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Synthesis and Evaluation of Antifungal Activity of N-Methyl-2-α-[(N^4-Aryl) Piperazin-1-yl] Ethyl Benzimidazoles

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GRAPHICAL ABSTRACT

The relative broad spectrum of activity of the azoles against common fungal pathogens, ease of administration and limited toxicity are highly attractive features. Antifungals work by exploiting differences between mammalian and fungal cells to kill off the fungal organism without dangerous effects on the host. Benzimidazoles and piperazines are the important pharmacophores in the field of medicinal chemistry, due to their widespread pharmacological activities, so to exploit their antifungal potential we have selected these two for our research work but they have several drawbacks and limitations like adverse drug reaction due to co-administration and occurrence of resistance of fungal organisms, especially Candida species, to fluconazole. These limitations of the azoles will become more problematic if fluconazole and other azoles continue to be used injudiciously. Many benzimidazole derivatives substituted with piperazines have been synthesized and evaluated for antifungal activity in our department. From the above limitations benzimidazole containing antifungal agents, we have synthesized different substituted aryl piperazines, N-methyl-2-[α-(chloro) piperazin-1-yl] ethyl benzimidazole nucleus, condensed it to offer targeted compounds, evaluated the structures of targeted compounds on the basis of Infra-red spectra, NMR spectra and mass spectra and further carried out antifungal activity against Candida albicans.

Keywords: Benzimidazole, Piperazines, Antifungal, *Candida albicans*

INTRODUCTION

The azoles, as a group of antifungal drugs, act by inhibiting the C-14 demethylation of sterols. In the last three decades, piperazine derivatives are dominating the bulk drug market. Today several thousand derivatives have reached advanced stages of drug discovery programs. This investigation relates to new compounds active as fungicidal and methods for their use. More specifically, this invention relates to 1-substituted benzimidazoles effective as fungicidal which possess a high degree of hydrophilic and lypophilic effects and demonstrate excellent systemic activity. An antifungal medication can also be termed as an antimycotic medication, is a pharmaceutical fungicide or fungistatic used to treat and prevent mycosis such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, dermatitis, subcutaneous, superficial, hair and nail. The organisms causing these types of infection are *E. Floccosum*, *M. Gypseum*, *T. Rubrum*, *T. Violaceum*, *T. Verrucosum*, *Malassazia furfur*, *Candia albicans* [1-10]. Nowadays, newer potent and less toxic triazoles and echinocandins are often recommended as first-line drugs for many invasive fungal infections. Fluconazole, and Itraconazole are the preferred oral agents. We are more likely to get a fungal infection if have a weakened immune system or take antibiotics. Fungi can be difficult to kill. For skin and nail infections, you can apply medicine directly to the infected area. Oral antifungal medicines are also available for serious infections. Antibiotics are mostly ineffective against fungal infections.

MATERIALS AND METHODS

Reagents

All chemicals used were of Ranbaxy Lab. Ltd. Delhi.

Equipment

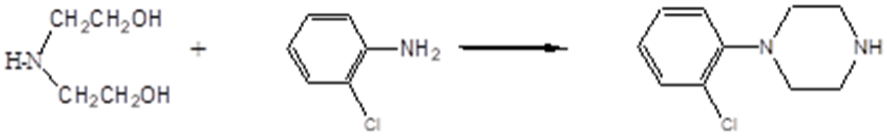
All the melting points were determined in thiel's tube and are uncorrected. Infrared spectrums were recorded using Nujol on Shimadzu Fourier-Transform Infrared Spectroscopy (FTIR)-84005 spectrophotometer [11-15]. Proton resonance magnetic spectra (^{1}H-NMR) were recorded on 300 MHz spectrophotometer and chemical shifts were expressed in parts per million (δ ppm), downfield from TMS as an internal standard. All the liquid intermediates were checked for their purity using gas chromatography, column SE-30 and carbowax 5% [except where mentioned].

TLC Analysis

Thin layer chromatography was performed using plates coated with Silica Gel G. Plates were visualized by UV light and iodine vapour.

*Synthesis of alkyl/aryl piperazine*s

a) Synthesis of 2-chlorophenyl piperazine:



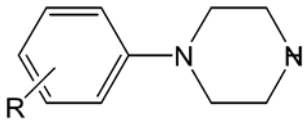
The following procedure was adopted for the synthesis of various aryl piperazines.

In a typical experiment, a mixture of 75 gm (0.71 moles) of Diethanolamine and 92 gm (0.7 moles) of 2-chloroaniline hydrochloride, in a three necked RBF, was heated with stirring at 215-225°C, where upon a continuous stream of anhydrous hydrogen chloride was passed into the mixture by means of perging tube placed below the surface of a molten reaction mass. The mixture was kept stirring under the foregoing condition for 60 min [3,8,16-18]. At the end of this time, the reaction mixture was cooled to 120°C, dissolved in cold water and then made alkaline to phenolphthalein at a temperature below 110°C by the slow addition of aqueous 50% sodium hydroxide (32 gm) with stirring. The organic layer was separated at 50°C and distilled under reduced pressure through fractionating column. Yield: 79 gm (54%) Rf: 0.67 [chloroform: methanol (9:1)].

Following general procedure was used for purification of aryl piperazines:

In a typical experiment, crude 4-chlorophenyl piperazine (6.8 gm, 0.04 moles) was added to 35 mL methanol and phosphoric acid (85%, 3.5 mL, 0.035 moles) was added drop wise. This mixture was refluxed on water bath, cooled to room temperature; the phosphate salt was filtered and washed with methanol. A test portion was made alkaline and checked for presence of aniline by GC. If aniline was found absent dephosphated by adding methanolic potassium hydroxide (28 gm in 100 mL methanol) till alkaline (pH- 11-12). It was then refluxed on water bath for 10 min. and then filtered. The filtrate was distilled to remove methanol and then upon molecular distillation under high vacuum gave pure 4-chlorophenyl piperazine (6.0 gm, yield 83%) at 1200-140°C (at 5 mm of Hg) [19-26]. If it found contaminated with aniline, the phosphate salt was recrystallized from boiling water and crystallization is repeated till free from aniline.

The purity of 4-chlorophenylpiperazine was analysed by Gas Chromatography (Ov101, 10%, 100 C/min, 300°C, injection temp. 280°C).

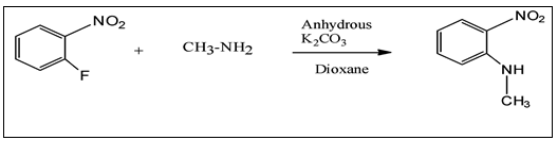


The piperazines thus synthesized are summarized in Table 1.

Compound			GC*	
S. No.	**R**	**Yield (%)**	**Purity (%)**	**Retention time (min)**
1	Phenyl	79	99.29	4.7
2	2-fluoro phenyl	87	96.2	8.2
3	4-Isoprpyl phenyl	76	98.67	5.3
4	2-Chlorophenyl	83	97.66	4.9
5	3-Trifluoromethyl phenyl	88	99.98	10.76

Table 1: Various piperazines synthesized

b) Synthesis of N-methyl-2-nitroaniline:

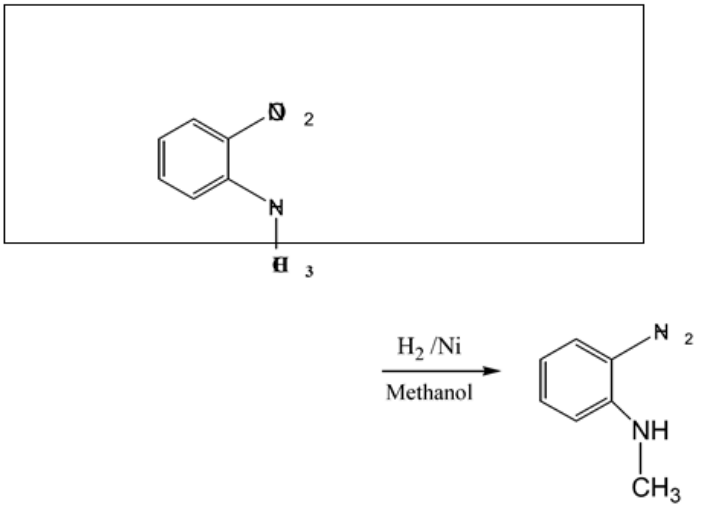


Following general method was adopted for the synthesis N-methyl-2-nitroaniline.

In a three neck 500 mL RBF (equipped with stirrer and condenser), placed 95.5 gm (0.67 moles) of 2-floronitrobenzene in Dioxane as a solvent and 110 gm (0.804 moles) anhydrous potassium carbonate, then 75 mL (2.5 moles, 40% solution) of methylamine was slowly added it through dropping funnel in cold condition, after complete addition of methylamine solution, reaction mixture was heated on a water bath for 3 hrs. The completion of reaction was checked by monitoring TLC [19,22,27-33]. After completion of reaction, dump the reaction mixture was dumped in ice cold water with stirring, and extracted with the product with ether, separated ether layer and dried it with sodium sulphate, then ether was

removed by distillation to get product. Melting point: 360-380 C (Checked by Freezing) Yield: 81 gm (79%) Rf: 0.86 [Chloroform: Methanol (8:2)].

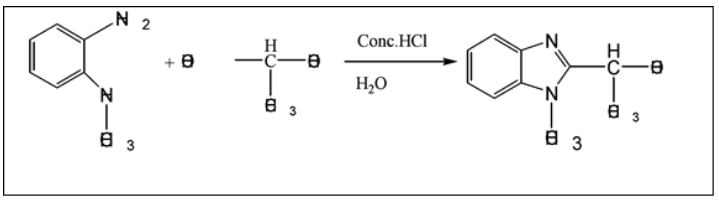
c) Synthesis of N^1-methyl-o-Phenylenediamine:



Following general method was adopted for N1-methyl-o-Phenylenediamine.

In a typical experiment, 30 gm (0.19 moles) N1-methyl-2-nitroaniline was dissolved in 200 mL methanol. To the clear solution 15 gm of Raney Ni W-2 catalyst (wet cake) was suspended. The reaction mixture was hydrogenated in 2 L. Stainless steel bottle at pressure of 3 kg till the hydrogen uptake is complete (4-5 hrs). The completion of reaction was checked by TLC [34-38]. The catalyst was filtered on a bed washed with 50 mL methanol. The solvent was distilled under diminished pressure to get the product. It was purified by high vacuum (0.2 mm of Hg) distillation. Boiling point: 118-121°C at 10 mm of Hg. Melting point: 188°C-190°C. Yield: 18 gm (77%) Rf: 0.76 [Chloroform: Methanol (8:2)]

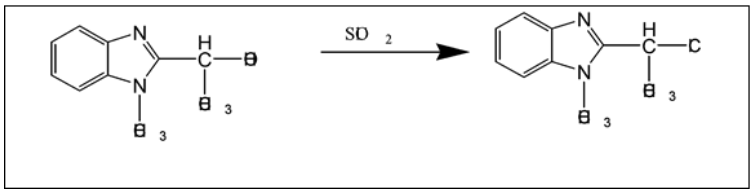
d) Synthesis of 2-α-hydroxy ethyl-N1-methylbenzimidazole:



Following general method was adopted for Synthesis of 2-α - hydroxy ethyl - N1 - methyl benzimidazole.

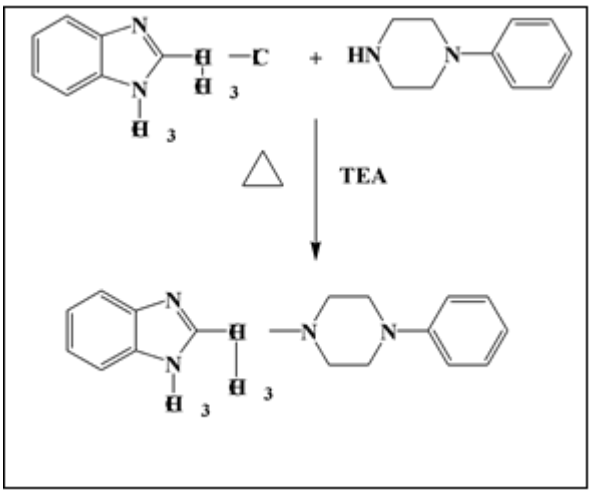
In a typical experiment, 20 gm (0.163 moles) 4-chloro-N1-methyl-o-phenylenediamine, 15 mL (0.170 moles) of lactic acid and 25 mL of conc. HCl was taken in a RBF and refluxed for 4 hrs. The reaction was monitored by TLC. A test portion was dumped in water and basified with ammonia solution [39-42]. The solid was extracted with Methylene dichloride (MDC) and TLC of this MDC extract was checked for the completion of reaction. After completion of the reaction, the reaction mixture was poured in ice-cold water. It was then basified with conc. ammonia solution. The solid precipitated was filtered immediately and dried. Melting point: 580-600°C Yield: 16 gm (55%) Rf: 0.61[chloroform: acetone (8:2)]

Synthesis of 2-α-chloro ethyl-N^1-methylbenzimidazole:



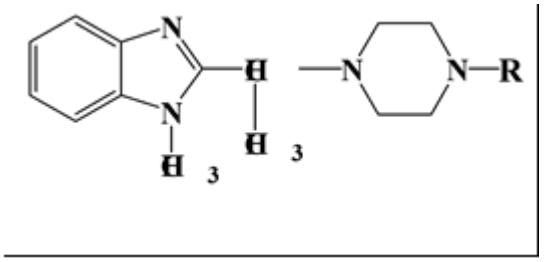
Following general method was adopted for Synthesis of 2-α-chloro ethyl-N1-methyl benzimidazole In a 250 mL three necks RBF, 75 mL thionyl chloride was transferred to a RBF and placed in an ice cold water bath. To it 15 gm of 2-α-hydroxy ethyl-N1- methyl benzimidazole was added slowly with occasionally shaking. Then RBF placed on heating mantel, fitted with a condenser and refluxed for 4 hrs. Excess thionyl chloride was recovered under vacuum on water bath. To the residue dry dioxane was added and stirred for half hour. Dioxane was recovered under vacuum to get product. Melting point: 640-660°C Yield: 12 gm (71%) Rf: 0.42 [Chloroform: Acetone (8:2)] [43].

Synthesis of 1-ethyl-2-[α-phenyl (4-N- alkyl/aryl piperazinyl)] ethyl Benzimidazole:



The following method was adopted for the synthesis of 1-ethyl-2-[α-phenyl (4-N- alkyl/aryl piperazinyl)] ethyl Benzimidazole. In a typical experiment, 1 gm (0.005 moles) of 2-α-chloro ethyl-N1-ethyl benzimidazole, 1.47 gm (0.005 moles) of N-Phenyl piperazine were dissolved in dry dioxane and mixed in an RBF. To it 0.76 mL (0.005 moles) of triethylamine was added and the reaction mixture was refluxed for 8 hrs.

The reactions was monitored by TLC. The reaction mixture was then dumped in ice cold water and the precipitate was collected by suction and dried. Melting point: 920 - 960 C Yield: 86+2 gm. TLC checked by mobile phase of [Chloroform: Acetone] (8:2)



The final compounds were purified and their structures were established by Infra-red, NMR and mass spectra. The final compounds were screened for antifungal activity.

Spectral Analysis

N-Methyl-2-α-[N 4-(phenyl) Piperazinyl] Ethyl Benzimidazole. (VP1) ^{1}H-NMR (DMSO): δ=2.14(d, 3H), 3.89 (s, 3H), 5.26 (q, 1H,), 7.27(s, 2H), 7.7 (s, 1H,) N-Methyl-2-α-[N 4-(2-fluorophenyl) Piperazinyl] Ethyl Benzimidazole; (VP2): IR (nujol): 3020.63 (CH), 1589 (C=N), 1481 (C=C), 1215 (C-N) cm^{-1}; ^{1}H-NMR (DMSO): δ=1.62(d, 3H), 2.71 (s, 4H), 3.03 (s, 4H), 3.95 (s, 3H), 4.10(q, 1H), 6.92-7.04 (m, 4H), 7.25 (s, 2H), 7.74 (s, 1H,).

N-Methyl-2-α-[N 4-(4-isopropylphenyl) Piperazinyl] Ethyl Benzimidazole. (VP3): IR (nujol) 3020.63 (CH), 1612 (C=N), 1465 (C=C), 1215(C-N) cm^{-1}; ^{1}H-NMR (DMSO): δ=1.59(d, 3H), 2.73 (s, 4H), 3.06 (s, 4H), 3.90(s, 3H), 4.14(q, 1H), 6.92-7.04(m, 4H), 7.25(s, 2H), 7.74(s, 1H,).

N-Methyl-2-α-[N 4-(2-chlorophenyl) Piperazinyl] Ethyl Benzimidazole. (VP4): IR (nujol) 3016(CH), 1612(C=N), 1465(C=C), 1215(C-N, vib), 1060(C-O, Aro), 1030(C-O, ali) cm^{-1}.

N-Methyl-2-α-[N 4-(3-trifluoromethylphenyl) Piperazinyl] Ethyl Benzimidazole. (VP5): IR (nujol) 3020(C-H), 1608(C=N), 1473(C=C), 1388 (C-H bend), 1215(C-N, vibe) [44].

Antifungal activity

The targeted compounds synthesized were screened for the antifungal potential against *C. albicans* was found to be sensitive to Itraconazole, Fluconazole, Ketoconazole and Clotrimazole but developed resistance against common antifungal antibiotics such as Nystatin and Amphoterecin-B. For convenience the synthesized compounds were coded by alphabets (VP). A stock solution of 1 µmol/ml of each compound was prepared. The fungistatic assay was carried out using sabouraud's liquid medium [45] (Figure 1).

Compound sensitivity Testing

Disc Method: Sensitivity of compounds was tested towards organism by Disc method.

Depending upon diffusion of drug from filter paper disc through Sabourd's agar layer in Petri dish to an extent such that growth of added microorganism is prevented entirely in a zone around disc containing solution of drug.

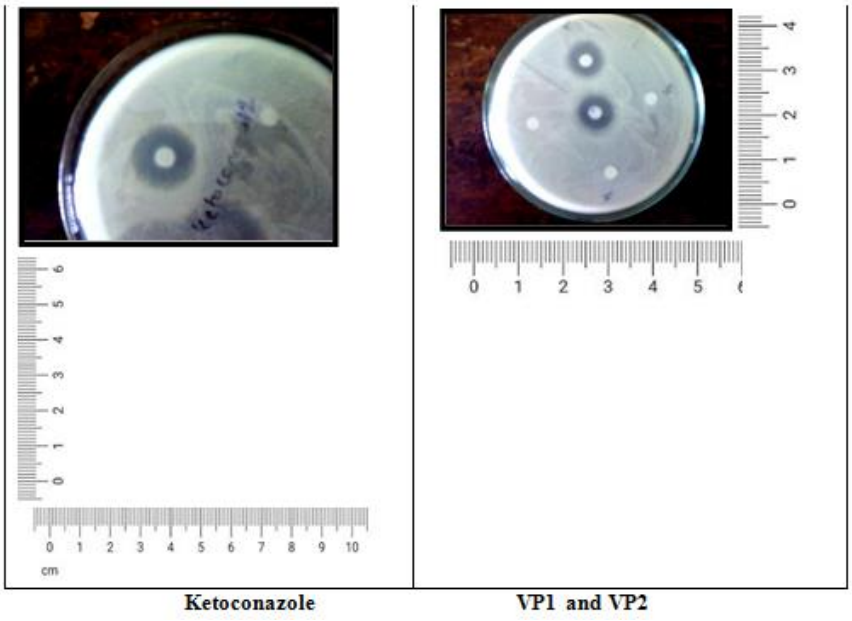


Figure 1: Antifungal activity against *Candida albicans*

Evaluation of antifungal activity

The antifungal activity was evaluated by the tube dilution method (turbidimetric method). The turbidimetric method depends upon the inhibition of growth of a microbial culture in a uniform solution of drug in a fluid medium that is favorable to its rapid growth. In this method, minimal inhibitory concentration (MIC) of the antifungal agent is determined. The MIC is the lowest concentration of an antimicrobial agent that inhibits the test organism. The growth in the tube was observed visually for turbidity and inhibition was determined by the absence of growth. MIC was determined by the lowest concentration of the sample that prevented the development of turbidity [46].

Procedure

The stock solution of (1 µmol/ mL) of compounds was prepared in DMSO and water. To each tube containing sterilized Sabouraud's liquid medium (1 mL), 1 mL of drug solution were added. Each tube was inoculated with the microorganism and was kept at 30°C for 14 days. Theserial dilutions were made to obtain concentrations (in µ mol/ mL) such as 0.125, 0.0625 and 0.0314. Positive control tubes (organism+broth+drug) and negative control tubes (Broth+DMSO+Drug) were also prepared (Figure 2).

Fungal strain used was *C. albicans.* All the tubes were incubated at 30°C for 14 days. The readings were taken and expressed as (-), if inhibition of growth is seen and (+), if inhibition of growth is not seen (Table 2).

S. No.	Compounds	Concentration of compound required for inhibition (µmol/ml)				
		0.125	0.0625	0.0312	0.015	0.007
1	VP1	-	-	+	+	+
2	VP2	-	-	-	+	+
3	VP3	-	+	+	+	+
4	VP4	-	-	+	+	+
5	VP5	-	+	+	+	+
6	Ketoconazole	-	-	-	-	-

(-) Indicates absenceof growth; (+) Indicates presence of growth

Table 2: Results of serial dilutions for *Candida albicans.*

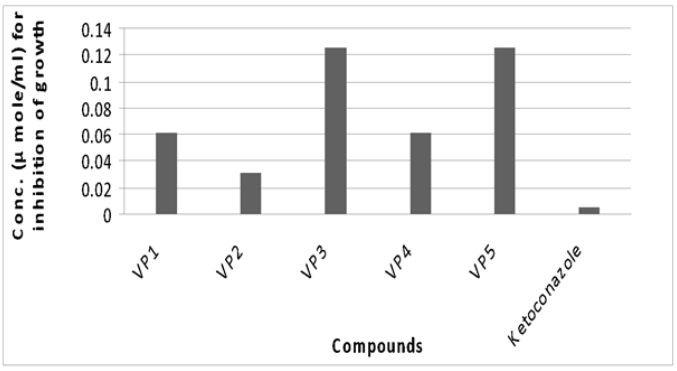


Figure 2: Antifungal activity against *Candida albicans*

RESULTS AND DISCUSSION

The present investigations were based upon following observations:

1. Unexploited 1-alkyl benzimidazole derivatives
2. Antifungal potential of N-aryl/alky piperazines.
3. Novel medicinal applications of simple N-alkyl benzimidazoles.

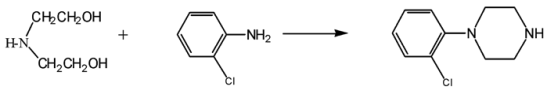
Many benzimidazole derivatives substituted with piperazines have been synthesized and evaluated for antifungal activity in our department previously .

From the limitations of therapeutically available benzimidazole containing antifungal agents and previous experiences of our department, we have synthesized different substituted aryl piperazines, N-methyl-2-[α-(chloro) piperazin-1-yl] ethyl benzimidazole nucleus and condensed them to offer targeted compounds [47].

The investigation was designed in following manner:

- Synthesis of various substituted aryl piperazines
- Synthesis of N-methyl-2-α-(chloro)-Ethyl-Benzimidazole nucleus.
- Synthesis of variousN-methyl-2-α-(N4-aryl)piperazin-1-yl)-Ethyl-Benzimidazole
- Establishment of structures of targeted compounds on the basis of Infra-red spectra, NMR spectra and mass spectra.
- Evaluation of targeted compounds for the antifungal activity against C. albicans. Following synthetic schemes was developed to synthesize the targeted compounds.

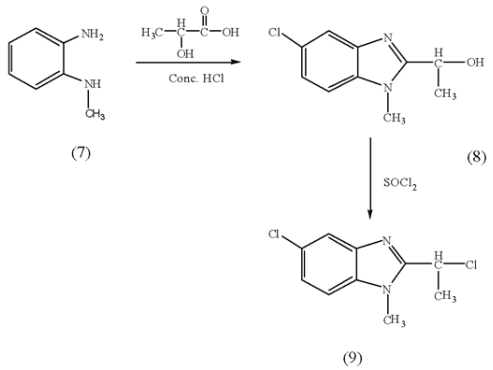
Scheme 1



(1) (2) (3)

Synthesis of aryl piperazines were planned and following method adopted. Diethanolamine hydrochloride (1) and substituted aniline (2) in equimolar quantities were heated for a period of 8 hr to give substituted N-phenylpiperazine (3). The diversified activities of benzimidazoles and piperazines reveal the importance of both in the field of medicinal chemistry. In the present work we have synthesized condensation products of aryl piperazines and N-methyl-2α-chloro-ethyl benzimidazole. Commercially ortho and para substituted anilines are synthesized by displacing chlorine by ammonia of ortho and p-nitrochlorobenzene at elevated temperatures and pressures. We have adopted similar methods by replacing ammonia with monoalkyl amines

Scheme 2

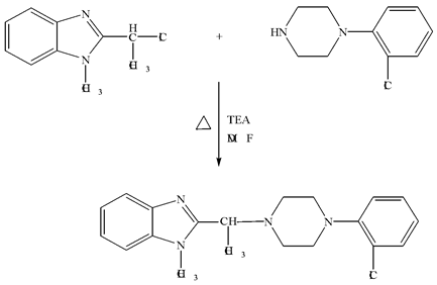


(7) (8) (9)

The N-methyl o-phenylenediamine converted to desired benzimidazole moiety by treatment with concentrated hydrochloric acid and lactic acid that give 2-α-hydroxyethyl-N1-methyl-benzimidazole which upon treatment with Thionyl chloride give 2-α-chloroethyl-N1-methyl benzimidazole. The method has got following advantages:

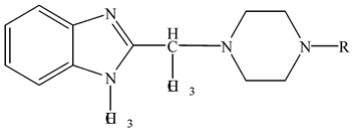
- Basic raw material like O-fluoronitrobenzene is used.
- The intermediate nitro aniline as well as product N-alkyl-o-phenylenediamine obtained in good yields and purities
- The reaction is eco-friendly and does not give color effluents.
- The method is applicable for most of the aliphatic amines.
- The method is possible at low temperature.

Scheme-3



The diversified activities of benzimidazoles and piperazines reveal the importance of both in the field of medicinal chemistry. In the present work we have synthesized the condensation products of 2-chloroethyl-N-alkyl-benzimidazoles with various substituted phenyl piperazines to synthesize a library of benzimidazole derivatives [48].

Scheme-4



S. No.	R	M.P (°C)	Yield (%)	R_f	Assay (%)
1	Phenyl	92-96	82	0.7	99.2
2	2-fluoro phenyl	112-116	65	0.6	98.38
3	4-Isoprpyl phenyl	80-84	71	0.5	98.02
4	2-Chlorophenyl	135-140	60	0.52	98
5	3-Trifluoromethyl phenyl	Low melting solid	75	0.64	97

Table 3: Various N-Methyl-2-[α-(N4-Aryl) Piperazinyl] Ethyl Benzimidazoles

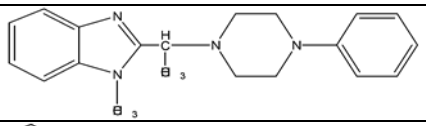
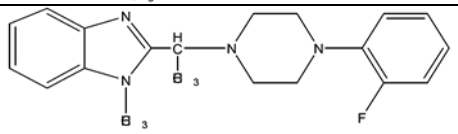
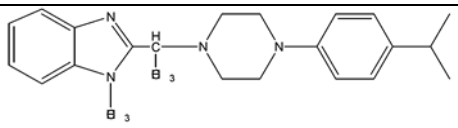
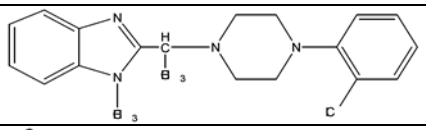
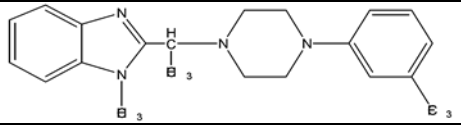
S. No.	Code	Structure
1	VP1	
2	VP2	
3	VP3	
4	VP4	
5	VP5	

Table 4: Various N-Methyl-2-[α-(N^4-Aryl) Piperazinyl] Ethyl Benzimidazoles

Spectral analysis

The IR spectral data of all synthesized compounds confirmed the structures as shown in spectral analysis. The major functional groups observed are C=N at 1670 cm^{-1}, C-N at 1153 cm^{-1}, C-H at 3016 cm^{-1}, C-Cl at 681 cm^{-1}, C-F at 1014 cm^{-1} (Tables 3 and 4). ^{1}H-NMR of two representative compounds (VP1 and VP2) was taken and the same fully supported the structures of that compound. The major functional groups splitting observed is alkane at 3.98 ppm (singlet Hb) for CH_3, 1.62 ppm (doublet Ha) for CH_3, Aromatic at 6.91-7.4 ppm, in piperazine -CH_2 at 2.7-3.2 ppm [48]. We failed to get mass spectra of our final compounds because

- In most of the antifungal drugs, long structure is present. The structure is much complex and needs a lengthy synthetic sequence to achieve the final product. This makes bulk drug expensive, which indirectly increases the cost of the treatment, also development of resistance and selectivity are problems associated with it. The generic market dominates the patented drugs in third world countries on the cost of treatment

issue. Therefore, producing a simple, inexpensive molecule with more or less identical activity to patented drug molecule has become a need for third world countries.

- This combination of benzimidazole and aryl piperazine was found to be active at 30-120 nano-mole /ml concentrations. VP2 was identified as highly active derivative and has shown MIC of 0.0312 µmole /ml against resistant strains of *C. albicans*. The compound VP1 and VP4 have shown MIC of 0.0625 µmole /ml, while the compounds like VP3 and VP5 (MIC-0.125 µmole /ml) has shown moderate antifungal activity.

CONCLUSION

This investigation opens new area of antifungal agents, which are cheaper and simple than the existing azoles antifungal agents.

FUTURE SCOPE

There is wide scope for further investigation in this area such as

1. 5-H can be substituted with chloro, alkoxy group and alkyl group.
2. The 1-alkyl substitution can be varied from C1-C4 according to hydrophobicity requirement.
3. Various alkyl or aryl substitutions can be made at N4 of piperazine.
4. Aryl piperazines can be substituted with 4-amino phenyl piperazine.

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