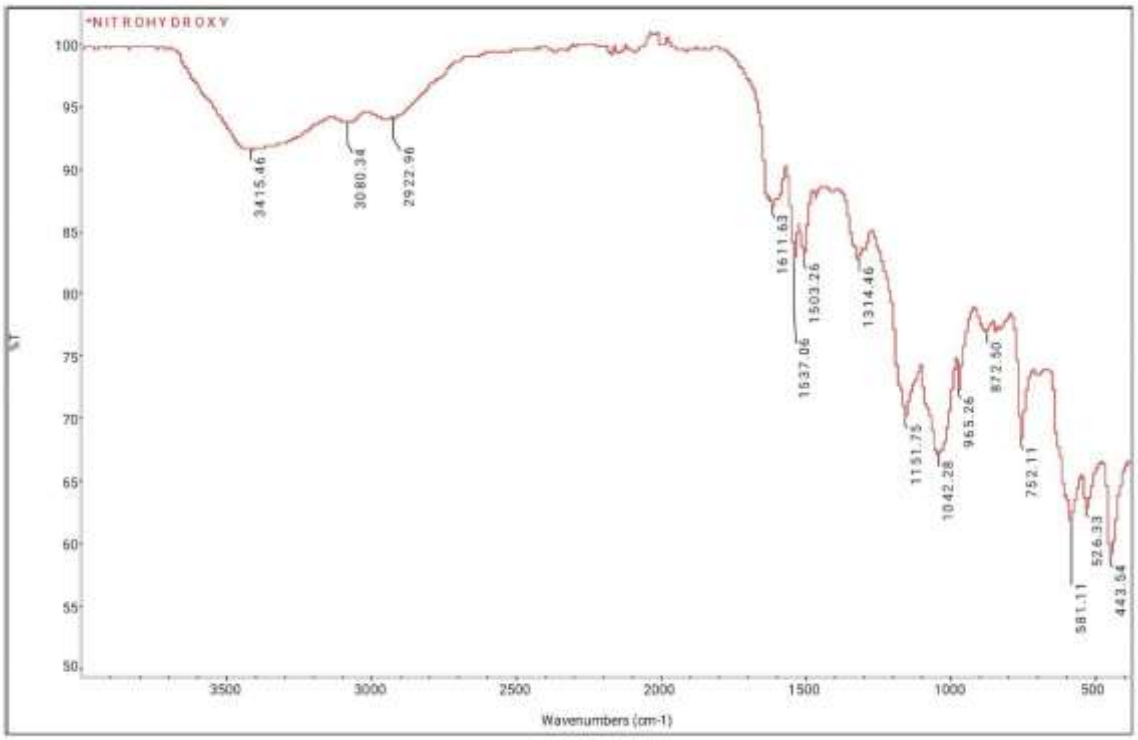
IR Spectrum of COM-2



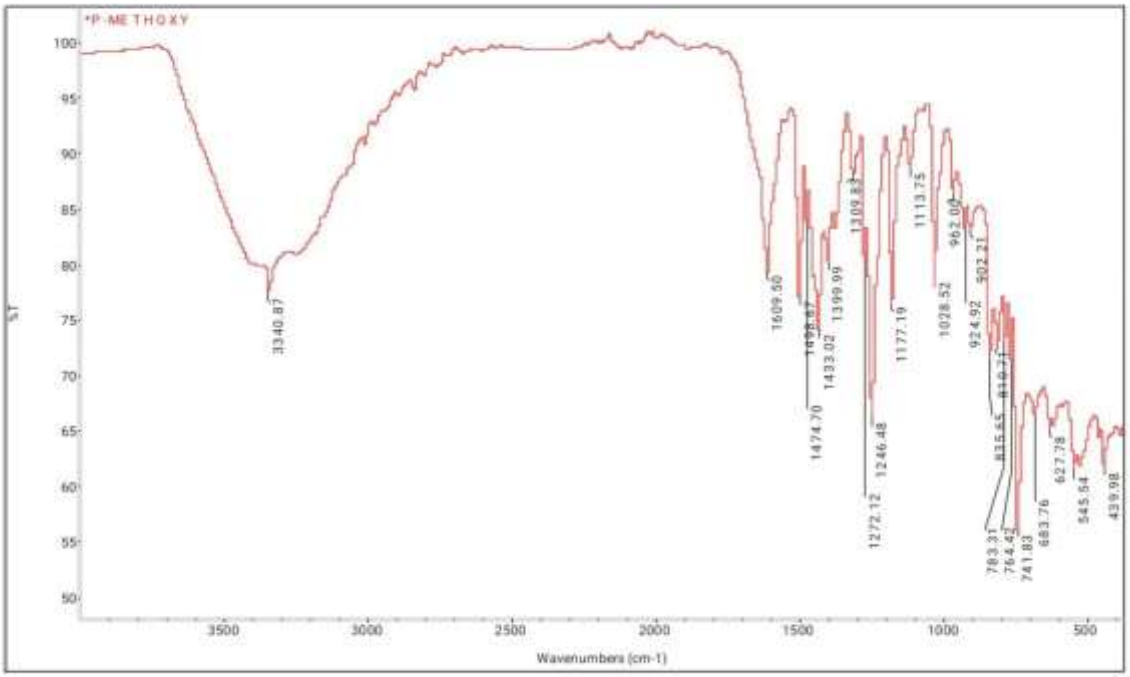
IR Spectrum of COM-2



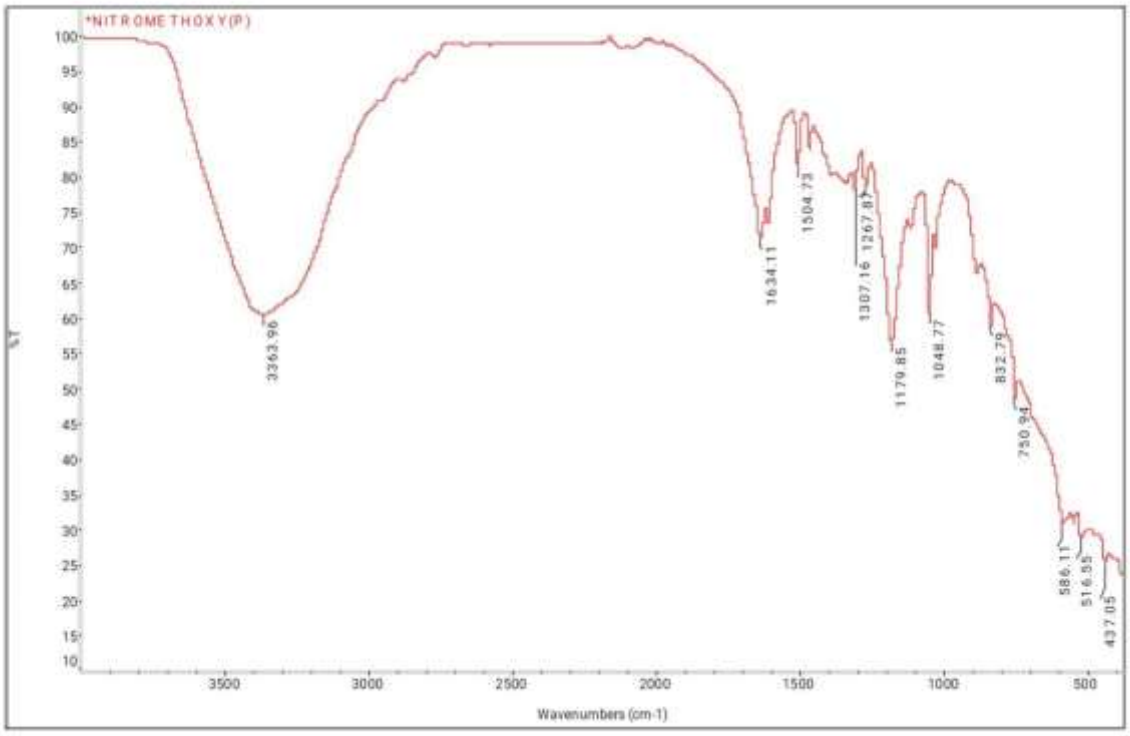
IR Spectrum of COM-3



IR Spectrum of COM-4



IR Spectrum of COM-5



IR Spectrum of COM-6

ANTIFUNGAL ACTIVITY :

The six synthetic compounds were screened against two fungal strains tested. The mean zones of inhibition ranged from 7.8 to 22.5 mm. The MIC values of the compounds between 15.5 to 31.25 µg/ml, while the MFC values are 31.25 to 62.5 µg/ml for all the fungal strains tested. The highest mean zone of inhibition (24.5 mm) was observed compound 6 against *C. krusei*. The lowest MIC (15.5 µg/ml) and MFC (31.25 µg/ml) values were obtained with the compound 3 against all the fungal strains tested.

Media Composition & its Preservation The antibacterial and antifungal activities was tested on solid (agar-agar) media in petriplates for bacterial assay nutrient agar (NA) (40gm/l) and fungus PDA (39gm/l) was used for developing surface colony growth. The suspension culture, for bacterial cell growth was performed by 2% (w/v) Lauria Broth and for fungus cell growth, 2.4% (w/v) PDB (Potato dextrose broth) was used. All media compositions were decontaminated by autoclaving at 125oCfor 15-25 min. (Chopra, et al., 1980).

Agar Well Diffusion methodThis method is widely used to examine the antimicrobialactivity. The 8-10 hold cultured broth plates were smeared forbacteria and fungi respectively with Nutrient agar (NA) andpotato Dextrose Agar (PDA) media. (Mann, et al., 1998) TheWells was digged in all the (10mm diameter and about 2 cm apart) plates with the help of sterile corn borer. Stock solution ofvarious samples taken out was made by taking concentration of1mg/ml in various dilutionsfrom various compounds was poured with decontaminated syringeinto

the well and maintained at 37.c for 2-3hrs. Further all theplates were incubated at 37^{o}C for 18-24hr for bacterial pathogenand 28oC for 46- 48 hr for fungal pathogen. The result wasrecorded nearby all the wells as measured of diameter of thezone of inhibition (mm). All the experimental process wasperformed in triplicate

MIC Values Analysis The analysis of MIC values was carried out with help of the broth serial dilution method. (Carson et al., 1999) After incubation of plates the reading was noted for calculation. All the different extracts are taken in serial dilution with Luria brothfor bacterium strains and PDB medium for the fungus strains. After the formation of media the test organism was poured in the serial dilution of the various extracts, further they all were incubated. After incubation the growth was measured. The extract no visible growth having minimum concentration is observed as the MIC Values.

Determination of MFC considered as least concentration of an antifungal drug which is needed to finish any fungal pathogen. To calculate the MFC value serial sub-cultivation of 2µLmicrotiter plates having 100µl of broth per well was used. After the formation of plates they were incubated for 72 hours at 280c. (Ratnasooriya, et al., 2005). The plate which shows the no visible growth and has the least concentration towards the growth was considered as MFC value. To compare the results Greisofluvin (1-3000ug/ml) which is the standard drug used as positive controls.

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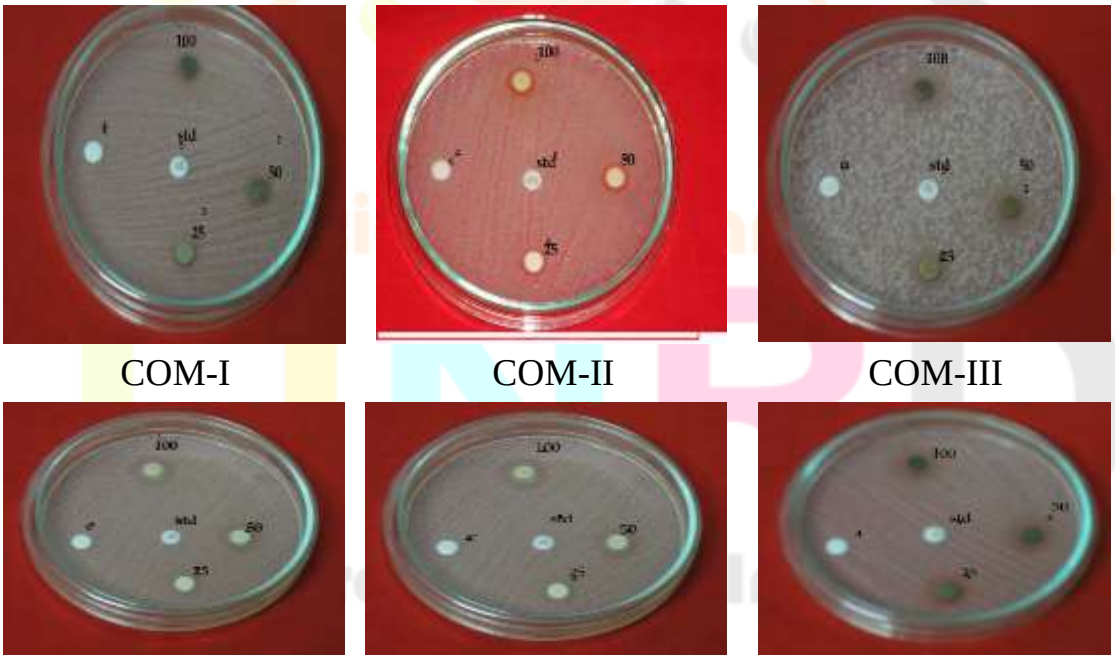
COM-I COM-II COM-III

COM-IV COM-V COM-VI

Fig 1 ***Candida albicans***



COM-I COM-II COM-III

COM-IV COM-V COM-VI

Fig 2 ***Candidakrusei***

Table1.In vitro antifungal activity of tested compounds in term of the zone of inhibition diameter (mm), Greisofluvin (1-3000ug/ml) are used as reference drugs.

S. No	Compounds	Zone of inhibition (in mm)					
		Candida albicans			*Candida krusei*		
		25 (µg)	50 (µg)	100 (µg)	25 (µg)	50 (µg)	100 (µg)
1.	COM I	9.8	11.6	12.8	13.8	15.5	18.8
2.	COM II	8.8	10.5	13.8	11.2	13.5	16.6
3.	COM III	7.8	10.8	12.8	14.3	18.8	22.5
4.	COM IV	13.8	15.5	18.8	13.3	15.8	19.5
5.	COM V	12.5	13.8	15.8	11.5	13.3	17.8
6.	COM VI	8.5	10.8	20.5	13.6	15.8	24.3

CONCLUSION:

The six benzimidazole derivatives with a fair amount of efficiency and face value. At compounds III and VI, an imidazole ring containing a phenyl moiety is present in the hybrid framework of the compounds. These substances exhibit strong antifungal properties. The entire study showed that substitution will change the compound's structure, and adding more rings to the benzimidazole nucleus could make it more potent. This will also change the compound's activity. Comparing the nitrated compound VI to other nitrated and benzimidazole derivatives, the former is more effective and highly active against *candida albicans*.

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