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Original Research Paper

DESIGN, SYNTHESIS AND ANTIFUNGAL ACTIVITY OF SOME BENZYL BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

Fungal infections are increasing rapidly due to an increase in number of immuno-compromised hosts. Azoles are first line drugs for treatment of fungal infections due to their high therapeutic index but development of resistance due to extensive use of azoles and toxicity has significantly reduced the efficacy of azole antifungal agents and led to the search for new azoles. Azoles act by competitive inhibition of cytochrome P 450 (CYP450) enzyme, Lanosterol 14α-demethylase a key enzyme in biosynthesis of sterol in fungi. From the study of active site of the enzyme and SAR of azole antifungal agents, benzyl benzimidazole derivatives were designed and synthesized. The structures of the synthesized compounds were elucidated by spectral data. The synthesized compounds were screened for antifungal activity against Candida species by serial dilution method using ketoconazole as a reference standard. Among the synthesized compounds, compounds having polar side chains were found to posses significant antifungal activity.

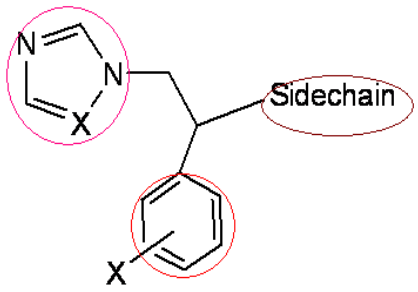
Keywords: Lanosterol 14α-demethylase, Benzyl benzimidazole, Azoles, Fungal infections, Antifungal.

INTRODUCTION

Fungal infections fall into two well-defined groups, the superficial and deep seated mycoses. The superficial mycoses include infections of the hair or hair follicles, the superficial infections of the intertriginous or flat areas of hairless skin and infections of nails. Deep seated mycoses include systemic infections of vital organs like lungs, bones, central nervous system which may develop into the severe and often fatal disease in some patients. Causative organisms for superficial infections include *Candida albicans, Microsporum trichophyton* while systemic infections are caused due to *Cryptococcus neoformans, Asperigillus fumigatus* etc. Fungal infections are increasing rapidly due to an increase in number of immuno-compromised hosts. Azoles are first line drugs for treatment of fungal infections due to their high therapeutic index unfortunately broad use of azoles has led to development of severe resistance. Toxicity associated with use of azoles has significantly reduced the efficacy of azole antifungal agents. This has led to the search for new azoles for treatment of fungal infections. Azoles act by competitive inhibition of cytochrome P 450 (CYP450) enzyme, Lanosterol 14α-demethylase a key enzyme in biosynthesis of sterol in fungi.

From SAR of Azoles, Common Features of Azole Antifungal Agents are[1,2]

- Weakly basic imidazole or 1, 2, 4-triazole ring bounded by N-C linkage to the rest of the structure.
- Two or three aromatic rings at least one of which is halogen substituted and nonpolar functional group.

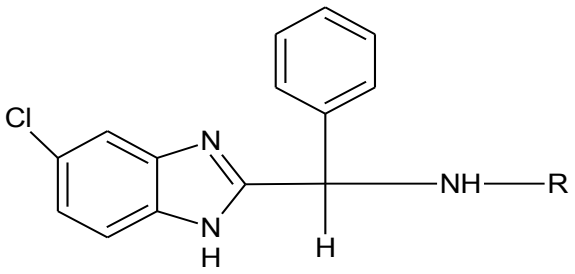


Active Site of CYP 450, Lanosterol l4α-demethylase[3,4]

Three dimensional models of cytochrome P 450, Lanosterol 14^{α}-demethylase of *Candida albicans*, *Aspergillus fumigates* and *Cryptococcus neoformans* and its binding with azole antifungal derivatives are studied. It is found that active site of the enzyme has four regions defined by specific amino acids,

- A hydrophilic hydrogen bond site,
- Site above heme portion representing core hydrophobic area. It is the site where N3(4) of the azoles bind by formation of coordinate bond with the iron atom of the heme.
- A narrow hydrophobic cleft, the diflurophenyl or dichlorophenyl group of azole derivatives fits in this and has hydrophobic interaction with corresponding amino acids.
- Hydrophobic hydrogen bond site, which interact with the side chain, forming strong hydrophobic and van der Walls interaction with enzyme. It gives specificity for the drug for fungal enzyme.

From the study of SAR of azole antifungal agents, active site of enzyme and docking studies of currently used antifungal agents with enzyme (with Shrodinger Maesto 9.2 software), it was decided to synthesize benzyl benzimidazole derivatives and evaluate its antifungal activity.



Where **R** is a triazole derivatives:

a) 5-Phenyl -2H 1,2,4 Triazole-3one
b) 5-(2-Methyl phenyl)- 2H 1,2,4 Triazole 3-one
c) 5-Phenyl-3,4 dihydro-2H 1,2,4 Triazole-3-ol
d) 1,2,4-Triazole 1H 5-thiol
e) 1H 1,2,4-Triazole.

MATERIALS AND METHODS

All chemicals used were of research lab Fine-Chem industry & Spectro-Chem Pvt. Ltd. Mumbai. 3-Amino-1,2,4-triazole-5-thiol and 3-Amino-1,2,4-triazole were purchased from Sigma-Aldrich laboratories. All the melting points were determined in Thiel's tube and are uncorrected. Infrared spectrums were recorded using KBr on Shimadzu FTIR-8400S spectrophotometer. Proton resonance magnetic spectra (1H NMR) were recorded and chemical shifts were expressed in parts per million (ppm), downfield from TMS as an internal standard. Thin layer chromatography was performed using plates coated with Silica Gel "G" to establish identity of reactants and products monitored in between reactions. The spots were visualized by iodine vapors in an enclosed chamber.

EXPERIMENTAL

Synthesis of N-Benzoyl ethylcarbazate

In an RBF 10 gm (0.096 mole) of ethyl carbazate was dissolved in methanol, to it added 10 ml (0.096 mole) of triethyl amine and the solution was cooled at 0-5°C, with constant stirring. To this solution 13.46 gm (0.096 mole) of benzoyl chloride was added dropwise using dropping funnel for 2.5-3 hrs. After this the mixture was refluxed on water bath for 3 hrs and monitored by TLC, after completion of reaction, the reaction mixture was dumped in cold water and extracted with dichloromethane. The solvent was then evaporated by Rotary Evaporator and the product was obtained as white solid crystals.

Synthesis of 5-Phenyl-4-amino-2H-1,2,4-triazole-3-one

A suspension of 5 gm (0.024mole) N-benzoyl ethyl carbazate, 5 ml 80 % hydrazine hydrate (0.040 mole) and water (2 mL) was refluxed with stirring for 0.5 to 1 hours. The reaction was

monitored by TLC and upon completion, the refluxed mixture was dumped in cold water, extracted with dichloromethane. The organic layer was evaporated by Rotary evaporator and the white needle shape crystals of 5-Phenyl-4-amino-1,2,4-triazole-3one were collected as product.

Similarly 5-(2-methyl phenyl)-4-amino-2H-1,2,4-triazole-3-one was prepared using tolyl chloride and 5-(4-nitro phenyl)-4-amino-2H-1,2,4-triazole-3-one was prepared using para nitrobenzoyl chloride.

Synthesis of 5-(4-amino phenyl)-2H-1,2,4-triazole-3-one

In a typical expriment 5 gm (0.024 mole) of 5-(4-nitro phenyl)-4-amino-2H-1,2,4-triazole-3-one was dissolved in dioxane and Raney Nickel catalyst 0.7-0.8 gm. was added to this solution and stirred by magnetic stirrer, and then 4.85 gm (0.097mole) hydrazine hydrate was added drop wise, the reaction was monitored by TLC, after completion of reaction catalyst was filtered carefully and the filtrate was concentrated by Rotary evaporator, after complete evaporation of solvent brownish needle shape crystals was obtained.

Synthesis of 5-chloro-2-(α-hydroxybenzyl) benzimidazole[5,6,7]

In a 500 ml of round bottom flask, equimolar amount of 4-chloro-1,2-phenylenediamine 10 gm (0.01mol) and mandelic acid 10.62 gm (0.01mol) were placed. 18 ml of 4N HCl was added followed by a few porcelain chips and the mixture was refluxed gently on an oil-bath at 135-140°C for 2 hours. The reaction mixture was then allowed to cool at room temperature, which was further neutralized with 10% sodium bicarbonate to obtain precipitate of 5-chloro-2-(α-hydroxy benzyl) benzimidazole.

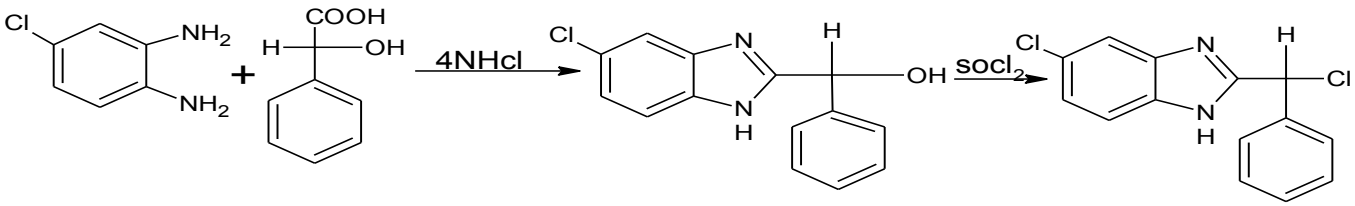
Synthesis of 5-Chloro-2-(α-chlorobenzyl) benzimidazole

In a 250 ml three neck RBF, 60 ml thionyl chloride was transferred and the RBF was placed in an ice cold water bath. To it 10 gm of 5-chloro-2-(α-hydroxybenzyl)benzimidazole was added slowly with occasionally shaking. Then placed RBF 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.

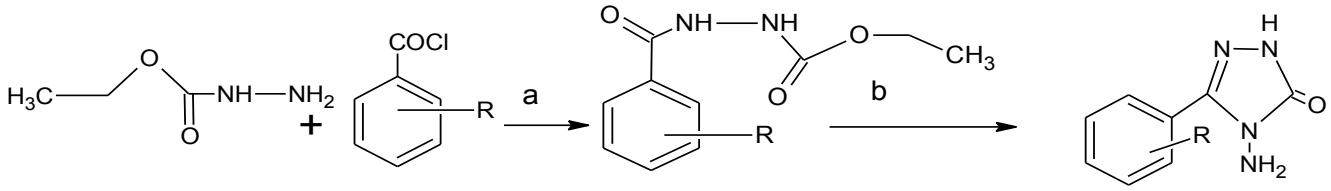
Synthesis of 4-((5-chloro-1H-benzo[d]imidazo-2-yl)(phenyl)methylamino)-5-phenyl-2H-1,2,4-triazol-3(4H)-one

In a RBF, 1 gm (0.005 mol) of 5-chloro 2-(α-Chlorobenzyl) benzimidazole, 1.47 gm (0.005 mol) of 5-phenyl-4-amino-1,2,4-triazole-3one were dissolved in dry dioxane and mixed. To it 0.76 ml (0.005 mol) of triethylamine was added and the reaction mixture was refluxed for 8 hrs. The reactions was monitored by TLC after completion the reaction mixture was then dumped in ice cold water and the precipitate was collected by suction and dried.

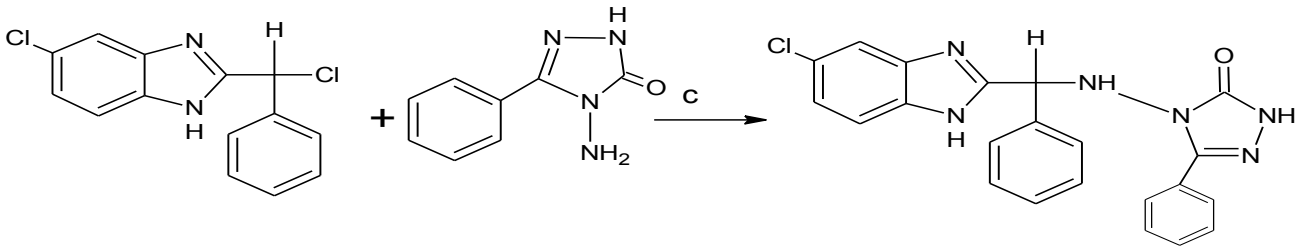
Similarly 4-((5-chloro-1H-benzo[d]imidazole-2-yl)(phenyl)methylamino)-5-o-tolyl-2H-1,2,4-triazol-3(4H)-one, 5-(4-((5-chloro-1H-benzo[d]imidazole-2-yl)(phenyl)methylamino)phenyl)-3,4-dihydro-2H-1,2,4-triazol-3-ol, 5-((5-chloro-1H-benzo[d]imidazole-2-yl)(phenyl)methylamino)-2H-1,2,4-triazole-3-thiol,N-((5-chloro-1H-benzo[d]imidazole-2-yl)(phenyl)methyl)-1H-1,2,4-triazol-3-amine were synthesized using corresponding triazoles.



Scheme 1: Synthesis of 5-Chloro-2-(α-chlorobenzyl) benzimidazole



Scheme 2: Synthesis of derivatives of 5-Phenyl-4-amino-1,2,4-triazole-3-one



Scheme 3: Condensation of 4-((5-chloro-1H-benzo[d]imidazo-2-yl)(phenyl)methylamino)-5-phenyl-2H-1,2,4-triazol-3(4H)-one (Triethylamine, Methanol at 0 to 5^{0}C b-NH_2-NH_2.H_2O Reflux c-Dioxane, Triethyl amine Reflux)

EVALUATION OF ANTIFUNGAL ACTIVITY[6]

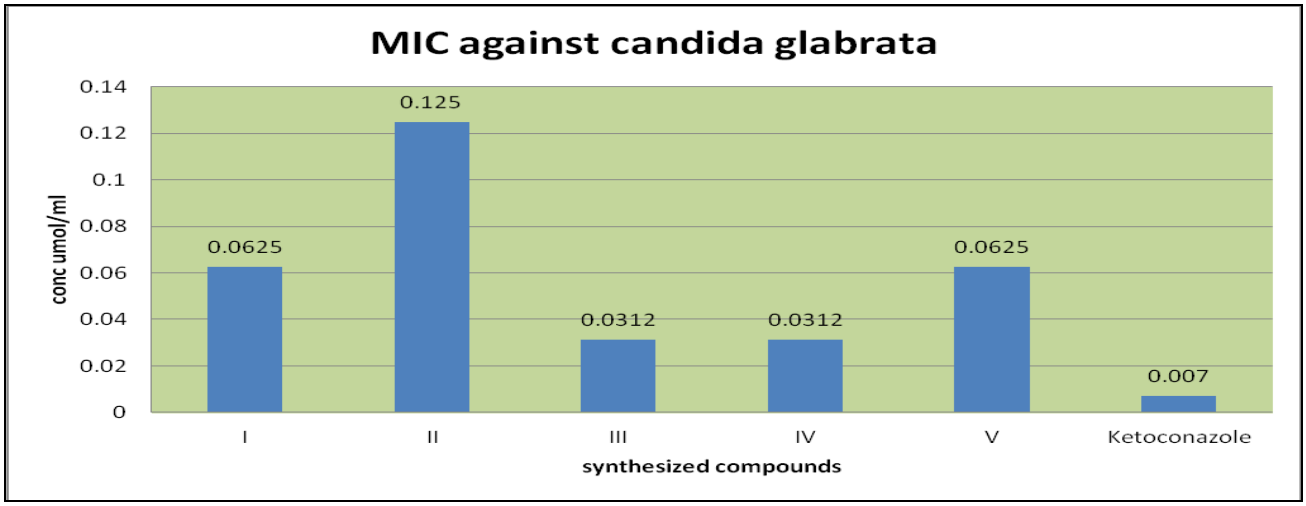
The antifungal activity of synthesized compounds I, II, III, IV and V was evaluated by the tube dilution method (Turbidimetric method) as minimum inhibitory concentration (MIC) against *Candida glabrata* NCIM 3236. Ketoconazole was used as a reference standard.

RESULT AND DISCUSSION

The derivatives of Benzylbenzimidazoles were synthesized. The identity of the products were confirmed by m.p., IR and NMR spectral data. The synthesized compounds were screened for antifungal activity.

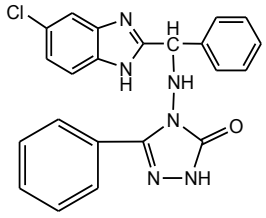
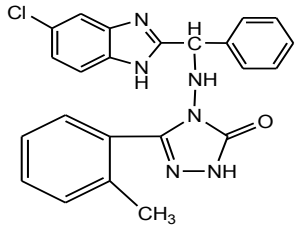
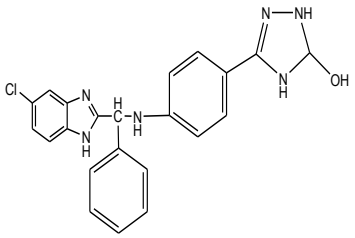
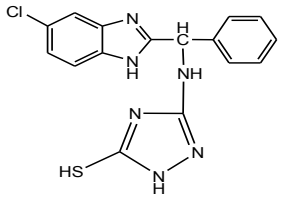
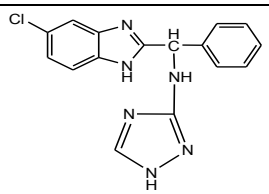
- *5-Phenyl-4-amino-1,2,4-triazole-3-one:* mp 108-110^{o}C, IR Wave Number (cm^{-1}) 3448.49- -NH_2, 1670.24 - -C=O; NMR(σ) ppm 2.480-2.5271, Broad (s) NH_2, 6.78-6.83(s) NH 7.34-7.55(m) Ar-H; Mass Interpretation m/e 176.12 (Molecular ion peak), 161, 105.03, 91.11
- *5-(2-Methylphenyl)-4-amino-2H-1,2,4-triazole-3-one:* mp 127-130^{o}C; IR Wave Number (cm^{-1}) 3373.27 --NH_2, 1718.43-- C=O; NMR (σ) ppm 2.35-2.47, (s) CH_3, 4.176, Broad (s) NH_2, 7.054 (s) NH, 7.22-7.54 (m) Ar-H; Mass Interpretation m/e191.15 (M+1) (Molecular ion peak) 133.14, 117.98, 91.13
- *5-(4-Nitro phenyl-4-amino-2H-1,2,4-triazole-3one:* mp 154-158; IR Wave Number (cm-1) 3344.34 -NH_2,1620.09 - C=O, 1541.02 - NO_2.
- *4-((5-Chloro-1H-benzo[d]imidazole-2-yl)(phenyl)methylamino)-5-phenyl-2H-1,2,4-triazol-3(4H)-one:* Mol.wt 416.5, mp 180-183 ^{o}C; IR Wave Number (cm^{-1}) 3311.55 NH, 1658.67 C=O, 1230.50 C-N, 2889.17 C-Haliphatic, 3029.96 C-H aromatic; NMR(σ) ppm 8.3-8.81,(s) NH 12.2,(s) NH, 7.2-7.8, (m) Ar-H.
- *4-((5-Chloro-1H-benzo[d]imidazole-2-yl)(phenyl)methylamino)-5-o-tolyl-2H-1,2,4-triazol-3(4H)-one:* Mol.wt 430.5**,** mp 156-158 ^{o}C; IR Wave Number (cm^{-1}) 3299.58 NH St, 1670.24 C=N, 1622.02 C=O, 1290.29 C-N, 2921.96 C-Haliphatic, 3018.39 C-H aromatic.
- *5-(4-((5-Chloro-1H-benzo[d]imidazole-2-yl)(phenyl)methylamino)phenyl)-3,4-dihydro-2H-1,2,4-triazol-3-ol***:** Mol.wt 416.5, mp 172-174 ^{o}C; IR Wave Number (cm-1) 3384.84 OH ,3209.33 NH, 1693.38 C=N, 1344.29 C-N, 2960.30 C-H aliphatic.
- *5-((5-Chloro-1H-benzo[d]imidazole-2-yl)(phenyl)methylamino)-2H-1,2,4-triazole-3-thiol:* Mol. wt 271.5, mp 270-275 ^{o}C; IR Wave Number (cm-1) 3379.5 NH, 1634.24 C=N, 1234.36 C-N, 2858.31 C-Haliphatic 3016.46 C-H aromatic 2615.29 SH; NMR(σ) ppm 12.9, (s) NH., 8.1, (s) Ar-CH 7.8-7.4 (m) Ar-H, 2.7-2.8,(s) SH

- *5-((5-Chloro-1H-benzo[d]imidazole-2-yl)(phenyl)methyl)-1H-1,2,4-triazol-3-amine:* Mol. wt 239, mp 173-175 ^{o}C; IR Wave Number (cm-1) 3355.91 NH St, 1652.88 C=N, 1325.01 C-N, 2918.10 C-Haliphatic 3039.60 C-H aromatic.



Graphical representation of MIC against *Candida glabrata* NCIM 3236

Table 1: Synthesized compounds and its antifungal activity

Code	Synthesised comp.	MIC µmol/ml against *Candida glabrata*
I		0.0625
II		0.125
III		0.0312
IV		0.015
V		0.0625

CONCLUSION

Compounds III and IV which has polar side chains (-OH, -SH) has shown remarkable antifungal activity among the five synthesized compounds. That is side chain linked to azole nucleus play important role in binding of the compound at the active site of fungal enzyme.

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