Turkish Online Journal of Qualitative Inquiry (TOJQI)
Volume 12, Issue 7 July 2021: 12803 – 12811

Research Article

Anti-Fungal Efficacy of Benzimidazole Analogues on Various Unicellular and Filamentous Fungi

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Abstract

The focal point of the present study is the synthesis of innovative antifungal medicines that are effective against various multicellular and filamentous fungi. Benzimidazole complexes containing various substitutions were prepared and screened using agar diffusion methods for their antifungal properties. The synthesized derivatives also get confirmed with chromatographic and spectroscopic techniques. Out of ten synthesized compounds, some show significant activity, some show moderate activity and two compounds show greater activity that is similar to the antifungal activity of standard compound i.e. ketoconazole.

Keywords: *Benzimidazole, antifungal, multicellular, filamentous, moderate.*

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Introduction

The large area of the universe is distributed with fungi. Some of them are observed in humans and animals. Through the infection, these fungi alter the host immune system and it becomes life-threatening, opportunistic, and pathogenic (Krasner, 2002). It is determined that almost 300 million people are in trouble worldwide with serious fungal infections and 25 million are in the danger zone of dying or losing their vision. The strength of this fungal infection in a sensitive population varies from acute, severe to chronic (Chen, 2013; Denning, 2013). In past decades, the infection rate of fungi was increased significantly. Morbidity and death rates in patients are continuously increased in current years due to unpleasant fungus infections. Most of these fungal infections are caused due to different species of fungi such as *Candida*, *Aspergillus*, etc. Some of the currently available antifungal medicines have reduced sensitivity towards few unusual fungi (Zhong, 2017). A scientist has defined glucan synthetase, ergosterol, and demethylase as a point of action for currently obtainable medications (Nafsika, 2017).

Nowadays there is remarkable growth in the expansion and applying new plans for the implementation of fresh medicines for various fungal treatments. The traditional screening methods of different synthetic and natural products have various drawbacks, to overcome these drawbacks molecular biological revolution is now emerging as the best method in new antifungal drug discovery. This technique involves the identification of gene expression under different conditions (Petrikkos, 2007; Perfect, 1996).

In the search for an antifungal drug, various possible objects are considered. In this pathway cell, the biology concept plays an important role. Different pathogenic fungi including unicellular and multicellular fungi are screened and analyzed under various conditions (Balkovec, 1994). The currently available antifungal medication has a different mechanism of action. The drug Amphotericin B acts on cyt. P450 and directly affects the biosynthesis of ergosterol. Another drug Echonochandins hampers 1,3-β-D glucan synthetase (Fox, 1996; Bowman, 1988).

Yet, presently good alternative for treating harmful infections of fungi has some restrictions due to resistance, toxicity, side effects that are undesirable (Vincent, 1999). We know that for the discovery of new medicines the certain type of compounds has passed through different stages of drug discovery and development. The antifungal class azoles also are currently in different processes of clinical trials. Lanosterol-14-α-demethylase and cyt. P450 is fixed as a target for this drug development stage (Giulia, 2004; Xiao, 2004).

Synthesis of six-membered heterocyclic molecules e.g imidazole, benzimidazole, pyridine ring is most important due to its synthetic conditions and pharmacological properties. It is observed that fused heterocyclic compounds having imidazole, benzimidazole and pyrimidine exhibited optimum biological activities such as antimicrobial, DNA cleavage, anti-inflammatory, antiviral, anti-HIV, and antitumor (Balzarini, 2002; Harinadhababu, 2004). Fight against the different species of microbes is not an ending battle. As far as the health and hygiene of society are concerned these harmful microbes pose the biggest problem. To conquer the shortage of current antifungal compounds and to produce more effective medicines the compounds which possess better action can be used in routine treatment for different fungal infections (Jantova, 2004; Shankar, 2017).

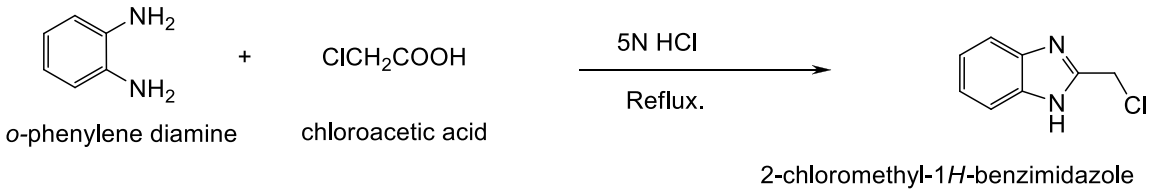
A drug such as voriconazole, Rauconazole, Itraconazole, Miconazole, Fluconazole belongs to azole aromatic heterocyclic compounds showing a wide range of antifungal activity. Based on this we get a certain clue for further alternatives in antifungal treatments. Systematic changes in the structure of azole which as well as shows herbicidal, insecticidal, antimalarial, anti-inflammatory, anticancer, anticonvulsant, and antibacterial properties (Bruch, 1996; Talwar, 1995).

Material and Methods

All the chemicals used for synthesis and antifungal activity purposes were of analytical reagent grade, commercially available from Merch, and were purified before use for further processing. The melting point was determined on the automatic melting point apparatus. The completion of all reactions was determined by using TLC techniques. The infrared absorption spectra of synthesized compounds were measured using a JASCO spectrophotometer.

Synthesis: Following scheme was carried out (see Figure 1 & Figure 2) for the synthesis of designed compounds. Different physicochemical properties of synthesized compounds were shown (see Table 1).

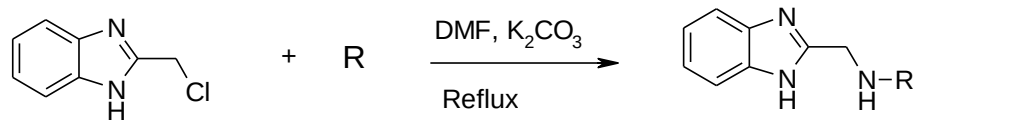
Scheme A: Synthesis of Benzimidazole as a basic moiety.



o-phenylene diamine chloroacetic acid 2-chloromethyl-1*H*-benzimidazole

Figure 1: General process for preparation of basic moiety.

Scheme B: Synthesis of 2-chlorometyl-1*H*-benzimidazole derivatives:



2-chloromethyl-1*H* -benzimidazole 2-chloromethyl-1*H* -benzimidazole derivatives

where, R = substituted aromatic/heteroaromatic amine

Figure 2: Scheme for synthesis of benzimidazole complexes

Table 1: *Characterization Studies of Synthesized Complexes.*

Comp. No.	Functional Group (R)	Molecular Formula	% Yield	M.P(^{0}C)	R_f Value
SA1	3-Aminobenzoic Acid	$C_{15}H_{13}N_3O_2$	70 %	146-148	0.65
SA2	2-Aminobenzoic Acid	$C_{15}H_{13}N_3O_2$	51 %	140-142	0.56
SA3	3-Chloroaniline	$C_{14}H_{12}N_3Cl$	53 %	155-157	0.58
SA4	4-Nitroimidazole	$C_{11}H_9N_5O_2$	65 %	118-120	0.62

SA5	4-aminotriazole	$C_{10}H_{10}N_6$	61 %	158-160	0.68
SA6	2-amino-3-methyl pyridine	$C_{15}H_{13}N_3O_2$	52 %	163-165	0.58
SA7	2-Chloroaniline	$C_{14}H_{12}N_3Cl$	56 %	98-100	0.62
SA8	o-Toluidine	$C_{15}H_{15}N_3$	69 %	82-84	0.55
SA9	2-amino-4-chloro benzoic acid	$C_{15}H_{12}ClN_3O_2$	53 %	180-182	0.70
SA10	2,6-xylidine	$C_{15}H_{15}N_3$	47 %	158-160	0.67

Antifungal Properties

The following section discusses, in brief, the various antifungal screening strategies (see Table 2) and their importance, which is then followed by the screening of the synthesized compounds (see Table 3) for their antifungal efficacy.

I. Primary Screening:

Detection of Anti-fungal potential
Method: Well plate method **(**Agar diffusion quantitative bioassay).
Medium: Sabouraud's dextrose agar.
Microorganism used: 1. *Penicillium notatum.*
2. *Aspergillus Niger*
3. *Aspergillus fumigatus*
4. *Candida albicans*

Inoculum: Sabouraud's dextrose agar on which 3-4 days old growth of fungi is suspended in normal saline solution. UV spectrophotometer is used to adjust the wavelength and absorbance of suspension.

Inoculum size: 10^7 CFU/ml.
Stock solution: DMSO is used for preparation of stock solution of synthesized and standard compound.
Drug concentration: 12.5, 25, 50, 100 μg/ml.
Incubation time: Two to Three days.
Incubation temperature: 30ºC
Interpretation: The synthesized compounds which show a zone of inhibition more than or equal to 9 mm or comparable to ketoconazole is said to be present a better antifungal activity.

II. Secondary Screening:

MIC value determination

Method: Well plate method **(**MIC value determination using Agar diffusion).
Medium: Hi Media.
Inoculum Preparation: Sabouraud's dextrose agar on which 3-4 days old growth of fungi is suspended in normal saline solution. UV spectrophotometer is used to adjust wavelength and absorbance of suspension.
Compound concentration: 12.5, 25, 50, 100 μg/ml.
Standard concentration: 12.5μg/ml.
Micro-organism used: *Candida albicans.*
Incubation time: Two to Three days at 30ºC
End point: Best MIC value is considered when at a specific concentration of compound at which there is complete vanishing or notable reduction in the growth of fungi, approximately 5-10 colonies per spot are observed.
Interpretation: Out of ten synthesized compounds, some compounds show MIC value which is equal to the MIC value of standard compound i.e. ketoconazole.

Table 2: *In vitro Antifungal Activity of Benzimidazole Derivatives in Term of MIC Values.*

Sr. No.	Compound Code	MIC value (μg/ml)
1	SA1	100
2	SA2	100
3	SA3	50
4	SA4	12.5
5	SA5	12.5
6	SA6	25
7	SA7	50

8	SA8	100
9	SA9	25
10	SA10	25
11	Ketoconazole	12.5

Table 3

In vitro Antifungal Property of Synthesized Compounds in Terms of Zone of Inhibition.

Sr. No.	Molecule Code	Zone of inhibition in mm at 25 µg/ml against			
		Aspergillus niger	*Aspergillus fumigatus*	*Penicillium notatum*	*Candida Albicans*
1.	SA1	30	NA	NA	21
2.	SA 2	35	20	NA	30
3.	SA 3	18	20	24	24
4.	SA 4	37	26	30	35
5.	SA 5	38	28	32	36
6.	SA 6	27	21	25	30
7.	SA 7	NA	18	29	28
8.	SA 8	35	20	NA	26
9.	SA 9	16	20	27	30
10.	SA10	31	23	30	25
11.	Ketoconazole (std.)	41	30	33	38

Result and Discussion

A new derivative of antifungal compounds containing benzimidazole basic skeleton was synthesized. These prepared derivatives then put for antifungal screening using *Aspergillus*

níger, Candida albicans, Aspergillus fumigatus, Penicillium notatum as a strain. After synthesis, it was found that compound SA1, SA2, SA8 (MIC Value 100 µg/ml), and compound SA3, SA7 (MIC Value 50 µg/ml) showed remarkable antifungal activity. The compounds SA6, SA9, SA10 (MIC Value 25 µg/ml) showed moderate activity. Compound **SA4** and **SA5** showed greater activity (MIC Value **12.5 µg/ml**) compared against the minimum inhibitory concentration of standard drug i.e. ketoconazole.

Conclusion

Based on various kinds of literature available on novel antifungal categories of medicine. It confirms that currently many pharmaceutical companies are in constant search of drugs that will show better activities than the compounds that are currently used for antifungal activities. Most of the scientific article contains benzimidazole as a basic skeleton in their structure. So, accordingly, we picked up benzimidazole for the invention of novel antifungal medicines.

Ten complexes of benzimidazole having aromatic and heteroaromatic structures were synthesized. These synthesized complexes were then confirmed using various chromatographic and spectroscopic methods. These derivatives afterward screened for antifungal activity using well plate (agar diffusion) methods. Various filamentous and multicellular fungal strains were used. The result of this activity was observed in terms of MIC values and zone of inhibition.

The synthesized complex SA1, SA2, SA8 (MIC Value 100 µg/ml), and compound SA3, SA7 (MIC Value 50 µg/ml) showed remarkable antifungal activity. SA6, SA9, SA10 (MIC Value 25 µg/ml) showed moderate activity. Compound **SA4** and **SA5** showed greater activity (MIC Value **12.5 µg/ml**) compared against the minimum inhibitory concentration of standard drug i.e. ketoconazole.

Acknowledgement

The authors are thankful to Vels Institute of Science Technology and Advanced Studies (VISTAS), Chennai Tamil Nadu for providing all the facilities to make this work complete. The authors are pleased to express sincere thanks to HSBPVT's, GOI, College of Pharmacy, Kashti, Maharashtra for valuable support.

Conflict of Interest

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

Funding Support

The authors declare that they have no funding support for this study.

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