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Synthesis, characterization and evaluation of antimicrobial activity of a series of 1,2,4-triazoles

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ABSTRACT

A series of 4H-1,2,4-triazoles was synthesized by using aromatic acid precursors via conversion to corresponding hydrazides, then thiosemicarbazides and finally to 1,2, 4-triazole derivatives. The synthesized compounds were characterized through various instrumental techniques viz., FTIR, FT-NMR, and MS. All the compounds were tested against six different strains of bacteria and two different strains of fungi. Compound no.s 2, 3, 4, 7 & 8 showed remarkable activities against the test microorganisms. The MICs of the compounds were also determined and reported.

Keywords: 1,2,4-triazole, minimum inhibitory concentration (MIC), hydrazides, agar diffusion.

INTRODUCTION

During the past decades, the human population affected with life-treating infectious diseases caused by multidrug-resistant Gram-positive and Gram-negative pathogen bacteria increased an alarming level around the world. Due to this reason, new classes of antibacterial agents with novel mechanisms are crucial need to combat with the multidrug-resistant infections. In the past years, some azole derivatives were developed as new antimicrobial agents, for instance, Linezolid and Eperezolid are currently used for the treatment of multidrug-resistant Grampositive infections [1–3]. There are a number of antimicrobial compounds containing a 1,2,4-triazole ring in their structures such as Fluconazole, Itraconazole, Voriconazole, Ravuconazole and Posaconazole that are important antifungal drugs [4].

Triazoles constitute an important class of biologically active heterocyclic compounds that have received a great deal of attention since their discovery. The considerable biological importance of triazoles has stimulated a lot of interest in its derivatives. 1,2,4-triazoles, being an important pharmacophores have a wide range of therapeutic properties like antibacterial [5-8], antifungal [9-14], antimycobacterial [15-20], antiviral [21], anti-inflammatory [22-25], anticonvulsant [26,27], antidepressant [28], antitubercular [20], antitumoral [29,30], antihypertensive [31], analgesic [32], enzyme inhibitor [33], hypoglycemic [34], sedative, hypnotic [35], antiparasitic, herbicidal [36], insecticidal37,38 and plant growth activities [39-42].

In the year 1855, bladin gave the name "Triazole" to the carbon-nitrogen ring system $C_2H_3N_3$ and described the derivatives of the class. Till date a number of triazole derivatives have entered the medicinal world as powerful therapeutic agents. Still, if we talk about broad spectrum triazole derivatives, we could not help to name even a few. So, the ongoing research is focused on developing triazole derivatives with broad spectrum of antimicrobial activity.

Here we report the synthesis, characterization and antimicrobial evaluation of some new 1,2,4-triazole derivatives.

MATERIALS AND METHODS

1.1 General Procedures:

Melting points were determined by open capillary tube method using Thiel's tube and are uncorrected. Progress of the reactions and purities of the compounds were determined by thin layer chromatography (TLC) using Silica gel G as stationary phase and iodine vapours for development. The IR spectra were obtained in a Shimadzu (Japan) 8400S FT-IR Spectrophotometer (KBr pellet method). The H-1 NMR spectra were recorded on a JEOL AL300 FTNMR Spectrometer using TMS as internal standard. The Mass spectra were recorded in a Q-Tof Micro from Waters corporation, UK .

1.2 Chemistry:

1, 2, 4-triazole derivatives have been synthesized in the laboratory as shown in **scheme (1)** via aromatic carboxylic acid hydrazide intermediate. The aromatic carboxylic acid is first esterified in the presence of conc. H_2SO_4 and absolute ethanol. The corresponding acid ester is then treated with 85% hydrazine hydrate in the presence of absolute ethanol as solvent that resulted in the formation of corresponding carboxylic acid hydrazide in equimolar proportions. The resulting hydrazide is treated with Potassium thiocyanate in acidic medium which resulted in the formation of thiosemicarbazide and the yields were quantitative. Cyclization of the thiosemicarbazide in the presence of NaOH resulted in the formation of the corresponding carboxylic acid-1,2,4-triazole-3-thiol derivative.

1.2.1 General Synthetic Procedure for compounds 1-8:

2.2.1.1: Synthesis of aromatic acid ester: The corresponding aromatic acid (0.1 mol) was refluxed with absolute ethanol (0.5 mol) in presence of 1-3 ml of conc. H_2SO_4 and few porcelain pieces in a round bottom flask for 4-5 hours. After refluxing, excess ethanol was removed by heating under reduced pressure. The resultant solution was diluted with 200 ml of distilled water and neutralized with the help of a solution of sodium bicarbonate. After complete neutralization, the product (aromatic acid ester) was extracted with carbon tetrachloride (CCl_4). The CCl_4 layer, after separation was dried over anhydrous sodium sulphate and concentrated under reduced pressure to give the product.

2.2.1.2: Synthesis of acyl hydrazide: The aromatic ester (0.05 mol) prepared above is refluxed with hydrazine hydrate (0.25 mol) in absolute ethanol for 4-5 hours. After refluxing is over, the mixture is cooled down. The cold mixture is dissolved in absolute ethanol and filtered. On cooling the filtrate white crystals of the acyl hydrazide separate out.

2.2.1.3: Synthesis of acyl thiosemicarbazide: The corresponding acyl thiosemicarbazide was prepared by refluxing a suspension of acyl hydrazide (0.02 mole), potassium thiocyanate (0.04 mole), hydrochloric acid (10ml) and water (200 ml) for 3 hours. On cooling the mixture a white solid separated out which was filtered, dried and then re-crystallized from ethanol.

2.2.1.4: Synthesis of 5-aromatic substituted-4H-1,2,4-triazole-3-thiol: The corresponding thiosemicarbazide (0.01 mole) obtained in the previous step was refluxed in sodium hydroxide solution (5%, 50 ml) for 3 hours. After 3 hours the resulting solution was treated with activated charcoal, filtered and cooled. The filtrate was neutralized with hydrochloric acid to pH 5-6. A solid crystalline product separated which was filtered, dried and recrystallized from dilute ethanol.

The physical and analytical data of the synthesized final products are given as follows:

5-phenyl-4H-1,2,4-triazole-3-thiol (C3_11): Yield: 75%; melting point: 218^0C; IR(KBr)cm^{-1}: 3380 (2^0 N-H str), 3006.16 (Ar C-H str), 2599 (S-H str), 1485 (Ar C-C str in ring), 769.52 (Ar C-H bend); 1H NMR (DMSO, 300 MHz): δ (ppm) 13.67 (1H, SH), 8.84 (1H, NH), 7.50-7.90 (5H, ArH); TOF MS ES+: $(M+H)^+$ 178.1.

4-(5-mercapto-4H-1,2,4-triazol-3-yl)phenol (C4_20): Yield: 65%; melting point: 300^0C; IR(KBr)cm^{-1}: 3432 (Phenolic O-H str), 3439 (2^0 N-H str), 3093 (Ar C-H str), 2610 (S-H str), 1490 (Ar C-C str in ring); 1H NMR (DMSO, 300 MHz): δ (ppm) 13.47 (1H, SH), 7.72-7.81 (4H, ArH), 6.86-6.88 (Ar OH), 10.059 (1H, NH) ; TOF MS ES+: $(M+H)^+$ 194.1.

5-(4-nitrophenyl)-4H-1,2,4-triazole-3-thiol (C5_14): Yield: 60%; melting point: 282^0C; IR(KBr)cm^{-1}: 3497 (2^0 N-H str), 3000 (Ar C-H str), 2575 (S-H str), 1502, 1347 (Ar NO_2 str); 1H NMR (DMSO, 300 MHz): δ (ppm) 12.34 (1H, SH), 8.086 – 8.236 (4H, Ar-H; TOF MS ES+: $(M-SH)^+$ 187.

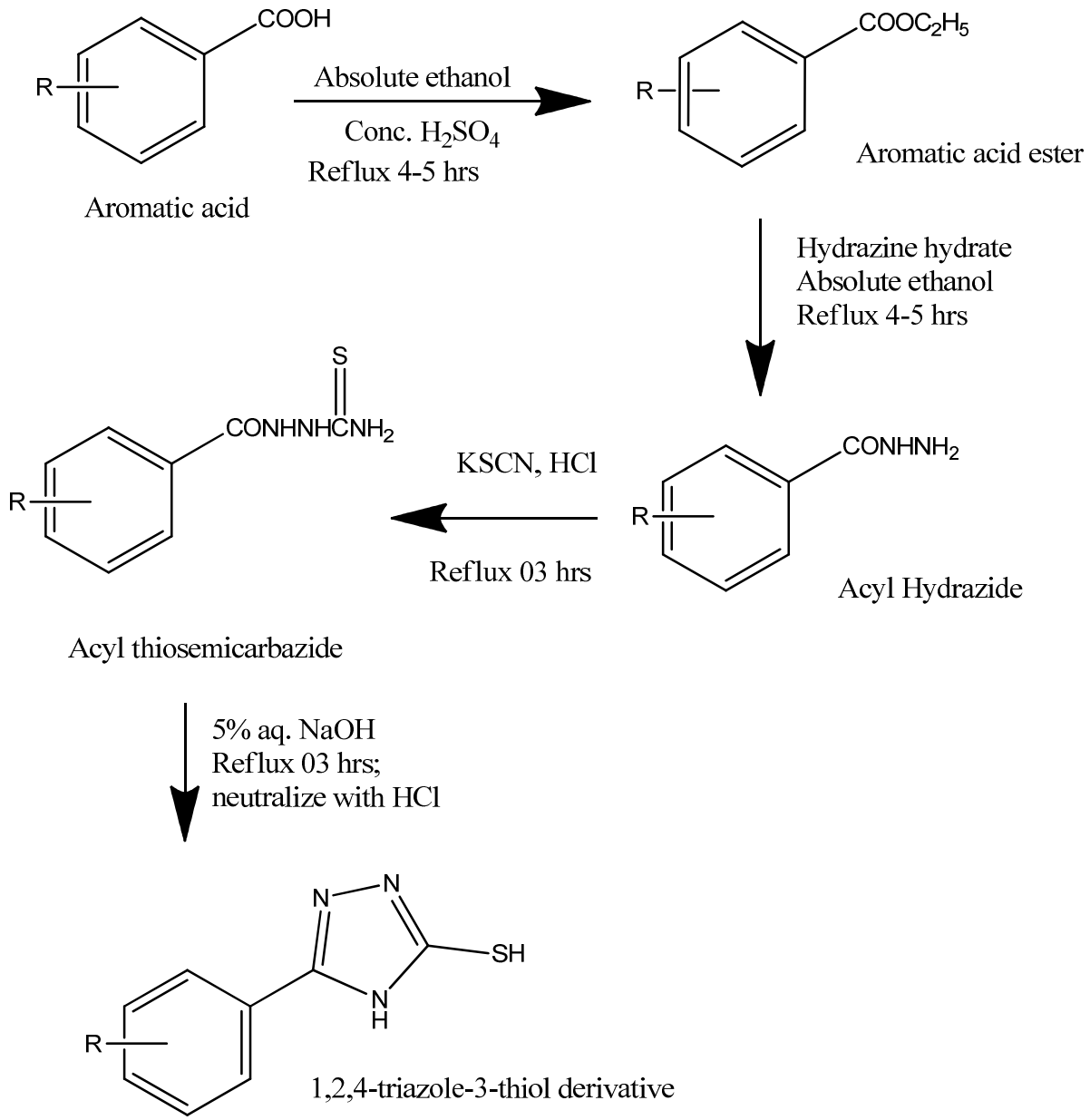
5-(phenoxymethyl)-4H-1,2,4-triazole-3-thiol (C6_3): Yield: 68%; melting point: 114^0C; IR(KBr)cm^{-1}: 3380 (2^0 N-H str), 3100 (Ar C-H str), 2550 (S-H str), 2950 (Methylene C-H str), 1241 (C-O-C str); 1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 13.05 (1H, SH), 6.889-7.716 (5h, Ar-H), 4.58 (2H, methylene); TOF MS ES+: $(M+H)^+$ 208.1.

5-((4-chlorophenoxy)methyl)-4H-1,2,4-triazole-3-thiol (C7_12): Yield: 70%; melting point: 142^0C; IR(KBr)cm^{-1}: 3400 (2^0 N-H str), 2914 (Ar C-H str), 2570 (S-H str), 2785 (Methylene C-H str), 1248 (C-O-C str), 1005 (Ar C-Cl str); 1H NMR (DMSO, 300 MHz): δ (ppm) 13.05 (1H, SH), 6.92-7.33 (4H, Ar-H), 4.68 (2H, methylene); TOF MS ES+: $(M+H)^+$ 242.

5-((2,4-dichlorophenoxy)methyl)-4H-1,2,4-triazole-3-thiol (C8_8): Yield: 62%; melting point: 132^0C; IR(KBr)cm^{-1}: 3497 (2^0 N-H str), 3100 (Ar C-H str), 2435 (S-H str), 2850-2900 (Methylene C-H str), 1080 (Aromatic O-CH_2 str), 1220 (Aromatic C-O str) 1035 (Ar C-Cl str); 1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 13.05 (1H, SH), 7.17-7.41 (3H, Ar-H), 4.73 (2H, methylene); TOF MS ES+: $(M+H)^+$ 277.1.

5-(2,4-dichlorophenyl)-4H-1,2,4-triazole-3-thiol (C9_2): Yield: 65%; melting point: 212^0C; IR(KBr)cm^{-1}: 3497 (2^0 N-H str), 3132 (Ar C-H str), 2550 (S-H str), 1030 (Ar C-Cl str); 1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 13.05 (1H, SH), 7.0-7.48 (3H, SH), ; TOF MS ES+: $(M+H)^+$ 247.1.

5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (C10_21): Yield: 51%; melting point: 309^0C; IR(KBr)cm^{-1}: 3480 (2^0 N-H str), 3048 (Ar C-H str), 2580 (S-H str), 1588, 1430, 832 (4-substitute pyridyl C-H str); 1H NMR (DMSO, 300 MHz): δ (ppm) 13.93 (1H, SH), 7.84-8.73 (4H, pyridyl-H); TOF MS ES+: $(M+H)^+$ 179.1.



Scheme (1)

2.3: Antimicrobial activity determination: The antimicrobial activities of the compounds were evaluated against six different strains of bacteria and two strains of fungi using well diffusion method and the Minimum Inhibitory Concentrations (MICs) of the compounds were determined using serial dilution method. Gentamycin was used as standard drug for antibacterial activity and Amphotericin B was used as standard drug for antifungal activity.

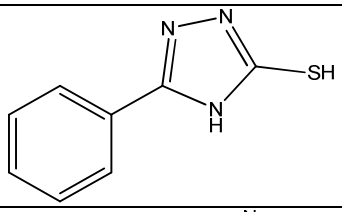
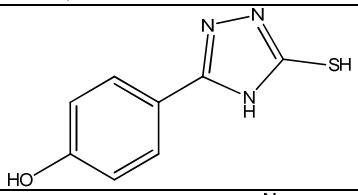
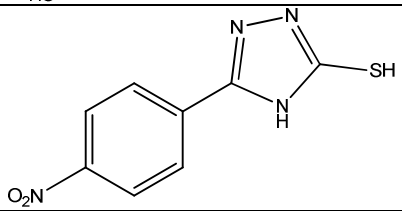
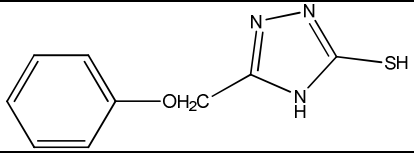
The compounds were screened *in vitro* for antibacterial activity against E. Coli (ATCC®10536), Staphylococcus aureus (ATCC ®25923), Staphylococcus cohni (MPCST 121), Proteus vulgaris (ATCC®6380), Klebsiella pneumonia (ATCC®13883) and Pseudomonas aerugenosa (ATCC®25619). Further all the compounds were screened for antifungal activity against Aspergillus niger (ATCC® 16404) and Candida albicans (ATCC®14053).

The antimicrobial screening was done by using agar plate diffusion method. This method depends upon the diffusion of the various samples from a cavity through the solidified agar layer of petri dish to an extent such that the growth of the added microorganism is prevented entirely in the circular area or zone around the cavity containing the sample. Using micropipette 0.5 ml of each of the seeded broth containing 10^{-5}-10^{-6} Efu/ml test organism were incubated on solidified agar and spreaded uniformly with a glass spreader. Then four wells were cut in the agar layer of each plate with an aluminium bore of 6 mm diameter to contain 0.5 ml each of sample solution, standard drug, DMSO and methanol. The plates were incubated for 24 hours at 37^0C in case of antibacterial activity. After the incubation period the mean diameter of the zone of inhibiton in mm was measured around the wells which are shown in Table **2** and **3**.

The antifungal activity determination was carried out in the same way as used in antibacterial study; only different nutrient medium was used i.e., Sabouraud-Dextrose Agar media (SDA media) instead of Nutrient agar medium which was used in antibacterial study.

Minimum inhibitory concentrations of the compounds were determined as follows: Two fold dilutions (six) of the samples were carried out starting from the concentration of 0.1 – 20 mg/ml. The tubes were inoculated with a microorganism suspension at a final density of 10^5 cells/ml. The tubes were incubated at 37^0C for 24 hours. The lowest concentration of the tubes which did not show any visible growth after macroscopic evaluation was considered as the MIC of the respective compound.

Table: 01 Details of compounds

S. No	Compound Name	Structure of Compound	IUPAC Name	Molecular weight	Melting Point	Yield
1	C3_11		5-phenyl-4H-1,2,4-triazole-3-thiol	177.23	218	75%
2.	C4_20		4-(5-mercapto-4H-1,2,4-triazol-3-yl)phenol	193.23	300	65%
3	C5_14		5-(4-nitrophenyl)-4H-1,2,4-triazole-3-thiol	222.22	282	60%
4	C6_3		5-(phenoxy- methyl)-4H-1,2,4-triazole-3-thio	207.25	114	68%

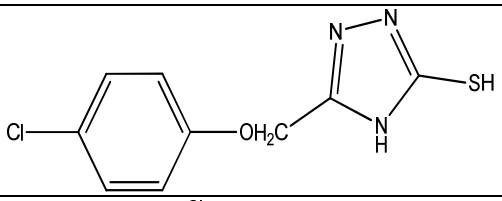
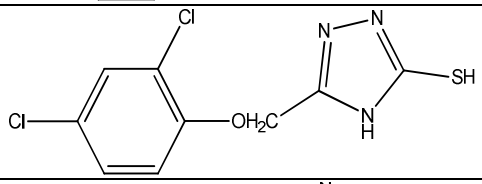
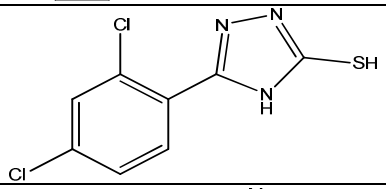
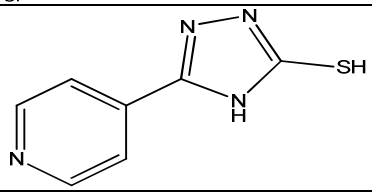
5	C7_12		5-((4-chloro phenoxy)methyl)-4H-1,2,4-triazole-3-thiol	241.7	142	70%
6	C8_8		5-((2,4-dichloro phenoxy)methyl)-4H-1,2,4-triazole-3-thiol	276.14	132	62%
7	C9_2		5-(2,4-dichlorophenyl)-4H-1,2,4-triazole-3-thiol	246.12	212	65%
8	C10_21		5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol	178.21	309	51%

Table 2: Antibacterial activity of the compounds

S. No.	Compound entry No.	Antibacterial activity against the bacterial strains used					
		E. Coli	*S. aureus*	*S. cohni*	*P. vulgaris*	*K. pneumonia*	*p. aerugenosa*
1	C3_11	-	-	+	+++	-	-
2	C4_20	-	-	+	+	+++	-
3	C5_14	++	+	+++	+++	+++	-
4	C6_3	+++	+++	++	-	+++	+++
5	C7_12	-	-	+	-	-	-
6	C8_8	-	-	-	-	-	-
7	C9_2	+++	-	-	-	++	+++
8	C10_21	-	-	+++	-	+++	++

Table 3: Antifungal activity of the compounds

S. No.	Compound entry No.	Antifungal activity against the fungal strains used	
		Aspergillus niger	*Candida albicans*
1	C3_11	-	-
2	C4_20	++	++
3	C5_14	-	++
4	C6_3	+++	+++
5	C7_12	-	-
6	C8_8	-	-
7	C9_2	+++	-
8	C10_21	++	+++

Table 4: MICs of the compounds against the microbes used:

S. No.	Compound Entry No.	Minimum Inhibitory Concentration (MIC) mg/ml							
		E. coli	*S. aureus*	*S. cohni*	*P. vulgaris*	*K. pneumonia*	*P. aerugenosa*	*A. niger*	*C. albicans*
1	C3_11	-	-	17-18	0.8-0.9	-	-	-	-
2	C4_20	-	-	15-16	17-18	0.2-0.3	-	0.8-1.2	2.0-2.1
3	C5_14	7-8	16-17	2.6-2.7	3.2-3.3	0.2-0.3	-	-	0.9-1.0
4	C6_3	0.1-0.15	1.2-1.6	10.8-11.2	-	2.8-3.2	0.1-0.11	0.1-0.2	0.1-0.2
5	C7_12	-	-	14-15	-	-	-	-	-
6	C8_8	-	-	-	-	-	-	-	-
7	C9_2	0.4-0.45	-	-	-	6.7-8.5	0.04-0.07	0.4-0.5	-
8	C10_21	-	-	0.6-0.7	-	0.2-0.3	7.8-7.9	0.8-1.2	0.7-0.8

RESULTS AND DISCUSSION

The compounds **1-8** synthesized as per the outlined scheme (1) were purified by re-crystallization and the purity was ascertained by TLC using Silica Gel G as stationary phase. The instrumental data suggested the formation of the compounds as desired. Antimicrobial screening of the compounds was performed using six different strains of bacteria and two different strains of fungi. Compounds **2,3,4,7 & 8** showed good activity against more than three different microbial strains (including bacteria as well as fungi). The structural make up of the compounds were thought of to be responsible of their antimicrobial activities. Compound **2** is having a 4-hydroxy group attached to an aromatic nucleus adjacent to 1,2,4-triazole which increases the hydrogen bonding of the of compound with bacterial and fungal cell wall proteins containing free sulfhydryl groups (-SH). Thus compound **2** showed activity against both bacteria and fungi to a good extent. The MICs for various strains of bacteria and fungi were determined and are depicted in table **4**.

Compound **3** possesses a 4-nitro group attached to the aromatic ring adjacent to 1,2,4-triazole, a structural feature which enables to penetrate the bacterial and fungal cell wall very easily. Compound **4** exhibited enhanced activity against *pseudomonas aerugenosa* due to the presence of phenoxymethyl group attached to 1,2,4-triazole nucleus. The phenoxy methyl group gives the compound extra stability while entering the bacterial cell membrane and the thiol group enhances penetration into fungal cell wall. Introduction of chloro (Cl) moiety at 4-position into the aromatic ring attached to the triazole nucleus resulted in poor antimicrobial activity with complete loss of antifungal activity, i.e. compound **7** showed poor activity. Further introduction of a chloro group at position 2 of the aromatic ring attached to the triazole nucleus leads to complete loss of antimicrobial activity of the compound i.e. compound **8**.

Compound **7** although containing 2,4-substituted aromatic moiety attached to the triazole ring showed good activity against bacteria and fungi both due to removal of the oxygen atom from between the aromatic ring and the methylene moiety. This leads to a compound with greater lipophilicity (Calculated Partition Coefficient = 3.458) enhancing penetration and binding to bacterial and fungal cell membranes.

Compound **8** containing a pyridyl moiety attached to the triazole nucleus at position 5 which further enhances the lipophilicity of the molecule enabling it to penetrate the microbial cell more easily, thus showing good activity.

CONCLUSION

Out of the eight compounds hereby reported compound **4** showed the best activity against seven out of eight microbial strains and compound **3** showed good activity against six out of eight strains tested. Compound 4 was found to be active against *Candida albicans* and *Aspergillus niger* with an MIC of 0.1-0.2 mg/ml in both the cases. The activity found is comparable to the standard drug Amphotericin B. Similarly compound **4** showed a MIC of 0.1-0.15 against E. coli which is the lowest of all the compounds being tested here showing that it is the most active compound of all. Compound **7** was found to be highly active against P. aerugenosa due to enhanced lipophilicity (Calculated LogP = 3.426). Other compounds showed moderate to week activities against the test microorganisms in vitro.

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