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Research Article

Synthesis Characterization and Antifungal Screening of Novel Substituted Bezimidazole Analogs

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Abstract

The various substituted ortho phenylenediamine and some other substituted cinnamic acid mixture were prepared in 10 mL of ethylene glycol contained in an RBF. This content was subjected to heat at 200°C for six-fourteen minutes on an oil bath. The product was recrystallized using ethanol. For scheme 2 Substituted ortho-phenylenediamine with substituted benzaldehyde, 0.01 mol each in the 100mL round bottom flask add 3mL hydrogen peroxide, and ceric ammonium nitrate (0.001 mol). The above content was subjected to heat maintaining the temperature at 50^0C for nine-thirty minutes. Antifungal studies of newly synthesized compounds were carried out against different unicellular and filamentous fungi. A qualitative evaluation of antifungal activity was resolved with the help of the well diffusion method. All the synthesized compounds confirm the presence of characteristic common groups as well as individual groups in ^{1}HNMR spectroscopy. ^{13}CNMR spectra of all the synthesized compounds show characteristic hydrogen as well as individual hydrogen of chemical structure. In experimental data denotes mass spectroscopic data of synthesized compounds. Well diffusion assay was performed for synthesized benzimidazole derivatives for determining the antifungal activity of compounds against various unicellular as well as filamentous fungi. Compounds 09, 02, 20, 16, 01 were evaluated for antifungal activity. Many of the compounds showed antifungal activity comparable to that of Amphotericin B (IC50= 0.84 -0.97 μg/ml), with compound 09 being the most active (IC50 =0.84 μg/ml less potent than amphotericin B).

Keywords: *Benzimidazole, cinnamic acid, anti-fungal, candida species.*

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Introduction

The benzimidazole compound (see Figure 1) is bicyclic which is made up of the combination of the benzene ring and imidazole ring (Wright, 1951). Benzimidazole having various pharmacological properties becomes a moiety of choice in the current situation. This benzimidazole ring acquires a high degree of stability. This mentioned compound is not influenced by concentrated sulfuric acid when heated under pressure to 270^{o}C or using different alkalies (Bahrami et al., 2008; Lambros et al., 1976). The benzene ring of benzimidazole only cleaves by oxidation under different conditions. The benzimidazole ring is also slightly unaffected by reduction; although tetrahydro and hexahydro benzimidazole in which the benzene ring is reduced possibly produce by catalytic reduction under definite conditions. The benzimidazole ring which has a hydrogen atom attached to nitrogen at the first position was easily tautomerized (Shingalapur et al., 2009; Trager et al., 1976). This may be represented as: This tautomerism is parallel to that present in the imidazole and amidines. The benzimidazole is perhaps considered as cyclic analogs of the amidines. On account of tautomerism in benzimidazoles precise derivatives which appear at first to be isomers are tautomers; however two non-equivalent structures can be written, only one compound is known (Nerya, et al., 2004; Casagrande et al., 2012).

Also, these merged heterocyclic were studied for their various antitumor, antiviral and antimicrobial activities as the new non-nucleoside topoisomerase I position, human immunodeficiency virus-I, reverse transcriptase inhibitors, and/ or potent DNA gyrase inhibitors. Furthermore, benzimidazole derivatives take part a crucial role in the theoretical development of heterocyclic chemistry and are also used comprehensively in organic synthesis (Nordlund et al., 2009; Berman et al., 2000)

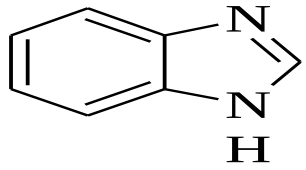


Figure 1. Benz imidazole Structure

Material and Methods

Reagents and Chemicals

The reagents and chemicals used for experimental work were of synthetic grade and purchased from TCI, SDFCL, Sigma Aldrich and Spectrochem.

Synthesis: Following scheme was carried out for synthesis of designed compounds.

Scheme 1- General method of synthesis of benzoimidazole derivatives:

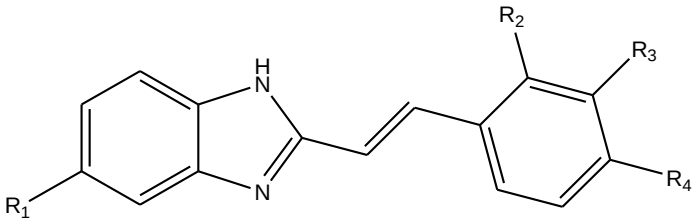


Figure 2. Structure of Benzimidazole Analog 1.

Table 1: *Designed Benzimidazoles Derivatives*

Comp No.	**Substitution**			
	R_1	**R_2**	**R_3**	**R_4**
01	H	H	H	H
02	H	H	H	NO_2
03	H	H	NO_2	H
04	NO_2	H	H	H
08	NO_2	H	NO_2	H
09	H	H	H	OCH_3
11	NO_2	H	H	OCH_3
15	NO_2	H	H	NO_2
16	NO_2	NO_2	H	H
18	NO_2	Cl	H	Cl
20	Cl	H	H	Cl

Scheme 2-

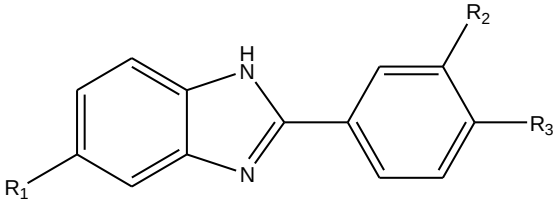


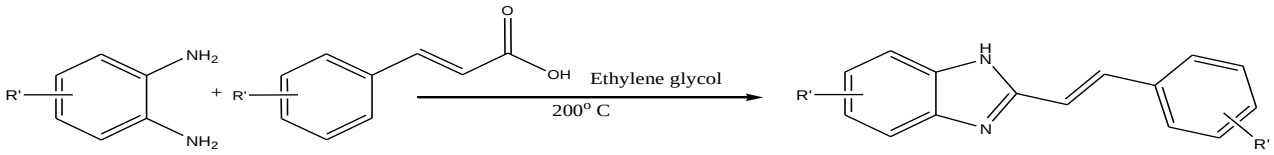
Figure 3. Structure of Benzimidazole Analog 2.

Table 2: Designed Benzimidazoles Derivatives

Comp No.	Substitution		
	R_1	**R_2**	**R_3**
07	H	OCH_3	OCH_3
10	NO_2	OCH_3	OCH_3
12	H	H	NO_2
14	NO_2	NO_2	H

Procedure Scheme 1

A procedure (see Figure 4) was employed to synthesize the designed compounds (see Figure 2). The various substituted ortho phenylenediamine and some other substituted cinnamic acid mixture were prepared in 10 mL of ethylene glycol contained in an RBF. This content was subjected to heat at 200 ^{0}C for six-fourteen minutes on an oil bath. After that this content was put into ice water and then filter using filtration assembly, washed with water and then extract with the help of ethyl acetate and water using separating funnel by phase separation then the product was passed from anhydrous sodium sulphate from funnel and the product was recrystallized using ethanol. The product was dried and used for further analysis. Eleven compounds was prepared by using this method (see Table 1). (Rieckmann et al., 1978; Desjardins et al., 1984).



Substituted ortho-phenylenediamine

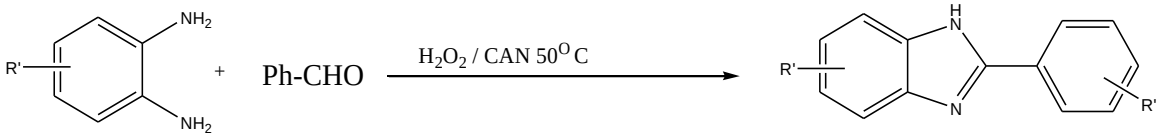
Substituted Cinnamic acid

Substituted 2-styryl-1*H*-benzo[*d*]imidazole

Figure 4. General Scheme for Synthesis of Benzimidazole Analogs 1.

Procedure Scheme 2

A procedure (see Figure 5) was employed to synthesize the designed compounds (see Figure 3). Substituted ortho-phenylenediamine 0.01 mol with various substituted Benzaldehyde 0.01 mol was taken in 100mL round bottom flask in that add 3mL hydrogen peroxide and ceric ammonium nitrate 0.001 mol. After that this content was heated for nine-thirty minutes at 50 ^{0}C. After that this solution was put into ice water and do filtration, washed with water after that do extraction by using ethyl acetate and water in the separating funnel by phase separation, then the product was passed from anhydrous sodium sulphate from funnel and the product was recrystallized using ethanol. The product was dried and used for further analysis. Four compounds were prepared by using this method (see Table 2). (Alasmary et al., 2015; Dixit et al., 2012).



Substituted ortho-phenylenediamine

Substituted Benzaldehyde

Substituted 2-phenyl-1*H*-indene

Figure 5. General Scheme for Synthesis of Benzimidazole Analogs 2

Physicochemical Characterization

Melting point

All synthesized compound was subjected for melting point determination by using melting point apparatus. These reported melting points were uncorrected.

Thin Layer Chromatography

Prepared aluminium sheet (0.2 mm thick) having precoated silica gel-G was used to perform thin layer chromatography. For this different solvent system were made to determine purity of the synthesized compounds. Iodine vapors were used as identifying agent.

Infrared Spectrum

IR spectrum was determined by using FTIR-8400S spectrometer instrument.

Nuclear Magnetic Resonance

^{1}H NMR and C^{13} NMR spectrums were obtained using $CDCl_3$ and DMSO as solvent.

Mass Spectral analysis studies

The mass spectra were recorded using ionization technique electro spray ionization.

***In vitro* Antifungal Screening**

The synthesized derivatives were put out for anti-fungal activity (see Table 3) in case of distinct filamentous as well as unicellular fungi (Ongarora et al., 2012). A qualitative evaluation of antifungal activity was resolved with the help of the well diffusion method (see Table 4). Malt extract agar (20 mL) was seeded in Petri dishes with two-three day's old culture of fungal inoculums (Singh et al. 1956., Paniker et al., 2007). 6 mm diameter wells were cut into agar with the help of a cork borer. 50 µL of synthesized compound diluted in DMSO were added at a concentration of 5 mg/mL. Depending upon the growth rate of each strain these Petri plates were incubated for three-seven days at 37 °C. Zone of inhibition was observed around each well, based on the diameter of the zone of inhibition antifungal activity was established. For this antifungal activity, Amphotericin B was used as a positive control. The purpose of this study was not to differentiate the activity on a molar basis since such comparison does not automatically have biological validity when comparing compounds with very different modes of action or solubility in diffusion assay. This permits us to recognize the compounds with the most promising activity, which could be looked at in more detail in upcoming studies. (Gut et al. 2014; Guantai et al., 2010)

Result and Discussion

Spectroscopic Interpretation of Synthesized Compounds:

With the help of FT-IR, ^{1}H- NMR, ^{13}CNMR and Mass spectroscopy techniques all the synthesized 2-styryl-1H-benzimidazole compounds were identified.

FT-IR Spectroscopic Analysis:

FTIR absorption values of 2-styryl-1H-benzimidazole derivative were reported in the experimental section. All the synthesized derivatives exhibited identical peaks of N-H stretch of in the region of 3286- 3100 cm^{-1} and N-H bending of the region 1650-1500 cm^{-1} this indicates the presence of imidazole moiety in all the synthesized compounds. Apart from that common peak of C=N hydrazones stretching is observed in the region of 1400-1600 cm^{-1} of frequency. A strong stretching band in the region of 1650-1700 was shown by all the compounds this wills denotes the C==C stretching of the compounds cm^{-1}. The NO_2, CH_3 group exhibited peaks 1600 & 1350 cm^{-1} and the Cl group exhibited peaks of 780- 550 cm^{-1}.

Mass Spectral Analysis:

In experimental data denotes mass spectroscopic data of synthesized compounds. In 01 and 20 compounds the M+ peak show at 219 and 288 this is due to the presence of isotopic carbon C^{13}. 02 and 11 show M+2 at 263 and 297. M+ peaks at 310 is also shown by08 compounds. 09, 12, 15 and 16 compounds show M+ peak at 250, 239, 310 and 310 respectively. 18shows M+2 on 337these M+ 2 peaks denotes the isotopic peak of – Cl which is present in 3rd and 4th position of aromatic ring.

^{1}HNMR Spectral Analysis

All the synthesized compounds confirm presence of characteristic common groups as well as individual groups in ^{1}HNMR spectroscopy. All the compounds show chemical shift value in the range of 7-8 and this particular value of δ denotes the proton of phenyl ring. The δ value in range of 4-6 denotes the more de shielded hydrogen of imidazole this value of H in this range is find in all the synthesized compounds. Hydrogen of butanol -CH system is found in the region of 7-8 because this is attached with electronegative atom N so this going in de-shielded region. All the compounds show presence of this type of proton in his spectra. All the aromatic hydrogen is denoted as multi-plate form. Apart from this common groups some individual peaks of different substituent of terminal phenyl ring was also observed. The proton of methyl group (-OCH_3) in 07, 09, 10, 11 if found at δ value of 3.79 and 4.12.

^{13}CNMR Spectral Analysis

^{13}CNMR spectra of all the synthesized compounds show characteristic hydrogen as well as individual hydrogen of chemical structure. The common carbon and grouped carbon show the range of chemical shift (δ) in ppm. More de shielded carbonyl carbon is found in the range of δ 160-195. All the synthesized compound shows the characteristic peak of aromatic ring ranging from δ to 115.4 - 139.1, N-C peak ranging from δ to 140 – 145, but - CH peak ranging from 110.2- 135.1 and the terminal aromatic ring peak ranging from δ to 125 – 137.

Biological Evaluation

The synthesized fifteen compounds were subjected for anti-fungal screening.

Table 3: *In-vitro* Antifungal Screening of Synthesized Derivatives

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S. No Compound IC_{50}

	ID	(µg /ml)
01	01	0.96
02	02	0.85
03	03	1.14
04	04	1.02
05	07	0.96
06	08	1.35
07	**09**	**0.84**
08	11	1.07
09	12	0.97
10	14	1.30
11	15	1.10
12	16	0.94
13	17	1.56
14	18	0.94
15	20	0.86
16	Standard	0.020

Table 4: *Result of Antifungal Activity of Prepared Benzimidazole Derivatives in the Well Diffusion Assay Techniques*

Strain of Fungi	**Zone of inhibition in terms of diameter (mm) around each compound**					
	01	**02**	**09**	**16**	**20**	**Am. B**
Candida albicans RCMB 05035	21	20	19	18	19	22
Candida krusei RCMB 05051	18	17	16	17	12	19
Candida parapsilosis RCMB 05065	13	14	13	12	14	18
Candida tropicalis RCMB 05049	19	18	17	21	20	25

*Good activity, in terms of zone of inhibition obtained and compared against amphotericin B.

The compound 09 shows an active potent as compared to the other synthesized compounds. The compound shows good effect on *Candida spec.* strain because of the substitution of the OCH_3 group at R^4 position which is electron donating group while the compound 11 does not shows good activity because the substitution of NO_2 group at the R^4 position on the (A) phenyl ring as we know that NO_2 is electron withdrawing groupin nature and the compound 20 is less active compound as compared to the 09. The compound 20 contains substitution of chloro group at (A&C) phenyl ring R^4 position the Cl group is electron withdrawing in nature and the compound 18 does not show good activity as compared to 20. The compound 18 contains the substitution of the NO_2 group at R^4 position at (A) phenyl ring and two Cl group at R^2 & R^4 position at (C) the

nitro group is electron withdrawing group in nature which decreases the anti-fungal activity of the compound.

Conclusion

The *In vitro* activity involved the exploration around the terminal phenyl ring (c) and variation in substitution at R^4 (including OCH_3 group).Compounds 09, 02, 20, 16, 01 were evaluated for anti-fungal activity. Many of the compounds showed anti-fungal activity comparable to that of Amphotericin B (IC_{50}= 0.84 -0.97 μg/ml), with compound 09 being the most active (IC_{50} =0.84 μg/ml less potent than amphotericin B).

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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