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Synthesis and biological evaluation of some novel 1,2,4-triazole derivatives

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ABSTRACT

*In order to search novel antimicrobial and antifungal agent series of new 5-phenyl-1-H-1,2,4-triazol-3-thione derivatives bearing different aldehydes moieties were designed and synthesized through multistep reactions. The newly synthesized compounds were evaluated for in vitro antibacterial activity against S. aureus, E. species, E. coli, P. auriginosa, and antifungal activity against A. niger, C. albicans using broth micro dilution method. Ciprofloxacin and Fluconazole were used as standard for antibacterial and antifungal screening respectively. Among the compounds **4a-4f**, particularly, compound **4b** and **4d** showed highest antibacterial activity as compared to the other derivatives against all bacterial species with a MIC of 200 µg/ml. Compound **4d** emerged as the most effective antifungal agent against C.albicans and A.niger.*

Keywords: Triazole, Antimicrobial, Antifungal, Ciprofloxacin, Synthesis

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INTRODUCTION

Since last few decades, there is tremendous growth of research in the synthesis of nitrogen containing heterocyclic derivatives because of their utility in various fields, such as pharmaceuticals, propellants,explosives, pyrotechnics and especially in chemotherapy, in the gravimetric estimation of silver, copper and lead [1-3].

Triazole heterocyclic compounds have been interestingly paid special attention due to wide potential applications as medicinal agents, agrochemicals, man-made materials, artificial acceptors, supermolecular ligands, biomimetic catalysts. While there are some known antimicrobial and antifungal drugs containing 1,2,4-triazole moiety like Triazolam, Alprazalam, Etizolam, Furacylin, Ribavirin, Hexaconazole, Triadimefon, Mycobutanil, Rizatriptan, Propicaconazole, Fluotrimazol and Fluconazole. 1,2,4-triazole also can act as intermediate for preparation of substituted caprolactum useful for treatment of HIV disease[4] and also find applications in the preparation of photographic plates, polymers and as analytical agent[5].

Thus in search of more potent antimicrobial and antifungal agent, herein we report the new, efficient and simple methodology for the synthesis of novel series of 5-phenyl-1-*H-1,2,4*-triazol-3-thione derivatives bearing different aldehydes moieties in good yields.

MATERIALS AND METHODS

Melting points were determined using microprocessor based melting point apparatus (Veego make) having liquid paraffin bath and are uncorrected. The IR spectra were recorded on a Perkin-Elmer Spectrum 100 FTIR spectrophotometer using KBr pellets and the wave numbers were given in cm^{-1}. The ^1H NMR spectra were recorded in DMSO-d_6/$CDCl_3$ on a Bruker Avance II spectrophotometer (400 MHz). All chemical shifts are reported in δ (ppm) using TMS as an internal standard. Chromatographic separations were performed on columns using silica gel

100-200 mesh and neutral alumina, activity grade I. The progress of all reactions were monitored by TLC on 2 cm X 5 cm pre-coated silica gel 60 F_{254} (Merck) plates of thickness of 0.25 mm by using chloroform : ethanol (4:1) solvent system. The chromatograms were visualized under UV (254 nm) and/or exposure to iodine vapours. All reagents used were of analytical reagent grade, obtained from S.D. Fine chemicals, Spectrochem, Qualigens and Sigma-Aldrich. Chemicals and solvents were purified by general laboratory techniques before use.

2.2 Synthesis:

2.2.1 Synthesis of 5-phenyl-1*H*-1,2,4-triazole-3-thione (1)

The cyclization of 1-benzoyl-3-thiosemicarbazide using aqueous sodium hydroxide or with aqueous ammonia, trimethyl amine and hydrazine yielded 5-substituted-1,2,4-triazole-3-thione. The yield was 75% [6]

2.2.2 Synthesis of ethyl [(5-phenyl-1*H*-1,2,4-triazole-3-yl)sulfanyl]acetate (2)

5-phenyl-3-mercapto-1*H*-1,2,4-triazole **1** (1mol) was refluxed with 1 equivalent of sodium in absolute ethanol for 2h. Then ethyl bromoacetate (1mol) was added and refluxed for an additional 3h. After evaporating the solvent under reduced pressure, a solid appeared **2**. The solid was recrystallised from ethanol. The yield was 68%

2.2.3 Synthesis of 2-[(5-phenyl-1*H*-1,2,4-triazol-3-yl)sulfanyl]acetohydrazide (3)

The above obtained ester (**2**) (0.005mole***)*** was dissolved in methanol (30 ml). To the clear solution, 99.9 % hydrazine hydrate (0.01mol) was added and heated under reflux. The progress of the reaction was monitored by thin layer chromatography using a mixture of chloroform and methanol (9:1) as eluent. The reaction mixture was cooled to 0-5^{0}C to crystallize the product. On filtration and washing with chilled methanol afforded acylated hydrazine derivative. The yield was 65% and melts at 56-58^{0}C. [7]

2.2.4 General procedure for Synthesis of (4a-f)

Equimolar quantity of hydrazide and different aldehydes were refluxed in alcohol for 5h in the presence of few drops of glacial acetic acid. The product was poured onto cold water, filtered and dried. Crude solid was recrystallized in ethanol. [8]

3. Biological Screening:

3.1 Antimicrobial activity:

The development of resistance among the various pathogenic organisms towards antibiotics stimulated the invention of newer antimicrobial agents [9]. The investigation of triazole derivatives as antimicrobial agents was initiated only in recent years. Within this short period of two or three decades, several triazole derivatives were found to possess such an activity.

It is evident from the above facts that several compounds possessing triazole moiety are capable of exhibiting broad spectrum antibacterial activity. When this ring system is fused with other heterocycles or coupled with other heterocycles directly or through bridges, the resulting compounds would exhibit enhanced antibacterial property. This fact was evident when such triazole compounds showed equipotent antibacterial activity in comparison with standard drug. Hence, selected triazole derivatives prepared in the course of present investigation are screened for antibacterial activity.

3.1.1 Materials and methods:

i. Nutrient broth.
ii. McFarland turbidity standards.
iii. Scrupulously clean, acid-washed borosilicate glass tubes.
iv. Micropipette.
v. Nutrient agar.

3.1.2 Evaluation of antibacterial activity:

The minimum inhibitory concentration (MIC) determination of the tested compounds were investigated in side by side comparison with Ciprofloxacin and Norfloxacin against gram-positive (*S.aureus, E.s*) and gram-negative bacteria (*E.coli, P.aeruginosa*) by broth micro dilution method. [10-11]

3.2 Antifungal activity:

Some triazole derivatives also have been subjected to antifungal screening against *Aspergillus niger* and *Candida albicans* as fungi organisms. Fluconazole was used as standard for comparing the observation.

3.2.1 Materials and methods:

i. Sabouraud dextrose broth.

ii. McFarland turbidity standards.
iii. Scrupulously clean, acid-washed borosilicate glass tubes.
iv. Micropipette.

3.2.2 Evaluation of antifungal activity:-
The antifungal activity of triazole derivatives was studied in comparison with that of standard antifungal drug fluconazole by broth micro dilution method.

RESULTS AND DISCUSSION

4.1 Chemical Studies:
The synthetic route leading to the desired compounds are shown in scheme. The cyclization of 1-benzoyl-3-thiosemicarbazide using aqueous sodium hydroxide or with aqueous ammonia, trimethyl amine and hydrazine yielded 5-substituted-1,2,4-triazole-3-thione **(1)** then it was reacted with ethyl bromoacetate in alkaline medium to get the corresponding ethyl ester **(2)** in yield (68%). Ester obtained was then converted to the hydrazide **(3)** after treatment with excess of hydrazine hydrate. (The reaction requires 2-3 hrs then it cooled) Triazole Schiff bases were synthesized by reaction of hydrazide **(4)** with various aromatic aldehydes in glacial acetic acid medium. In literature synthesis of Schiff base was catalyzed with acid or base in ethanol medium. Here we used glacial acetic acid as catalyst. The reaction require of 3-5 hrs and the product purified by crystallization using ethanol.

The infrared spectrum of hydrazide (**3**) showed strong absorption band at 1600 cm^{-1} characteristic of C=O, and two bands in the range of 3193-3320 cm^{-1} are the stretching modes of NH_2 and C=N. The ^1H NMR spectra of hydrazide showed a singlet at 9.4 ppm (1H, HNC=O) and broad singlet at 4.41 ppm (2H) assigned to the hydrazide NH_2. The absence of SH proton confirmed that the ester was converted into hydrazide.

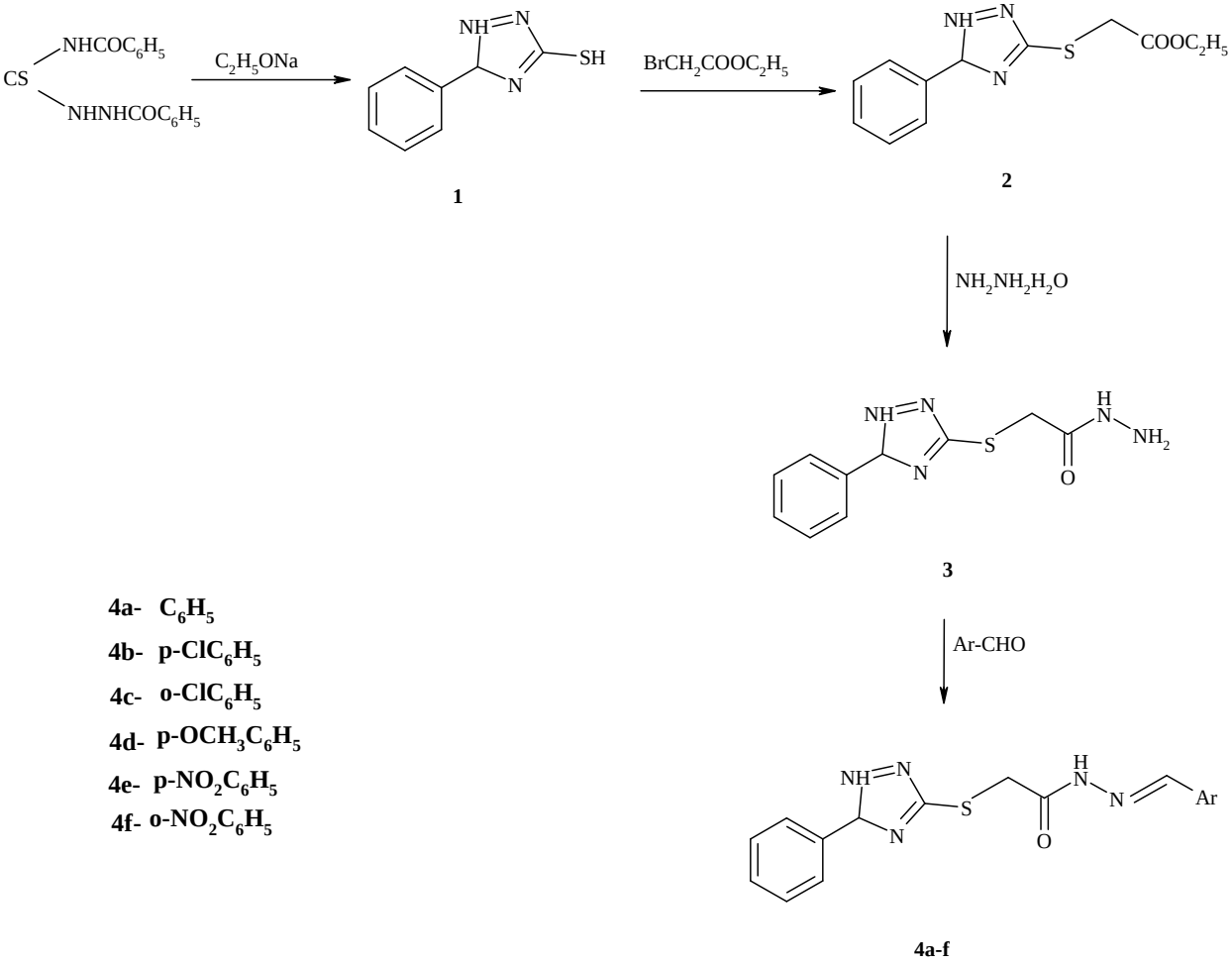
The formation of Schiff bases was indicated by the presence of the CH=N stretching band near to the 1559cm^{-1}, combined with the disappearance of the NH_2 stretching band. The ^1H NMR spectra of compounds **4a-f** displayed additional signals due to the aromatic ring derived from aldehyde moiety at aromatic region, while the signal belonging to $–NH_2$ group of hydrazide structure did not appear. In the ^1H NMR spectra of compounds **4a-f** two sets of signals each belonging to the $–SCH_2$ group, -N=CH group were observed between 4.03 and 8.50 ppm.

In addition, compound **4b** and **4c** showed absorption band at 1091 and 1065 cm^{-1} respectively, characteristic of C-Cl. Methoxy group of compound **4d** resonated at 3.83 ppm integrating three protons as a singlet in the ^1H NMR spectrum. Moreover, the absorption band from NO_2 group in compound **4e** and the signals derived from CH=CH group in compound **4f** were recorded at 5.1 and 7.05 ppm as doublet in the ^1H NMR spectrum.

4.2 Biological Activity:
4.2.1 Antimicrobial activity:
The antibacterial activity studies of the newly synthesized triazole derivatives (**4a-4f)** have been carried out against four pathogenic organisms, viz., *Staphylococcus aureus* (G^+), *Enterococcus species* (G^+), *Escherichia coli* (G^-), and *Pseudomonas aeruginosa* (G^-). Ciprofloxacin was used as standard drug for antibacterial screening. Minimum inhibitory concentration for ciprofloxacin is approximately 10 µg/ml against all microbes used.

Among the compounds **4a-4f**, particularly, Compound **4b** and **4d** showed highest antibacterial activity as compared to the other derivatives against all bacterial species with a MIC of 200 µg/ml. compound **4e** and **4f** showed the lowest antibacterial activity against all bacterial species with a MIC of 400 µg/ml. Compound **4a and 4c** yielded moderate activity with a MIC of 300 µg/ml in comparison to other compounds synthesized. The results of antibacterial activity showed that compounds with a unsubstituted aromatic ring showed good antibacterial activity among all the synthesized compounds. The compounds **4a and 4c** exhibited somewhat less activity where as compound **4e** and **4f** exhibited poor activity in comparison to standard, ciprofloxacin. From the above discussion it is evident that compound **4b** and **4d** emerged as the most active antibacterial triazoles with a MIC of 200 µg/ml.

4.2.2 Antifungal activity:
The antifungal activity studies of the newly synthesized triazole derivatives **4a - 4f** have been carried out against two fungal strains Viz., *Candida albicans* and *Aspergillus niger*. Fluconazole was used as standard drug for antifungal screening. The results of antifungal activity showed that substitution at position 4 on the aromatic ring by a methoxy group increase in activity. Substitution by 3-nitro, 2-chloro, 4-chloro group decrease the activity. The compounds **4a, and 4c** exhibited somewhat less activity where as compound **4e, 4b** and **4f** exhibited poor activity in comparison to standard, fluconazole. Thus compound **4d** emerged as the most effective antifungal agent against *C.albicans*.



4a- C_6H_5
4b- p-ClC_6H_5
4c- o-ClC_6H_5
4d- p-$OCH_3C_6H_5$
4e- p-$NO_2C_6H_5$
4f- o-$NO_2C_6H_5$

Scheme

Table 1: Physical data of 5-phenyl-1-*H-1,2,4*-triazol-3-thione derivatives

Compound No.	Ar	Molecular Formula	Molecular Weight	% Yield	M.P(^{0}C)
4a	C_6H_5	$C_{17}H_{15}N_5OS$	337.400	67%	90-92 ^{0}C
4b	p-Cl –C_6H_5	$C_{17}H_{14}ClN_5OS$	371.845	73%	180-184 ^{0}C
4c	O-Cl- C_6H_5	$C_{17}H_{14}ClN_5OS$	371.845	73%	162-168 ^{0}C
4d	OCH_3-C_6H_5	$C_{18}H_{17}N_5O_2S$	367.426	66%	112-114 ^{0}C
4e	p-NO_2-C_6H_5	$C_{17}H_{14}N_6O_3S$	382.398	69%	138-140 ^{0}C
4f	O-NO_2-C_6H_5	$C_{17}H_{14}N_6O_3S$	382.398	71%	158-160 ^{0}C

Table 2: Spectral data of 5-phenyl-1-*H-1,2,4*-triazol-3-thione derivatives

Comp.	Ar	IR(KBr) cm^{-1}	^{1}H NMR (DMSO) δppm	Elemental Analysis Found(cacld)% C H N
4a	C_6H_5	3430(NHstr), 1688(C=Ostr), 721(C-Sstr), 1315(C-Nstr)	4.1(s, 2HCH$_2$), 7.2-7.9(m, 5Har), 8.1(m, 1H CH), 11.4(s, 1H NH)	61.32 4.52 19.87 (60.52) (4.48) (20.76)
4b	*p*-Cl-C_6H_5	3419(NHstr), 1686(C=Ostr), 630(C-Sstr), 1319(C-Nstr)	4.2(s, 2HCH$_2$), 7.1-7.8(m, 5Har), 8.3(m, 1H CH), 11.5(s, 1H NH)	52.86 3.11 20.11 (54.91) (3.79) (18.83)
4c	*o*-Cl- C_6H_5	3385(NHstr), 1687(C=Ostr), 685(C-Sstr), 1335(C-Nstr)	4.0(s, 2HCH$_2$), 7.2-7.7(m, 5Har), 8.3(m, 1H CH), 11.07(s, 1H NH)	55.63 3.01 20.32 (54.91) (3.79) (18.83)
4d	*o*-CH_3-C_6H_5	3446(NHstr), 1659(C=Ostr), 788(C-Sstr), (1386 C-Nstr)	4.2(s, 2HCH$_2$), 7.1-7.8(m, 5Har), 8.2(m, 1H CH), 11.07(s, 1H NH)	56.16 2.33 17.11 (58.84) (4.66) (19.06)
4e	*p*-NO_2-C_6H_5	3425(NHstr), 1625(C=Ostr), 619(C-Sstr), 1342(C-Nstr)	4.4(s, 2HCH$_2$), 7.3-7.5(m, 5Har), 8.3(m, 1H CH), 11.8(s, 1H NH)	52.88 4.77 23.11 (53.40) (3.69) (21.98)
4f	*o*-NO_2-C_6H_5	3399(NHstr), 1601(C=Ostr), 701(C-Sstr), 1375(C-Nstr)	4.1(s, 2HCH$_2$), 7.1-7.5(m, 5Har), 8.1(m, 1H CH), 11.1(s, 1H NH)	51.76 4.92 22.91 (53.40) (3.69) (21.98)

Table 3: Biological activity data 5-phenyl-1-*H-1,2,4*-triazol-3-thione derivatives

Comp.	Ar	MIC values (µg/ml)					
		Antibacterial activity				Antifungal activity	
		Gram-positive		Gram-negative			
		Sa	Es	Ec	Pa	An	Ca
4a	C_6H_5	300	400	300	300	300	300
4b	p-Cl-C_6H_5	200	200	200	200	300	400
4c	o-Cl-C_6H_5	200	300	400	300	300	300
4d	p-OCH_3-C_6H_5	200	200	200	200	200	200
4e	p-NO_2-C_6H_5	400	300	300	400	400	400
4f	p-NO_2-C_6H_5	300	400	300	300	400	400
Ciprofloxacin	-	<5	<1	<1	<5	-	-
Fluconazole	-	-	-	-	-	0.25	0.25

Sa- S.aureus, Es-Enterococcus species, Ec-E.coli, Pa- P.aeruginosa, An-Aspergillus niger,Ca-Candida albicans.

CONCLUSION

Summarizing, a series of triazole derivatives have been synthesized successfully, in appreciable yields and screened for their in vitro antimicrobial activity against bacterial strains *S.aureus*, *E.coli* and fungal species *C.albicans* and *A.niger*. From the activity studies, it was concluded that among all the triazole derivatives , antibacterial activity decrease when there is a substitution on aromatic ring where as compound **4d** showed overall maximum activity against *C.albicans* and *A.niger*

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