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Synthesis of some 4-Amino-5-(substituted-phenyl)-4H-[1, 2, 4] triazole-3-thiol derivatives and Antifungal activity

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Abstract

Despite the remarkable progress in diagnostic and antifungal drugs research in past 10 years ,still complexity of the characteristics of patients continue to make the management of fungal infection is a great challenge .1,2,4-triazole compounds not only offer an interesting chemistry but also their various derivatives possesses diverse chemotherapeutic activity. The recent literatures are enriched with the progressive finding about the synthesis and biological activity of 1,2,4-triazole heterocyclic compounds .4 amino -1,2,4-triazole -3-thiol are known to exhibit diverse pharmacological profile including analgesic anti-inflammatory ,anti allergic ,anti viral ,anti–hiv, anti microbial ,anticonvulsant ,anti depressant ,antifungal, anti cancerous ,anti bacterial and anti tubercular activities. A series of 4 amino -5-(substituted phenyl-4H[1,2,4]-triazole -3-thiol were synthesized by cyclization of thiocarbohydrazide and substituted benzoic acid by fusing method.4-(4-substitutedbenzylideneamino)-5(substitutedphenyl)-2H-1,2,4-triazole-4(4H)thione was prepared by condensation of primary amine of 4-Amino-5-(substituted phenyl)- 4H[1,2,4]-triazole -3-thiol with various substituted aromatic aldehyde through a single step and 4-(4- substituted benzylideneamino)-2-(morpholinomethyl). 5(substituted phenyl)-2H-1,2,4-triazole-4(4H)thione were afforded by the reaction of corresponding Schiff base with formaldehyde and morpholine with the formation of Iminium ion. Elemental analysis, IR,1H NMR & MASS spectral data confirmed the structure of newly synthesized compounds .Synthesized triazole investigated for antifungal activity .some of tested compounds show good and moderate antifungal activity against various antifungal strains.

Key-Words: 1, 2, 4-Triazole, Thiocarbohydrazide, Anti fungal activity

Introduction

Systemic fungal infections are important problems in physiopathology and especially in medicine, the care of patient's immune suppressed by infectious diseases, chemotherapy, or age. Infections caused by fungal species are common in immune compromised patients and carry significant treatment costs and mortality.1Standard systemic antibiotic therapy alone is frequently unsatisfactory in certain circumstances. Also, more attention is being focused on addressing the problem of multi drug resistant bacteria and the staggering costs and consequences resulting from this. The emerging resistance of microorganisms to some synthetic antifungal agents makes it necessary to continue the search for new antifungal substances[1].

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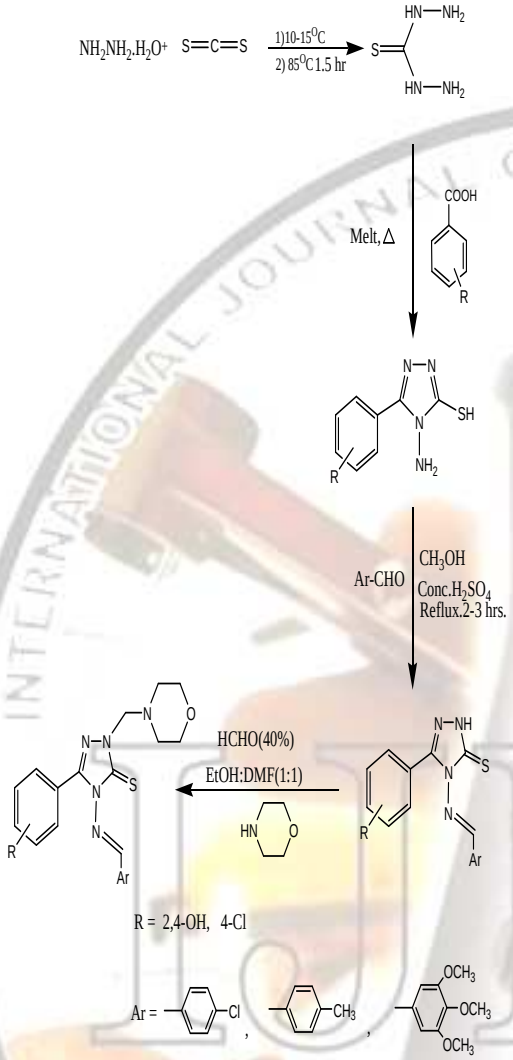
The main objective of this paper is to study the antifungal property of various triazole derivatives. The biological activities of various triazole derivatives have been extensively studied. It is known from the literature that the triazole moiety has great versatility in fusing to various ring systems and possesses a broad spectrum of biological activities. Among the most important effects, triazole derivatives have been reported to exhibit antifungal properties[2].

Chemistry and pharmacological activity of such substituted triazole compounds prompts us to synthesize a series of new potentially active group bearing the 1, 2, 4-triazole nucleus. Prompted by these observations, it was contemplated to synthesize some 5-sustituted 1, 2,4-triazole-3-thiol derivatives containing Schiff base with the view to explore their potency as better chemotherapeutic agent.

Chemistry

The synthetic pathway followed for the preparation of the title compounds was accomplished as shown in Scheme 1



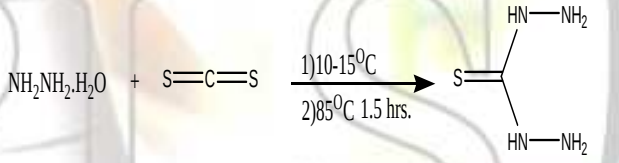
Scheme 1

The Mannich reaction is an organic reaction which consists of an amino alkylation of an acidic proton placed next to a carbonyl functional group with formaldehyde and ammonia or any primary or secondary amine. The final product is a β-amino-carbonyl compound also known as a Mannich base. The mechanism of the Mannich reaction starts with the formation of an iminium ion from the amine and the formaldehyde. 1, 2, 4-triazole system containing Schiff base significantly shows diverse biological activities. Schiff base is a compound formed by condensation reaction between aromatic primary amines with aldehyde or ketone. Imine (Schiff base) formation is one of the mechanisms used by enzymes, to catalyze reaction between organic molecules through the formation of imines Schiff base. The reaction of an amine with either aldehyde or ketone proceeds through the carbinolamine intermediate formation and reaction usually requires acid catalyst Different substituted benzoic acids were used as starting material, which on melting with thiocarbohydrazide, gave triazole nucleus. This substituted triazole nucleus on refluxing with substituted benzaldehyde in presence of catalytic amount of conc. H_2SO_4 for 3-4 h was resulted Schiff base. The Schiff base was dissolved in a mixture of ethanol and DMF and react with formaldehyde (40%) and primary/secondary amine for 2–3 h was resulted end product mannich base. All the synthesized compounds were characterized by elemental analysis, IR, 1H NMR, 13C NMR and mass spectrometry.

Material and Methods

Synthesis of Thiocarbohydrazide (TCH)

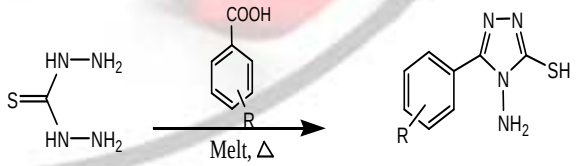
In a round bottom flask hydrazine hydrate (1.0 mol) was placed, which was equipped with thermometer, efficient agitator, and reflux condenser. The temperature was lowered to 10°C and 0.2 mol of carbon disulfide (15.2 g, 12.1 mL) was added dropwise into the flask while maintaining the temperature below 15°C and the temperature was raised gradually to 85°C for 1.5 hr. reaction mixture was cooled to 10°C, precipitate was filtered and washed with water.



Hydrazine Hydrate Carbon Disulphide Thiocarbohydrazide

Synthesis of 4-Amino-5-(substituted-phenyl)-4H-[1,2,4]-triazole-3-thiol

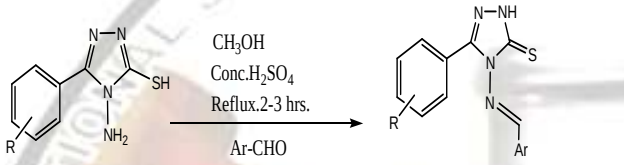
A mixture of substituted benzoic acid (0.01mol) and thiocarbohydrazide (0.015mol) were taken in a round-bottomed flask and heated on a mantle until the content of the flask was melted. The product obtained on cooling was treated with sodium bicarbonate solution to neutralize the unreacted carboxylic acid, if any. It was then washed with water and collected by filtration. The product was recrystallized with ethanol to afford the title compounds.



Thiocarbohydrazide 4-Amino-5-(substituted-phenyl)-4*H*-[1,2,4]triazole-3-thiol

General Procedure for Derivative Preparation Containing Schiff base

To a suspension of substituted amino mercapto triazole (0.2 mol) in methanol, an equimolar amount of the corresponding substituted benzaldehyde with 3 to 4 drops of sulphuric acid was added. The reaction mixture was refluxed for 2-3h at 80-90°C. The precipitate was obtained which was washed with water, filtered and dried.

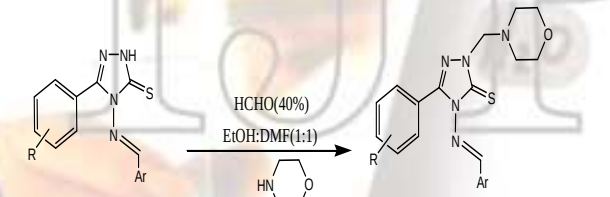


4-Amino-5-(substituted-phenyl)-4*H*-[1,2,4]triazole-3-thiol

4-(4-substitutedbenzylideneamino)-5(substituted-phenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione

General Procedure for Preparation of Mannich Base

The Schiff base (0.01 mol) was dissolved in a mixture of ethanol and DMF. Then formaldehyde (40%, 1.5 mL) and primary/secondary amine (0.01 mol) were added to this solution. The mixture was stirred for 2–3h and kept overnight at room temperature. The resulting solid was collected by filtration, washed with cold ethanol and recrystallized from ethanol and DMF to yield the title compound.



4-(4-substituted benzylideneamino)-5(substituted-phenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione

4-(4-substituted benzylideneamino)-2-(morpholinomethyl)-5(substituted-phenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione

Biological Evaluation

Antifungal activity of the synthesized compounds was carried out through tube dilution/turbidity method against different fungal strains (Table 1).

Tube dilution/turbidity method

This method is used to determine the susceptibility of an organism to antibiotics/antifungal as well as Minimal Inhibitory Concentration (MIC) of that antibiotic/antifungal. The MIC is the lowest concentration of an antimicrobial agent that inhibits the growth of the test microorganisms. A clinician to establish effective antimicrobial regimen for the treatment of bacterial/fungal infections may use quantitative data of this nature. This method is also used to compare the activity of an unknown crude antibiotic with the known antibiotic/antifungal.

Fungal Strain

Table 1: List of Fungal Strain Considered During Study

Fungal Strain
Candida albicans ATCC 10231
Candida albicans ATCC 24433
Candida tropicalis ATCC 13803
Aspergillus niger ATCC 9029

Preparation of Sample

25.6 mg of synthesized compounds were dissolved in 2mL of DMSO and made up to 100 mL with sterilized double distilled water in a volumetric flask. So that it gave 256 µg/mL. All the sample solution had been prepared in aseptic condition.

Procedure

- MIC tubes were taken and labelled with numbers I to IX.
- Different concentrations of drug were prepared from tube I to IX by serial dilution as shown in the Table 9.
- In the tube I only 2 mL of test comound was added, while tube II, 2 mL medium and 2 mL test compound (256 µg/mL) were taken and mixed well. 2ml of the inoculums was transfer to higher number test tube (n+1). 2 mL of inoculum was discarded from the last test tube. To each test tube 2 mL of test culture was added.
- All tubes were incubated at 25°C for 48h.
- Turbidity was observed after 48h for MIC determination.

Results and Discussion

The investigation of antifungal screening data revealed that the compounds A-1, A-2, A-3, A-4, A-7 are more potent than fluconazole (standard antifungal drug) for *A. niger with* MIC value 64 µg/mL. The compound A-3 is more potent than fluconazole for *C. tropicalis*(3556*)* with MIC value 32 µg/mL. The synthesized compounds A-1, A-2 and A-3 are equipotent as fluconazole against *C. albican* (3557) and *C. albican* (3471) with MIC value of 32 µg /mL. Similarly the synthesized compounds A-1, A-2 and A-4 are equipotent as fluconazole for *C. tropicalis* with MIC value of 64 µg/mL and compounds A-5, A-6 are equipotent for *A.niger* with MIC value 128 µg/mL. The compounds A-5, A-6 and A-7 are less potent against *C .albican* (3557), *C. albican* (3471) and *C. tropicalis* (3556) with MIC value in the range of 64-128 µg/mL. The good activity is attributed to the presence of electronegative group, 4-chloro and 2,4-dichloro

groups at aryl moiety attached to 5th position of triazole nucleus. The presence of 2,4-hydroxy group at aryl moiety attached to 5th position are responsible for decrease in activity. The enhanced activity is also attributed to the presence of electronegative chloro group at benzylidene nucleus attached to 4th position. The presence of 3,4,5-methoxy and 4-methyl at benzylidene nucleus attached to 4th position of triazole system are responsible for decrease in activity. The preliminary SAR of synthesized compound revealed that electronegative group on 2nd and 4th position of phenyl ring favour for the activity, at the same time electron releasing group unfavourable for the activity. The presence of electron withdrawing group at 4th position of benzylidene nucleus is favourable, while electron releasing groups are unfavourable for the anti-fungal activity.

The present study has resulted in the identification of 5-substituted-4-amino-1,2,4-triazole-3- thiol analogs as lead nucleus for antifungal activity with improved potency against *A. niger* and *C. tropicalis*.

The following compounds were prepared by an analogous procedure

4-(4- chlorobenzylideneamino)-5-(4-chlorophenyl)-2*H*-1, 2, 4-triazole-3(4*H*)-thione (A-1): Molecular Formula: $C_{15}H_{10}Cl_2N_4S$, **Yield:** 56.60%, M.P 220-222 **°C,** IR v cm-$^{-1}$: 1696 (C=N), 1206(C-N), 1238(C=S), 1474, 1589(Aromatic (C=C), 628 (C-Cl) ,molecular weight : 348.00, **λ_{max} (nm):** 231.5, *m/z:349, δ-7.0-8.1*

4-(4-chlorobenzylideneamino)-5-(4-chlorophenyl)-2-(morpholinomethy)-2*H*-1,2,4-triazole-3(4*H*)-thione (A-2): molecular formula: $C_{20}H_{19}C_{l2}N_5OS$, **Yield:** 52.54,M.P 275-278**°C,** IR v cm-$^{-1}$: 1677(C=N), 1093(C-O-C), 1153(C-N), 1260(C=S), 1458, 1592(Aromatic (C=C), 766(C-Cl), molecular weight : 447.07, **λ_{max} (nm)** 231.5, *m/z:449, δ-7.3-8.1*

4-(4-chlorobenzylideneamino)-5-(2,4-dichlorophenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione(A-3): Molecular Formula: $C_{15}H_9Cl_3N_4S$, **Yield:** 61.33%, M.P : 120-122**°C,** IR v cm-$^{-1}$: 1640(C=N), 1210(C-N), 1263(C=S), 1471, 1582(Aromatic (C=C), 573(C-Cl), molecular weight : 381.96, **λ_{max} (nm):** 271.4, *m/z:* 380.9, δ 7.27-8.024

4-(4-chlorobenzylideneamino)-5-(2,4-dichlorophenyl)-2-(morpholinomethy)-2*H*-1,2,4-triazole-3(4*H*)-thione (A-4):Molecular Formula:$C_{20}H_{18}Cl_3N_5OS$, **Yield:** 49.12%,M.P : 198-200**°C,** IR v cm-$^{-1}$: 1690(C=N), 1099(C-O-C), 1169(C-N), 1260(C=S), 1475,1580(Aromatic (C=C), 591(C-Cl), molecular weight : 481.03, **λ_{max} (nm):** 268.6,*m/z:485,* δ:7.2-8.1

4-(3,4,5-trimethoxybenzylideneamino)-5-(4-chlorophenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione(A-5): Molecular Formula: $C_{18}H_{17}ClN_4O_3S$, **Yield:** 76.23%, M.P : 210**°C,** IR v cm-$^{-1}$: 1686(C=N), 1123(C-O-C), 1188(C-N), 1231(C=S), 1459,1590(Aromatic (C=C), 619(C-Cl), molecular weight : 404.07**λ_{max} (nm):** 284.2,*m/z:406.1,* δ:7.0-8.1

4-(4-methyl benzylideneamino)-5-(4-chlorophenyl)-2*H*-1,2,4-triazole-3(4*H*)thione(A6): Molecular Formula: $C_{16}H_{13}ClN_4S$,**Yield:**55.54%, M.P : 110-112**°C,** IR v cm-$^{-1}$: 1702(C=N), 1191(C-N), 1237(C=S), 1487,1600(Aromatic (C=C), 676(C-Cl), molecular weight : 328.05,**λ_{max} (nm):** 277.8,*m/z:330.2,* δ:7.0-8.1

4-(4-chlorobenzylideneamino)-5-(2,4-dihydroxyphenyl)-2*H*-1,2,4-triazole-3(4*H*)thione(A7) :Molecular Formula: $C_{15}H_{11}ClN_4O_2S$, **Yield:** 67.60 %, M.P : 190-192**°C** ,IR v cm-$^{-1}$: 1648(C=N), 1196(C-N), 3396(O-H), 1429,1616(Aromatic (C=C), 774(C-Cl), molecular weight : 346.03, ,**λ_{max} (nm):** 290,*m/z:3480,* δ:6.3-8.1

1,2,4-triazoles cover a wide range of application and their importance for the chemical industry is remarkable. Among the many application, they are used in medicinal chemistry as part of more complex molecules or as fungicides. Literature survey reveals that piperazine and morpholine ring is important for antimicrobial activity.

Many advances in the development of antifungal agents have been made in last decade. Whilst the availability of extended spectrum triazole and the study reports the synthesis of some heterocyclic compounds containing 1,2,4-triazole along with morpholine ring and such attempt is made to improve potency of existing drug with minimum side effect and toxicity. The scientific literature reveals that 1,2,4-triazole are effective in treating fungal infection and this activity is due to the presence of NH-c(s)-NH function of group in a molecule and change in activity depends on nature of substitute. On the basis of literature survey seven 1,2,4-triazole derivatives were designed and synthesised.

Thiocarbohydrazide were subjected to intermolecular cyclization with substituted benzoic acid to give1,2,4-triazole. Since, 1,2,4-triazole-3-thione may exist in thiol-thione tautomeric forms. Our investigation showed that triazole gave significant IR band at 1230-1263 cm^{-1} and no peaks was observed around 2550-2600 cm^{-1} indicating thione form. Schiff base obtained from the 5-substituted-4-amino-1,2,4-triazole-3-thiol have high ability to form metal complex. Mannich reaction is an important tool for synthesis of novel compounds. Mannich bases are physiologically reactive because of the basic function rendering the molecule soluble in aqueous solvents when it is transformed into iminium salt.

The investigation of antifungal screening data revealed that the compounds A-1, A-2, A-3, A-4, A-7 are more potent than fluconazole (standard antifungal drug) for *A. niger with* MIC value 64 µg/mL. The compound A-3 is more potent than fluconazole for *C. tropicalis*(3556*)* with MIC value 32 µg/mL. The synthesized compounds A-1, A-2 and A-3 are equipotent as fluconazole against *C. albican* (3557) and *C. albican* (3471) with MIC value of 32 µg /mL. Similarly the synthesized compounds A-1, A-2 and A-4 are equipotent as fluconazole for *C. tropicalis* with MIC value of 64 µg/mL and compounds A-5, A-6 are equipotent for *A.niger* with MIC value 128 µg/mL. The compounds A-5, A-6 and A-7 are less potent against *C .albican* (3557), *C. albican* (3471) and *C. tropicalis* (3556) with MIC value in the range of 64-128 µg/mL. The good activity is attributed to the presence of electronegative group, 4-chloro and 2,4-dichloro groups at aryl moiety attached to 5^{th} position of triazole nucleus. The presence of 2,4-hydroxy group at aryl moiety attached to 5^{th} position are responsible for decrease in activity. The enhanced activity is also attributed to the presence of electronegative chloro group at benzylidene nucleus attached to 4^{th} position. The presence of 3,4,5-methoxy and 4-methyl at benzylidene nucleus attached to 4^{th} position of triazole system are responsible for decrease in activity. The preliminary SAR of synthesized compound revealed that electronegative group on 2^{nd} and 4^{th} position of phenyl ring favour for the activity, at the same time electron releasing group unfavourable for the activity. The presence of electron withdrawing group at 4^{th} position of benzylidene nucleus is favourable, while electron releasing groups are unfavourable for the anti-fungal activity.

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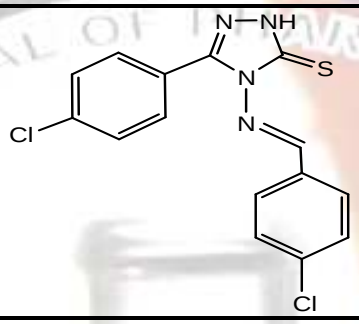
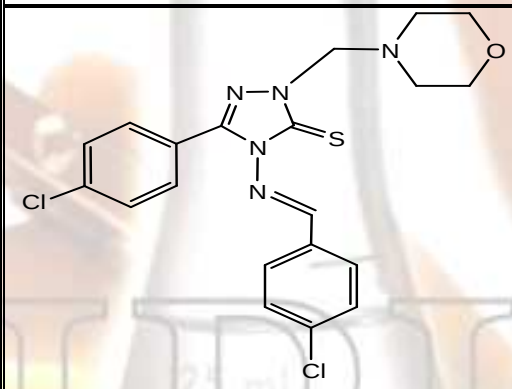
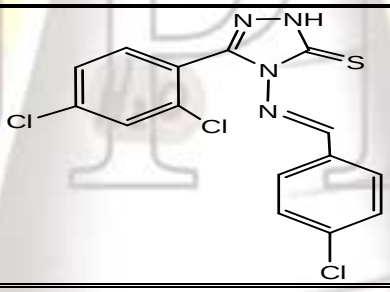
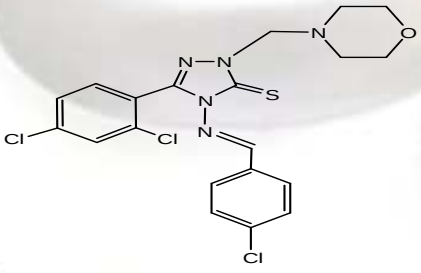
The following compounds were prepared by an analogous procedure

S/No.	Compound Code	Structure	Chemical Name
1	A-1		4-(4- chlorobenzylideneamino)-5-(4-chlorophenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione
2	**A-2**		4-(4-chlorobenzylideneamino)-5-(4-chlorophenyl)-2-(morpholinomethy)-2*H*-1,2,4-triazole-3(4*H*)-thione
3	A-3		4-(4-chlorobenzylideneamino)-5-(2,4-dichlorophenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione
4	A-4		4-(4-chlorobenzylideneamino)-5-(2,4-dichlorophenyl)-2-(morpholinomethy)-2*H*-1,2,4-triazole-3(4*H*)-thione

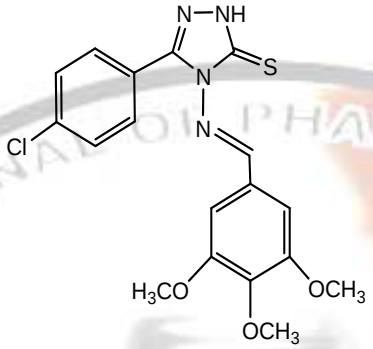
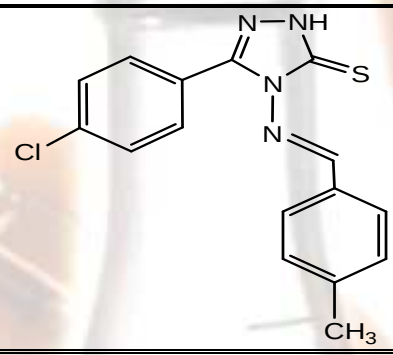
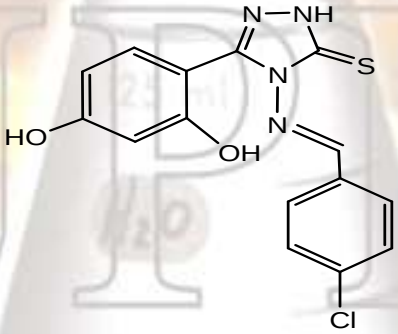
5	A-5		4-(3,4,5-trimethoxybenzylideneamino)-5-(4-chlorophenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione
6	A-6		4-(4-methyl benzylideneamino)-5-(4-chlorophenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione
7	A-7		4-(4-chlorobenzylideneamino)-5-(2,4-dihydroxyphenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione

Table 2: Procedures for MIC Determination of Synthesized Compounds

Tube No.	I	II	III	IV	V	VI	VII	VIII	IX
Medium (mL)	0	2	2	2	2	2	2	2	2
Synthesized compounds/ Fluconazole (mL)	2	2	Serial dilution was carried out from Tube III to IX using 2mL inoculums from n-1 TT (2mL inoculums from the tube IX had been discarded)						
Test culture (mL)	2	2	2	2	2	2	2	2	2
Total volume (mL)	4	4	4	4	4	4	4	4	4
Concentration of drug (µg/mL)	128	128	64	32	16	8	4	2	1

Table 3: Experimental MIC Values of Synthesized Compounds and Standard Drug (Fluconazole)

S/No.	Comp.Code	MIC value (µg/mL)			
		Candida albicans (3557)	*Candida albicans* (3471)	*Candida tropicalis* (3556)	*Aspergilus niger* (545)
1.	A1	32	32	64	64
2.	A2	32	32	64	64
3.	A3	32	32	32	64
4.	A4	64	64	64	64
5.	A5	128	128	128	128
6.	A6	128	64	128	128
7.	A7	128	128	128	64
8.	Fluconazole	32	32	64	128

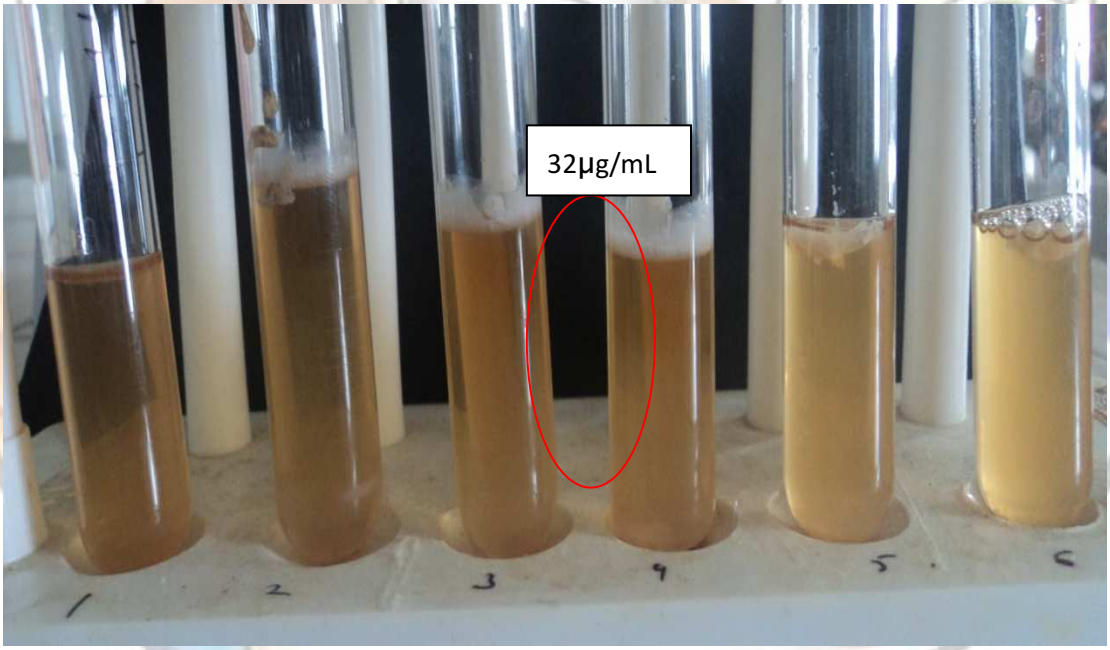


Fig. 3.1: *In vitro* Antifungal Activity of A-3 against *Candida Albicans* (3557)

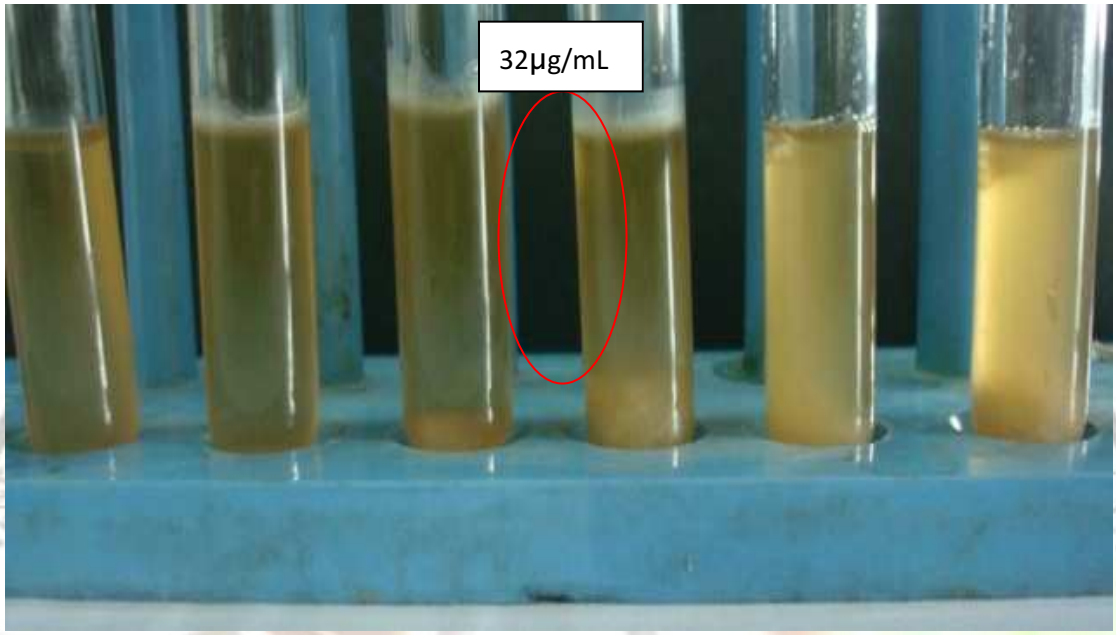


Fig. 3.2: *In vitro* Antifungal Activity of A-3 against *Candida Albicans* (3471)

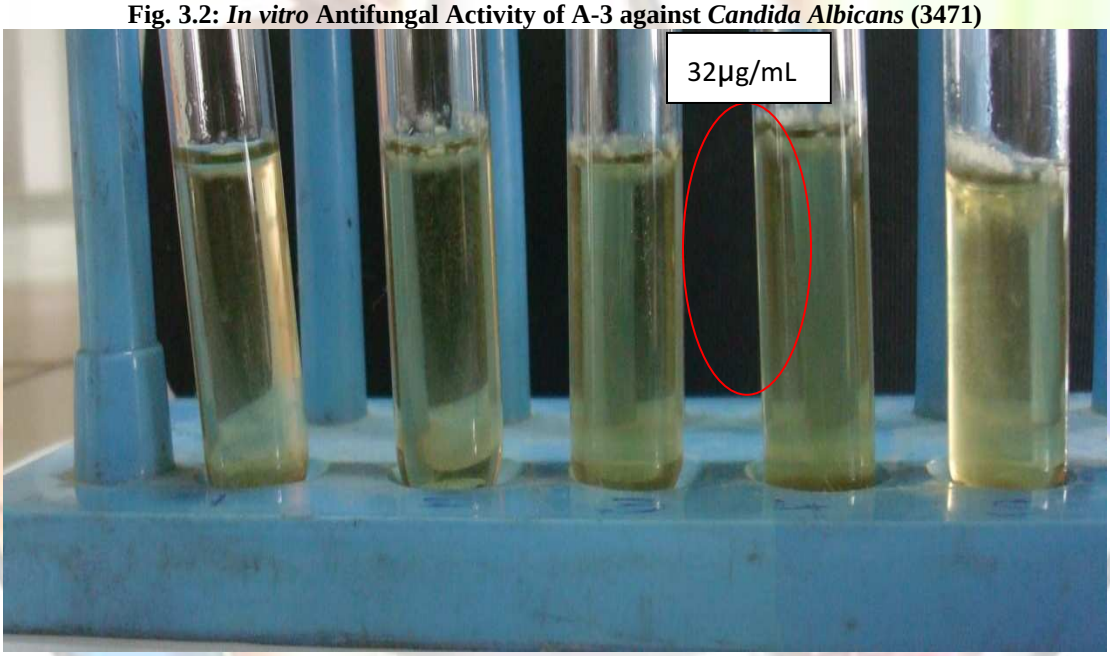


Fig. 3.3: *In vitro* Antifungal Activity of A-3 against *Candida Tropicalis* (3556)

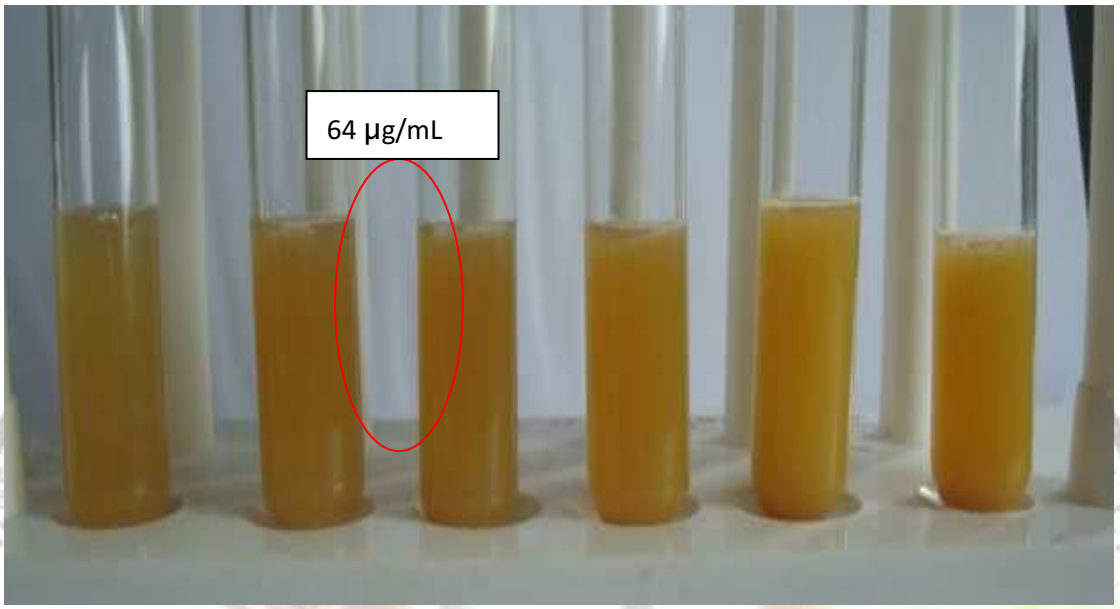


Fig. 3.4: *In vitro* Antifungal Activity of A-3 against *Aspergillus Niger* (545)

A comparative MIC plot of synthesized compounds and standard drug is shown in fig. 3.4.5

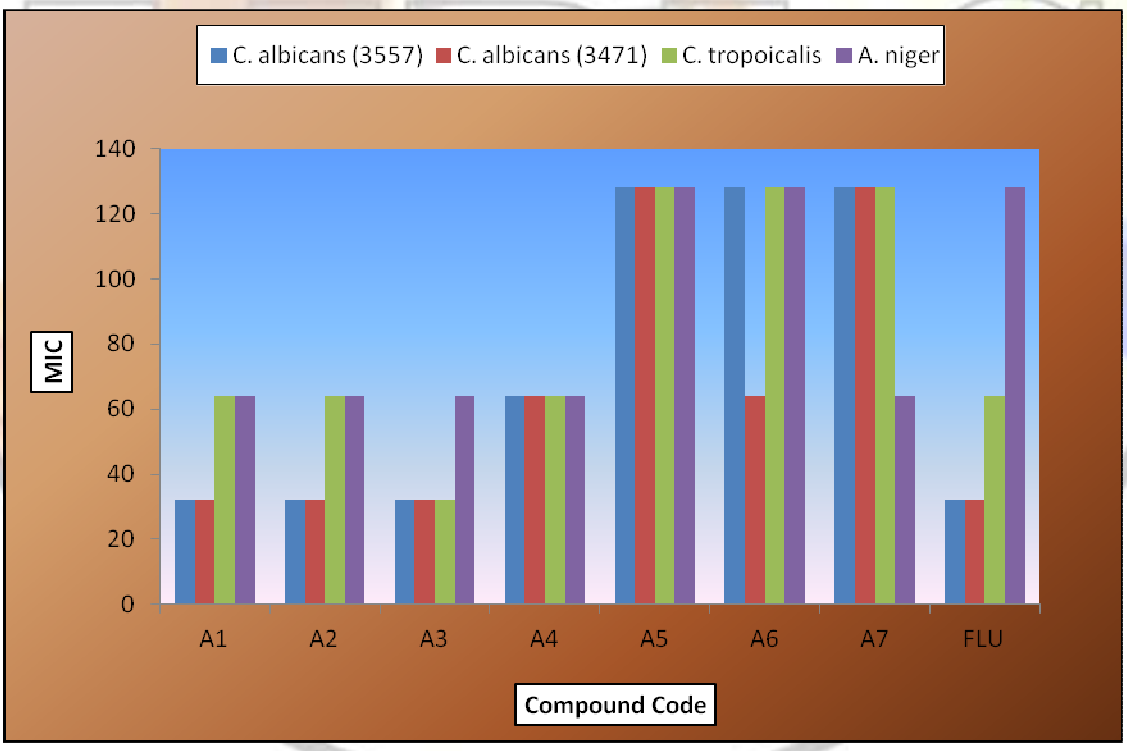


Fig.3. 5: MIC Plot of Synthesized Compounds and Standard Drug