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Synthesis and evaluation of antifungal activity of 5-chloro-1-methyl-2-α-[(N4-aryl) piperazin-1-yl] ethyl benzimidazoles

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ABSTRACT

The availability over the past 2 decades of the azole antifungal agents represents a major advance in management of systemic fungal infections. Like miconazol, fluconazole itraconazlole. These drugs having some drawbacks like interaction with co administered drugs, emergence of resistance of fungal organisms. From the limitations of therapeutically available benzimidazole containing antifungal agents and previous experiences of our department, we have synthesized different substituted aryl piperazines, 5-chloro-1-methyl-2-[α-(chloro) piperazin-1-yl ethyl benzimidazole nucleuses and condensed them to offer targeted compounds. Compounds were characterized by IR, 1H NMR. All the compounds were tested for their antifungal activity against fungal strain of Candida albicans. Most of the compounds showed moderate to good anti-fungal activity.

Key words: Antifungal, Benzimidazole, Piperazines, *Candida alicans,*

INTRODUTION

The availability over the past 2 decades of the azole antifungal agents represents a major advance in management of systemic fungal infections. Miconazole, the first azole drug approved and now recently withdrawn from the market, was available only as a highly toxic IV formulation; consequently, it was only rarely used [1-7]. By contrast, the three oral azoles, ketoconazole, an imidazole, and, especially, itraconazole and fluconazole (both triazoles), have become frequently used therapeutic alternatives to amphotericin B. The relative broad spectrum of activity of the azoles against common fungal pathogens, ease of administration and limited toxicity are highly attractive features. Fluconazole and itraconazole are better tolerated and more effective than ketoconazole. These agents have several drawbacks and limitations also. One potential limitation of the azole antifungal agent is the frequency of their interaction with co administered drugs, which results in adverse consequences. A second limitation of the azoles is the emergence 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 [8-12].

The present investigations were based upon following observations:
Unexploited substituted 1-alkyl benzimidazoles. Attractive biological profile of N-aryl piperazines. Novel medicinal applications of simple N-alkyl-2-piperazinyl benzimidazoles. Enantiomer selectivity towards antifungal activity. 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, 5-chloro-1-methyl-2-[α-(chloro) piperazin-1-yl] ethyl benzimidazole nucleus and condensed them to offer targeted compounds.

The investigation was designed in following manner

- Synthesis of various substituted aryl piperazines
- Synthesis of 5-Chloro-1-methyl-2-α-(chloro)-Ehyl-Benzimidazole nucieus.
- Synthesis of targeted compounds
- 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.

MATERIALS AND METHODS

Reagents

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

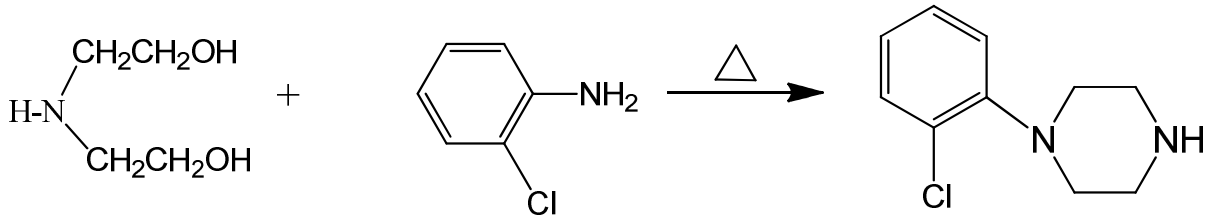
Equipment

All the melting points were determined in thiel's tube and are uncorrected. Infrared spectrums were recorded using Nujol on Shimadzu FTIR-84005 spectrophotometer. Proton resonance magnetic spectra (1H NMR) were recorded on 300MHz 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.

1. Synthesis of aryl piperazines:



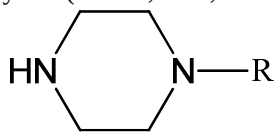
Synthesis of 2-chlorophenyl piperazine: -

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

In a typical experiment, a mixture of 75g (0.7mol) of diethanolamine and 92g (0.7mol) of 2-chloroaniline hydrochloride, in a three necked RBF, was heated with stirring at 215-225^{0}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 60min. At the end of this time, the reaction mixture was cooled to 120^{0}C, dissolved in cold water and then made alkaline to phenolphthalein at a temperature below 110^{0}C by the slow addition of aqueous 50% sodium hydroxide (32g) with stirring. The organic layer was separated at 50^{0}C and distilled under reduced pressure through fractionating column. Yield: 79g (54%) Rf: 0.67 [chloroform: methanol (9:1)]

Following general procedure was used for purification of aryl Piperazines:

In a typical experiment, crude 2-chlorophenylpiperazine (6.8g, 0.04mol) was added to 35ml methanol and phosphoric acid (85%, 3.5ml, 0.035mol) 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 (28g in 100ml methanol) till alkaline (pH- 11-12). It was then refluxed on water bath for 10min. And then filtered. The filtrate was distilled to remove methanol and then upon molecular distillation under high vacuum gave pure 2-chlorophenylpiperazine (6.0g, yield 83%) at 120-140^{0}C (at 5mm Hg). If found contaminated with aniline the phosphate salt was recrystallised from boiling water and crystallization is repeated till free from aniline. The purity of 2-chlorophenylpiperazine was analyzed by GC (Ov101, 10%, 10^{0}C/min, 300^{0}C, injection temp. 280^{0}C)



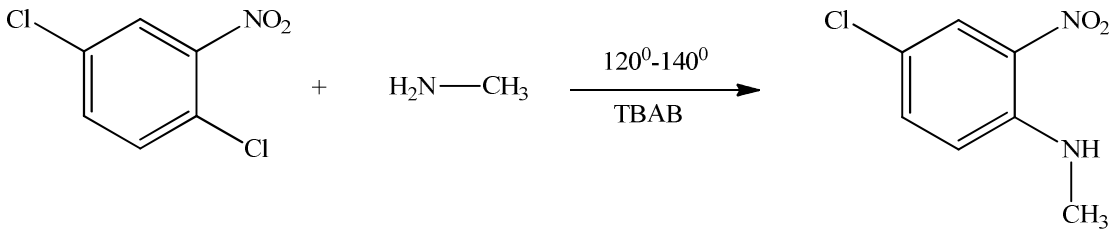
The piperazines thus synthesized are summarized in table no.1

Table 1: Various piperazines synthesized

Sr.No	R	Yield(%)	GC* Purity (%)
1	2-Chlorophenyl	79	99.29
2	2- fluorophenyl	87	96.20
3	2-methoxyphenyl	76	98.67
4	4-isopropylphenyl	83	97.66
5	3-trifluromethylphenyl trifluromethylphenyl trifluromethylphenyl	88	99.98

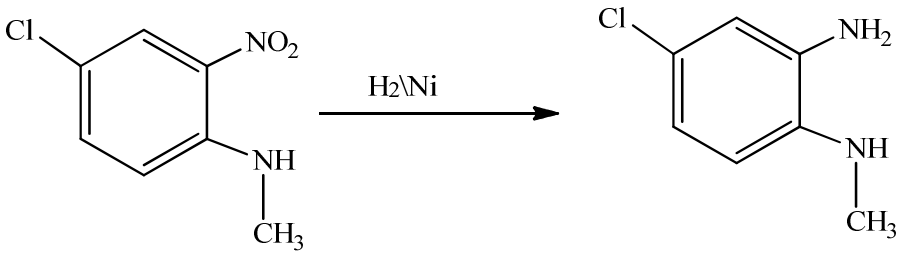
** (Column Ov 101, 7^{0}C/min, 300^{0}C, injection temp. 280^{0}C)*

1. Synthesis of 4-chloro2-nitro-N-methyl aniline:



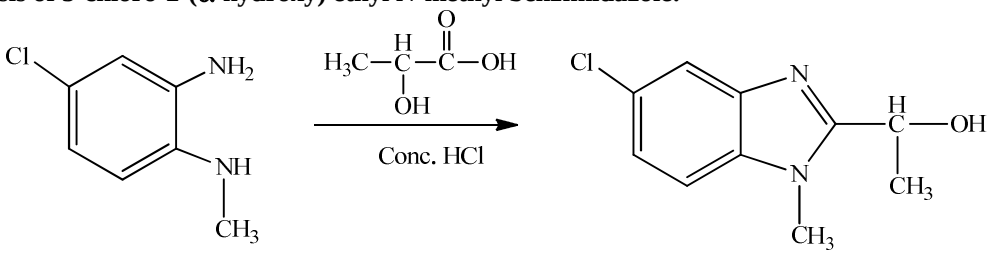
In an autoclave reactor vessel, 100 gm (0.52mol) of 2, 5- dichloronitrobenzene, 40.35 gm (0.52mol, 40% solution) of monomethyl amine and 10 gm of TBAB, a phase transfer catalyst was added. Then reaction was well maintained between temperature range of 120^0-140^0 and pressure of 3-4 Kg for 7-8 hrs. The completion of reaction was checked by monitoring TLC. After completion of reaction, the reaction mixture was filtered and the above solid product is collected, dried and recrystallised to get pure 4-chloro2-nitroN-methyl aniline. Melting Point: 86^0-89^{0}C Yield: 78gm (80%) R_f: 0.6 [Toluene: methanol (8:2)]

2. Synthesis of 4-chloro-N-methyl-o-phenylenediamine:



In a typical experiment, 25g (0.133mol) of p-chloro–o-nitro N-Me aniline was dissolved in 200ml methanol. To the clear solution, 15gm of Raney Nickel W-2 catalyst (wet cake) was suspended. The reaction mixture was hydrogenated in 2lit. stainless steel bottle at pressure of 3kg till the hydrogen uptake was completed (6-8hrs.). The completion of reaction was also checked by TLC. The catalyst was filtered on a hyflow bed and washed with 50ml methanol. The solvent was distilled under diminished pressure. Melting Point: 90^{0}C-95^{0}C Yield: 18gm (85%) R_f: 0.56 [Toluene: methanol (8:2)]

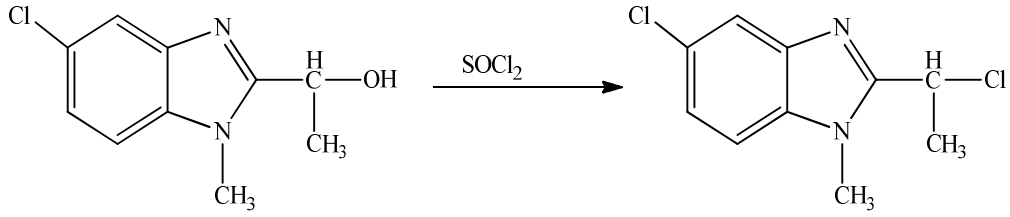
3. Synthesis of 5-chloro-2-(α-hydroxy) ethyl N-methyl benzimidazole:



In a typical experiment, 25g (0.159moles) 4-chloro-N-methyl-0-phenylenediamine, 14.94 gm (0.166moles) of lactic acid and 25 ml of conc. Hydrochloric acid was taken in an RBF and refluxed for 8hrs. The reaction was monitored by TLC. A test portion was dumped in water and basified with ammonia solution. The solid was extracted with ether and TLC of this ether 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

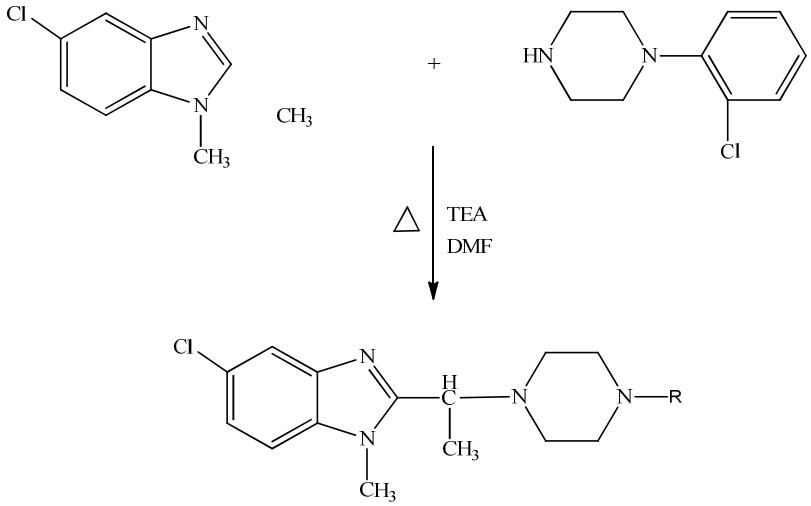
precipitated was filtered immediately and dried. Melting Point: 110^{0}C Yield: 26gm (77%) R_f: 0.44 [Toluene: methanol (8:2)]

4. Synthesis of 5-chloro- 2-(α-chloro) ethyl N-methylbenzimidazole:



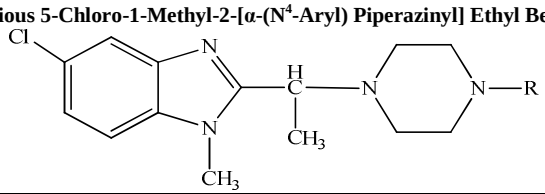
In a 250 ml three necked RBF, 75ml thionyl chloride was taken, cooled in an ice bath .To it, 15g of 5-chloro-2-(α-hydroxy)ethyl N-methyl benzimidazole was added slowly with occasionally shaking. The RBF fitted with condensor was placed on heating mantle and refluxed for 10 hrs. Later excess thionyl chloride was recovered under water vacuum on a waterbath. To the residue dry dioxane was added and stirred for half hour and dioxane was removed under vacuum to get product. Melting point: 136^{0}C Yield: 12gm (74%) R_f: 0.68 [chloroform: acetone (8:2)]

5. Synthesis of 5-chloro-2-α [(N^{4}aryl) piperazinyl] ethyl N- methylbenzimidazole:



In a typical experiment, 1g (0.004mol) of 5-chloro-2-α-chloro ethylN-methyl benzimidazole, 0.85gm (0.004mol) of substituted phenyl piperazines were separately dissolved in dry dioxane and mixed in a RBF. To it 0.6ml (0.004mol) of triethylamine was added and the reaction mixture was refluxed for 12hrs.The reaction was monitored by TLC. The reaction mixture was then dumped in ice cold water and the precipitate was collected by suction and dried. The solid was recrystallised from acetone. TLC checked by mobile phase of Chloroform: ethyl acetate (8:2)

Table 2: Various 5-Chloro-1-Methyl-2-[α-(N^4-Aryl) Piperazinyl] Ethyl Benzimidazoles.



Sr.No	Code	R	m.p. (^{0}C)	Yield *(%)*	R_f*	Assay (%)
1.	RG-1	2-Chlorophenyl	195	82	0.6	89
2.	RG-2	2- fluorophenyl	145	65	0.52	90
3.	RG-3	2-methoxyphenyl	150	71	0.48	89
4.	RG-4	4-isopropylphenyl	110	57	0.5	82
5.	RG-5	3-trifluromethylphenyl	120	66	0.64	86

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

5-Chloro-1-Methyl-2-α-(Chloro) Ethyl Benzimidazole. (RG) 1H NMR (DMSO): δ = 2.14(d, 3H,), 3.89(s, 3H), 5.26 (q, 1H,), 7.27(s, 2H), 7.7(s, 1H,)

5-Chloro-1-Methyl-2-α-[N^4-(2-chlorophenyl) Piperazinyl] Ethyl Benzimidazole. **(RG1)** IR (nujol): 3020.63(C-H), 1589(C=N), 1481(C=C), 1215(C-N) $^{cm-1}$
1H 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,)

5-Chloro-1-Methyl-2-α-[N^4-(2-fluorophenyl) Piperazinyl] Ethyl Benzimidazole. (RG2) IR (nujol) 3020.63(C-H), 1612(C=N), 1465(C=C), 1215(C-N) $^{cm-1}$
1H 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,)

5-Chloro-1-Methyl-2-α-[N4-(2-methoxyphenyl) Piperazinyl] Ethyl Benzimidazole,(RG3) IR (nujol) 3016(C-H), 1612(C=N), 1465(C=C), 1215(C-N, vib), 1060(C-O, Aro), 1030(C-O, ali) $^{cm-1}$

5-Chloro-1-Methyl-2-α-[N^4-(4-isopropylphenyl) Piperazinyl] Ethyl Benzimidazole. (RG4) IR (nujol) 3016(C-H), 1612(C=N), 1465(C=C), 1215(C-N, vib) $^{cm-1}$

5-Chloro-1-Methyl-2-α-[N^4-(3-trifluoromethylphenyl) Piperazinyl] Ethyl Benzimidazole. (**RG5)** IR (nujol) 3020(C-H), 1608(C=N), 1473(C=C), 1388 (C-H bend), 1215(C-N, vibe) $^{cm-1}$

Biological evaluation

ANTIFUNGAL ACTIVITY

The targeted compounds synthesized were screened for the antifungal potential against *Candida albicans*., isolated from sputum sample of patient and 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 (**RG**). A stock solution of 0.250µmol/ml of each compound was prepared. The fungistatic assay was carried out using sabouraud's liquid medium.

The media used for the microorganism was the double strength Sabouraud's broth. The composition of the Sabouraud's broth is given below

Table 3: Composition of Sabourauds broth

Sr. no.	Ingredient	Quantity (gm)
1	Glucose	40
2	Peptone	10
3	Water	q.s. to 1000ml

Glucose and peptone were dissolved in water with heating, cooled and the pH was adjusted to 5.4 with lactic acid and filtered. Total matrix was sterilized at 120^0C for 15 minutes.

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.

PROCEDURE

The stock solution of (1µmol/ml) of compounds was prepared in DMSO and water. To each tube containing sterilized Sabouraud's liquid medium (1ml), 1ml of drug solution were added. Each tube was inoculated with the microorganism and was kept at 30^0C for 14 days. The serial dilutions were made to obtain concentrations (in µmol/ml) such as 0.125, 0.0625 and 0.0314. Positive control tubes (organism + broth + DMSO) and negative control tubes (broth + drug) were also prepared.

Fungal strain used was *Candida albicans***.**
All the tubes were incubated at 30^0C 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 4: Results of serial dilutions for *Candida albicans*

Sr. no.	Compounds	Concentration of compound required for inhibition (µmol/ml)				
		0.125	0.0625	0.0312	0.015	0.007
1	**RG1**	-	-	+	+	+
2	**RG2**	-	-	-	+	+
3	**RG3**	-	+	+	+	+
4	**RG4**	-	+	+	+	+
5	**RG5**	-	+	+	+	+
6	**Ketoconazole**	-	-	-	-	-

(-) Indicates absence of growth. (+) Indicates presence of growth.

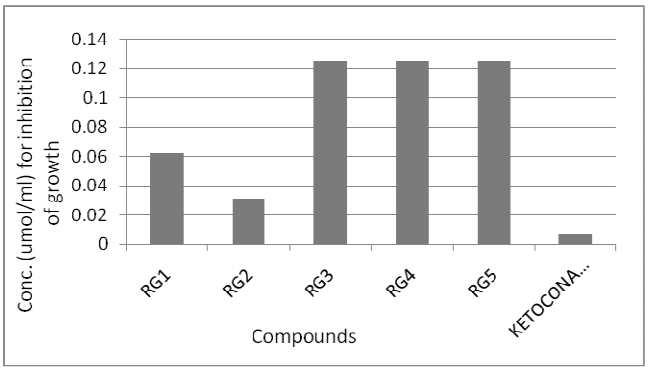


Fig 1: Antifungal activity against *Candida albicans*

RESULTS AND DISCUSSION

All the synthesized compounds were screened for antifungal activity against *candida albicans* ATCC10231. The results of antifungal activity are quite good but compounds were comparatively less active than standard. RG2 was identified as highly active derivative and has shown MIC of 0.0312 µmole /ml against resistant strains of *C.albicans*. The compound RG1 has shown MIC of 0.0625 µmole /ml, while the compounds like RG3, RG4 and RG5 (MIC-0.125 µmole /ml) has shown moderate antifungal activity.

CONCLUSION

The combination of benzimidazole and aryl piperazine was found to be active at 30-120 nanomole/ml concentrations. Our prediction for the use of benzimidazole for heme-ligation and the aryl piperazine fragment for selectivity in the inhibition of fungal Cytochrome P_{450} iso-enzymes was found to be successful. This investigation opens new area of antifungal agents, which are cheaper and simple than the existing azoles antifungal agents.

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