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Research Article

SYNTHESIS, CHARACTERIZATION, AND EVALUATION OF ANTIFUNGAL PROPERTIES OF SUBSTITUTED BENZIMIDAZOLE ANALOG

VIJEY AANANDHI M*, ANBHULE SACHIN J

Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu, India. Email: hodpchemistry@velsuniv.ac.in

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ABSTRACT

Objective: The aim of the present study is to synthesize novel benzimidazole derivatives as potent antifungal agents.

Methods: 2-chloromethyl-1H-benzimidazole has been synthesized by refluxing o-phenylenediamine with chloroacetic acid in the presence of 5N HCL. Further, N-substituted benzimidazole derivatives containing various heteroamines and aromatic compounds were synthesized. These, finally, prepared derivatives were kept for *in vitro* antifungal property.

Results: A novel series of antifungal agents, containing benzimidazole nucleus as a basic skeleton, has been synthesized.

Conclusion: The synthesized novel benzimidazole derivatives exhibit moderate activity (VMSA 1, 2, 8), some compounds showed significant activity (VMSA 6, 9, and 10), and compound VMSA 4 and 5 exhibits maximum activity.

Keywords: Benzimidazole, *In vitro*, Significant, Antifungal activity, Aromatic.

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INTRODUCTION

The fungi are more gradually developed form of organism when compared to unicellular organism such as bacteria, viruses, and prions. The fungi are classified as eukaryotes which have nuclear membrane that surrounds the nucleus. The fungi have complex structural features due to genetic and morphological complexity [1,2].

It is observed that, among all the different microbes available, fungi have a tremendous impact on the immunocompromised patients and which is emerged as a major infection [3].

There has been significant progress in developing and implementing strategies for the development of new antifungal agents. One strategy for antifungal drug development besides the classical screening of many classes of synthetic or natural products against a variety of fungi involves the use of the molecular biological revolution. It is now possible to identify molecular targets that are essential for fungal virulence. The identification of unique genes and their signaling network, important to the path biology of fungi, could translate into their use as molecular targets for drug development. There are, now, a series of molecular techniques to identify gene expression(s) under certain conditions, and the field of molecular pathogenesis has matured to the point that validation for these potential "virulence" targets can be achieved [4-7].

Furthermore, area of cell biology is now being focused, within pathogenic fungi, and uses all the molecular and biochemical tools and understanding to dissect it, and thereby, attempt was taken for selecting specific fungicidal objects having different selectivity with mammalian cells. Many potential objects for antifungal drug development have been recognized [8].

These can vary from the azoles which inhibit cyt.P450-dependent endoplasmic reticulum where ergosterol gets biosynthesized. A certain antifungal agent such as Amphotericin B which attach to ergosterol inducing conformational change with formation of ionic pores. Echinocandins inhibit 1, 3-β-D glucan synthesis induced by β-1, 3 glucan synthesis [9,10].

Although currently therapeutic choice for the use of invasive fungal infection has some limits, most of antifungal compounds are not too specific due to resistance development, toxicity of host, and side effects which are undesirable [11]. The azole category of antifungal drugs has different agents which are under various clinical phases. These azole category drugs inhibit membrane-bound enzyme. The selected objects of azoles are cyt.P450 and lanosterol-14 demethylase [12]. Ergosterol biosynthesis was inhibited due to azoles which directly reduce the fungal growth by the binding of active sites with azoles [13]. The biosynthesis of plant phytosterol, animal cholesterol, and fungal sterol create an intermediate step due to hemoprotein [14].

As azoles and their derivatives are the most important class of the aromatic heterocyclic compounds exhibiting a wide range of biological applications, especially as active moieties in first-, second-, and third-generation azoles which act as antifungal agents such as miconazole, econazole, fluconazole, itraconazole, and voriconazole, we were now in position for exploring further possibilities in azoles compounds [15,16].

Structural modification has shown that the azole compounds also exhibit properties such as antibacterial [17], anticonvulsant [18,19], anticancer [20,21], anti-inflammatory [22,23], antimalarial [24], antineoplastic [25], insecticidal, and herbicidal [26].

METHODS

1. The chemicals necessary for the study were purchased from Merck and they were purified before use.
2. Campbell melting point apparatus was used for determining melting point of synthesized compounds.
3. The thin-layer chromatographic method was used where Silica Gel-G was selected for the preparation of thin-layer chromatography (TLC) plates.
4. TLC plates gets visualized under iodine vapors or observed below UV light.
5. "SHIMADZU 3100" and "JASCO 530V" were used for determining infrared spectra. $CDCl_3$/dimethyl sulfoxide (DMSO) was used for

determining ^{1}H-nuclear magnetic resonance spectra where standard selected is tetramethylsilane (Schemes 1 and 2).

Antifungal property of synthesized compound

The antifungal activity of the synthesized compounds was carried out using well-plate method, and minimum inhibitory concentrations (MIC) values were calculated as µg/ml. The compounds were tested against *Candida albicans*, *Penicillium notatum, Aspergillus niger*, and *Aspergillus fumigatus.*

Stock solutions of the synthesized derivatives were prepared in DMSO. Dilution series using sterile water were prepared from 12.5 µg/ml to 100 µg/ml in microtest tubes. Suspension of test organism was freshly prepared in 1 ml of sterile normal saline solution and was standardized spectrophotometrically to 10^7 CFU/ml.

RESULTS AND DISCUSSION

A novel series of antifungal agents, containing benzimidazole nucleus as a basic skeleton, has been synthesized. The antifungal testing of synthesized compounds was carried out using *A. niger, C. albicans, A. fumigatus,* and *P. notatum as* a strain.

Among synthesized compounds, VMSA 1, 2, and 8 (MIC=100 µg/mL) and 3 and 7 (MIC=50 µg/mL) exhibit significant activity against *C. albicans*, whereas compounds VMSA 6, 9, and 10 (MIC=25 µg/mL) showed significant activity. VMSA 4 and 5 show greater activity (MIC=12.5 µg/mL) compared with MIC of the ketoconazole (Tables 1 and 2).

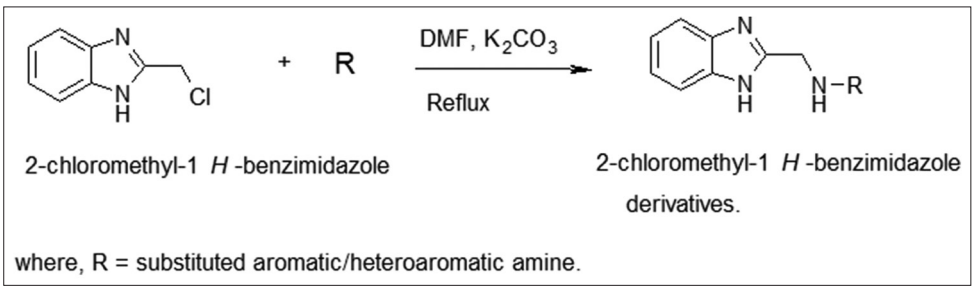
CONCLUSION

From the literature survey, it was found that there has been a lot of research going in the field of novel antifungal drug development. It was found that many antifungal agents contain benzimidazole moieties in their structure. Therefore, benzimidazole was selected for the development of new chemical entities as potential antifungal agents.

Ten benzimidazole derivatives containing heteroamines and aromatic compounds were prepared. The prepared compounds were confirmed by different spectroscopic and chromatographic techniques.

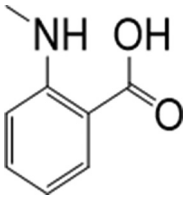
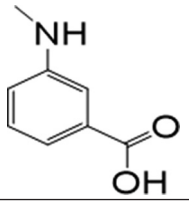
Well plate method was used for testing *in vitro* antifungal activity of ten synthesized compounds. These activities were observed in agar culture plates in the form of zone of inhibition which was recorded in

Scheme 1: Preparation of chloromethyl substituted benzimidazole compound



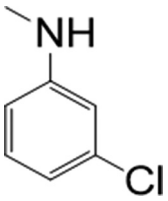
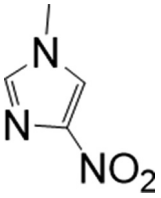
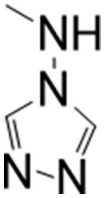
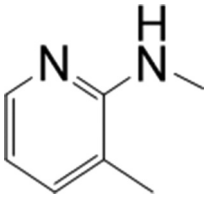
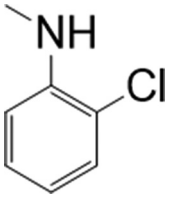
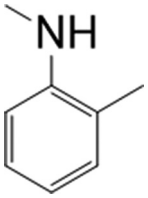
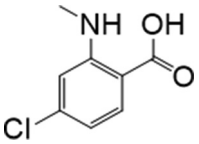
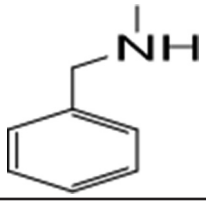
Scheme 2: Synthesis of 2-chloromethyl-1*H*-benzimidazole derivatives

Table 1: Physicochemical study of 2-chloromethyl-1H-benzimidazole derivatives

Code	R	Molecular formula	% Yield	Molecular pressure (°C)	Rf value
VMSA1		$C_{15}H_{13}N_3O_2$	70	146–148	0.65
VMSA2		$C_{15}H_{13}N_3O_2$	51	140–142	0.56

(Contd...)

Table 1: ***(Continued)***

Code	R	Molecular formula	% Yield	Molecular pressure (°C)	Rf value
VMSA3		$C_{14}H_{12}N_3Cl$	53	155–157	0.58
VMSA4		$C_{11}H_9N_5O_2$	65	118–120	0.62
VMSA5		$C_{10}H_{10}N_6$	61	158–160	0.68
VMSA6		$C_{15}H_{13}N_3O_2$	52	163–165	0.58
VMSA7		$C_{14}H_{12}N_3Cl$	56	98–100	0.62
VMSA8		$C_{15}H_{15}N_3$	69	82–84	0.55
VMSA9		$C_{15}H_{12}ClN_3O_2$	53	180–182	0.70
VMSA10		$C_{15}H_{15}N_3$	47	158–160	0.67

centimeters. Compounds VMSA 1, 2, and 8 and VMSA 3 and 7 showed intense activity against *C. albicans*, whereas compounds VMSA 6, 9, and 10 exhibit greater activity. VMSA 4 and 5 exhibit maximum activities with MIC compared to that of the ketoconazole.

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Table 2: *In vitro* antifungal activity of benzimidazole derivatives (MIC data for prepared derivatives)

S. No.	Mol. Code.	MIC (µg/ml)
1	VMSA1	100
2	VMSA2	100
3	VMSA3	50
4	VMSA4	12.5
5	VMSA5	12.5
6	VMSA6	25
7	VMSA7	50
8	VMSA8	100
9	VMSA9	25
10	VMSA10	25
11	Ketoconazole	12.5

MIC: Minimum inhibitory concentrations

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