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Synthesis, characterization and antimicrobial activity of some new pyrimidines containing tetrazole

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ABSTRACT

In attempt to find new pharmacologically active molecules, we report here the synthesis and the *in vitro* antimicrobial activity of various new pyrimidines containing 5-phenyl tetrazole. The 3-(substituted phenyl) -1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one[2a-f] were prepared by the reaction of 5-phenyl 1-acetyl tetrazole with different aromatic aldehydes in the presence of an alkaline medium. Reaction of [2a-f] with urea and thiourea resulted in 4-(substituted phenyl)-6-(5-phenyl-1H-tetrazol-1-yl)pyrimidin-2-ol [3a-f] and 4-(substituted phenyl)-6-(5-phenyl-1H-tetrazol-1-yl)pyrimidine-2-thiol [4a-f] respectively. The synthesized compounds were identified by spectral data and evaluated for their *in vitro* antibacterial and antifungal properties.

Keywords: *Pyrimidine, Tetrazole, Anti-microbial Activity*

1. INTRODUCTION

Derivatives of pyrimidine have played a crucial role in the history of heterocyclic chemistry being used extensively as important pharmacophores and synthons. Owing to their versatile chemotherapeutic importance, a significant amount of research effort has been focused on these nuclei [1]. Many classes of chemotherapeutic agents containing pyrimidine nucleus are in clinical use such as antibacterial (sulfadiazine, sulfamerazine and sulpfamethazine), anticancer (5-flurouracil and ftorafur), antiviral (iodoxuridine, trifluridine and zidovudine), antifungal (flucytosine) and antimalarial (pyrimethamine) agents [2]. Nitrogen containing hetrocycles such as pyrimidine and tetrazole is a promising structural moiety for drug designing. Pyrimidine based heterocycles are potential bioactive molecules and exhibit antibacterial, anti-inflammatory, antioxidant [3], antitumoral [4], analgesic [5], antitubercular and antiviral [6], antihypertensive [7], anticonvulsant [8], antimicrobial [9] agents and also act as enzyme inhibitors [10]. Tetrazole also possess a broad spectrum of biological activities, such as antimicrobial [11], anti-inflammatory[12], anticancer [13], antifungal [14] and analgesic activities [15]. Inspired from these facts, in the present work an attempt is being made to synthesize pyrimidines containing tetrazole and evaluate them for the antimicrobial activity which has not been reported yet. Hence the present work deals with the reaction of 5-phenyl 1-acetyl tetrazole (1) with different aromatic aldehydes in the presence of alkaline medium to form 3-(substituted phenyl) -1-(5-phenyl-1*H*-tetrazol-1-yl) prop-2-en-1-one (2a-

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f). Reaction of (2a-f) with urea and thiourea resulted in 4-(substituted phenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl)pyrimidin-2-ol (3a-f) and 4-(substituted phenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl)pyrimidin-2-thiol (4a-f) respectively. The structures of all various synthesized compounds were assigned on the basis of IR, ^{1}H NMR, MS spectral data and elemental analysis.

2. EXPERIMENTAL SECTION

2.1. Synthesis procedure.

2.1.1. General procedure for the preparation of 3-(substituted phenyl)-1-(5-phenyl-1*H*-tetrazol-1-yl) prop-2-en-1-one [2a-f][11]**.** A solution of 5-phenyl 1-acetyl tetrazole (8.5g, 0.005 moles) and aromatic aldehydes (0.005 mole) in ethanol (12 ml) was cooled to 5 to 10^{o}C on an ice bath. The cooled solution was treated with drop wise addition of aqueous sodium hydroxide (2.5 ml, 50%). The reaction mixture was magnetically stirred for 30 min and then left over night. The resulting dark solution was diluted with ice water and carefully acidified using diluted hydrochloric acid. The tetrazole analogues of chalcone which crystallized were collected by filtration after washing with sodium bicarbonate and water. It was further purified by crystallization from ethanol.

2.1.2. Synthesis of compounds 4-(substituted phenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl)pyrimidin-2-ol [4a-f]. To a solution of 3-substituted phenyl-1-(5-phenyl-1*H*-tetrazol-1-yl) prop-2-en-1-one 2a-f (0.01mole) in anhydrous ethanol (50 mL), urea (0.01 mole) and aqueous sodium hydroxide (0.01 mole). The reaction mixture was refluxed for 5 hrs and poured into ice cold water the product obtained was filtered, washed with water and crystallized from aqueous ethanol. The progress of the reaction was monitored by TLC using a mixture of hexane and ethyl acetate (7:3) as a mobile phase.

2.1.3. Synthesis of compounds 4-(substituted phenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl)pyrimidin-2-thiol [5a-f]. To a solution of 3-(substituted phenyl)-1-(5-phenyl-1*H*-tetrazol-1-yl) prop-2-en-1-one 2a-f (0.01mole) in anhydrous ethanol (50 mL), thiourea (0.01 mole) and aqueous sodium hydroxide (0.01 mole). The reaction mixture was refluxed for 5 hrs and poured into ice cold water the product obtained was filtered, washed with water and crystallized from aqueous ethanol. The progress of the reaction was monitored by TLC using a mixture of hexane and ethyl acetate (6:4) as a mobile phase.

2.2. Antibacterial and antifungal activity assay. All the newly synthesized compounds were screened for their antimicrobial activity against both gram positive *S. aureus* and gram negative *E. coli* bacteria and antifungal activity against *C. albicans* and *A. niger* according to cup plate method [16] at a concentration 100ug/0.1ml respectively. Streptomycin and clotrimazole were used as standard for comparison of antibacterial and antifungal activity [17]. Solvent dimethyl sulphoxide (DMSO) was used as control.

3. RESULTS SECTION

3.1. Synthesis and spectral characterization. A series of 4-(substituted phenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl) pyrimidin-2-ol and 4-(substituted phenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl) pyrimidin-2-thiol were synthesized from chalcones of 5-phenyl tetrazole. All synthesis steps are presented in scheme 1. Melting points were determined with open capillary and were uncorrected. FT-IR spectra were recorded on a Shimadzu FT-IR model 8010 spectrophotometer using KBr pellets in cm^{-1}, ^{1}H NMR spectra were recorded in DMSO in ppm on a Varian mercury FT-NMR model YH- 300 instrument using TMS as internal standard. Mass spectra were recorded on GC-MS auto tune EI instrument using DMSO as solvent as (m/z).The physical data of compounds were presented in Table 1.The FT-IR spectra shows 1542 cm^{-1}(C=N), 1445 cm^{-1}(C=C) and 3054 cm^{-1} (Ar-CH) providing the strong evidence for pyrimidine aromatic ring respectively. ^{1}H NMR spectrum shows

7.14-7.50 ppm for aromatic protons and 9.6 ppm for OH protons and 13.6 for SH protons were observed at expected signals.

3a: 4-phenyl-6-(5-phenyl-1*H*-tetrazol-1-yl) pyrimidin-2-ol:IR in cm^{-1}: 3747 (OH), 3058 (Ar-CH), 1542(C=N), 1445(C=C), 1286(N-N=N-),1120 and 1145(Tetrazole ring) ,^{1}H NMR:6.5-6.8(d,1H,CH=CH) ,7.14-7.50 (m ,11H, Ar-H), 9.6 (1H, OH).Mass spectrum (m/z) molecular ion peak at 316 and isotopic peak at 317.

3b: 4-(2-chlorophenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl)pyrimidin-2-ol:IR in cm^{-1}: 3742 (OH), 3055 (Ar-CH), 1540(C=N), 1446(C=C), 1285(N-N=N-),1120 and 1145(Tetrazole ring) , 684(C-Cl).,^{1}H NMR:6.5-6.8(d,1H,CH=CH) ,7.14-7.50 (m ,10H, Ar-H), 9.5 (1H, OH). Mass spectrum (m/z) molecular ion peak at 351.

3c: 4-(4-methoxyphenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl)pyrimidin-2-ol:IR in cm^{-1}: 3748 (OH), 3056 (Ar-CH), 1545(C=N), 1442(C=C), 1286(N-N=N-),1245(-OCH3) ,1120 and 1145(Tetrazole ring) ,^{1}H NMR: 3.9(s, 3H, OCH3),6.5-6.8(d,1H,CH=CH) ,7.14-7.50 (m ,10H, Ar-H), 9.4 (1H, OH), Mass spectrum (m/z) molecular ion peak at 346.

3d: 4-(4-nitrophenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl)pyrimidin-2-ol:IR in cm^{-1}: 3740 (OH), 3050 (Ar-CH), 1560(-NO2),1540(C=N), 1441(C=C), 1282(N-N=N-),1120 and 1145(Tetrazole ring) ,^{1}H NMR:6.5-6.8(d,1H,CH=CH), 7.14-7.50 (m ,10H, Ar-H), 9.4 (1H, OH), Mass spectrum (m/z) molecular ion peak at 361.

3e: 4-(4-dimethylaminophenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl)pyrimidin-2-ol:IR in cm^{-1}: 3742 (OH), 3048 (Ar-CH), 1544(C=N), 1445(C=C), 1331(-N(CH3)2,1286(N-N=N-),1120 and 1145(Tetrazole ring) ,^{1}H NMR: 2.9(s, 6H, CH3),6.5-6.8(d,1H,CH=CH) ,7.14-7.50 (m ,10H, Ar-H), 9.6 (1H, OH), Mass spectrum (m/z) molecular ion peak at 359.

3f: 4-(4-methylphenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl)pyrimidin-2-ol:IR in cm^{-1}: 3741 (OH), 3058 (Ar-CH), 1548(C=N), 1446(C=C), 1355(CH3),1288(N-N=N-),1120 and 1145(Tetrazole ring) ,^{1}H NMR: 2.8(s, 3H, CH3),6.5-6.8(d,1H,CH=CH) ,7.14-7.50 (m ,10H, Ar-H), 9.5 (1H, OH), Mass spectrum (m/z) molecular ion peak at 330.

4a: 4-phenyl-6-(5-phenyl-1*H*-tetrazol-1-yl) pyrimidin-2-thiol:IR in cm^{-1}:3056 (Ar-CH), 1538(C=N), 1436(C=C), 1286(N-N=N-),1120 and 1145(Tetrazole ring) ,^{1}H NMR:6.5-6.8(d,1H,CH=CH) ,7.10-7.55 (m ,10H, Ar-H), 13.6 (s, 1H, SH), Mass spectrum (m/z) molecular ion peak at 332.

4b: 4-(2-chlorophenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl)pyrimidin-2-thiol:IR in cm^{-1}: 3050 (Ar-CH), 1535(C=N), 1436(C=C), 1286(N-N=N-),1120 and 1145(Tetrazole ring) ,680(C-Cl).,^{1}H NMR:6.5-6.8(d,1H,CH=CH) 7.14-7.50 (m ,10H, Ar-H), 13.6 (s, 1H, SH), Mass spectrum (m/z) molecular ion peak at 366.

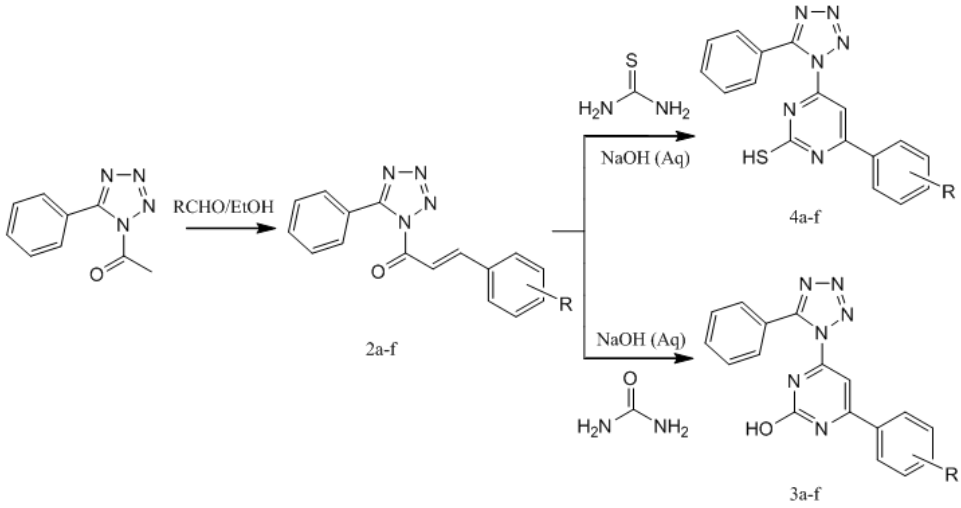
4c: 4-(4-methoxyphenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl)pyrimidin-2-thiol:IR in cm^{-1}: 3052 (Ar-CH), 1544(C=N), 1448(C=C), 1286(N-N=N-),1245(-OCH3) ,1120 and 1145(Tetrazole ring) ,^{1}H NMR: 3.9(s, 3H, OCH3),6.5-6.8(d,1H,CH=CH) ,7.14-7.50 (m ,10H, Ar-H), 13.5 (s, 1H, SH), Mass spectrum (m/z) molecular ion peak at 362.

4d: 4-(4-nitrophenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl)pyrimidin-2-thiol:IR in cm^{-1}: 3058 (Ar-CH), 1543(C=N), 1560(-NO2),1442(C=C), 1286(N-N=N-), 1120 and 1145(Tetrazole ring) ,^{1}H NMR:6.5-6.8(d,1H,CH=CH) ,7.14-7.50 (m ,10H, Ar-H), 13.4(s, 1H, SH), Mass spectrum (m/z) molecular ion peak at 377.

4e:4-(4-dimethylaminophenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl)pyrimidin-2-thiol:IR in cm^{-1}:3050 (Ar-CH), 1542(C=N), 1442(C=C), 1331(-N(CH3)2,1286(N-N=N-),1120 and 1145(Tetrazole ring)

,^{1}H NMR: 2.9(s, 6H, CH3),6.5-6.8(d,1H,CH=CH) ,7.14-7.50 (m ,10H, Ar-H), 13.3(s, 1H, SH), Mass spectrum (m/z) molecular ion peak at 375 and isotopic peak at 377.

4f:4-(4-methylphenyl)-6-(5-phenyl-1*H*-tetrazol-1-yl)pyrimidin-2-thiol:IR in cm^{-1}: 3051(Ar-CH), 1540(C=N), 1442(C=C), 1355(CH3),1286(N-N=N-),1120 and 1145(Tetrazole ring) ,^{1}H NMR: 2.8(s, 3H, CH3),6.5-6.8(d,1H,CH=CH) ,7.14-7.50 (m ,10H, Ar-H), 13.4(s, 1H, SH), Mass spectrum (m/z) molecular ion peak at 346.



Scheme 1: Synthesis of pyrimidines

Table 1: Physical Data of the obtained Compounds

Comp no	R	Mole. Formula	MW	% Yield	M.P.^{0}C	R_f.	Found (Calcd) %		
							C	H	N
3a	H	$C_{17}H_{12}N_60$	316	70	145	0.65	64.53 (64.55)	3.80 (3.82)	26.57 (26.57)
3b	2-Cl	$C_{17}H_{11}ClN_60$	351	63	174	0.60	58.20 (58.21)	3.14 (3.16)	23.94 (23.96)
3c	4-OCH_3	$C_{18}H_{14}N_60_2$	346	62	184	0.75	62.40 (62.42)	4.05 (4.07)	24.25 (24.27)
3d	4-NO_2	$C_{16}H_{11}N_50_3$	361	55	165	0.65	56.50 (56.51)	3.05 (3.07)	27.09 (27.11)
3e	4-$(CH_3)_2N$	$C_{19}H_{17}N_70$	359	71	150	0.55	63.49 (63.50)	4.75 (4.77)	27.80 (27.82)
3f	4-CH_3	$C_{18}H_{14}N_60$	330	52	138	0.71	65.42 (65.44)	4.26 (4.27)	25.42 (25.44)
4a	H	$C_{17}H_{12}N_6S$	332	59	198	0.65	61.40 (61.43)	3.62 (3.64)	28.32 (28.34)
4b	2-Cl	$C_{17}H_{11}ClN_6S$	366	75	185	0.61	55.64 (55.66)	3.00 (3.02)	22.88 (22.91)
4c	4-OCH_3	$C_{18}H_{14}N_60S$	362	74	180	0.75	59.62 (59.65)	3.88 (3.89)	23.18 (23.19)
4d	4-NO_2	$C_{16}H_{11}N_50_2S$	377	63	163	0.58	54.10 (54.11)	2.92 (2.94)	25.95 (25.98)
4e	4-$(CH_3)_2N$	$C_{18}H_{17}N_50S$	375	64	175	0.55	60.75 (60.78)	4.54 (4.56)	26.10 (26.11)
4f	4-CH_3-	$C_{18}H_{14}N_6S$	346	58	190	0.71	62.39 (62.41)	4.05 (4.07)	24.24 (24.26)

3.2. Antibacterial and antifungal activity. The results of preliminary antibacterial bioassays were compared with the standard drug ciprofloxacin. Most of the synthesized compounds showed antibacterial

activity against the tested bacteria. It is evident that most of the compounds are very weakly active and few are moderately active against *S. aureus* and *E. coli,* but however compounds 3c, 3b and 3a and compounds 4b, 4c and 4a possess very good activity against *S. aureus* and *E. coli* at a concentration of 100ug/0.1ml. Similarly, the results of preliminary antifungal bioassays were compared with the standard drug clotrimazole. Most of the synthesized compounds showed antifungal activity against the tested fungi. The compounds 3e, 3c and 3b and compounds 4e, 4b and 4c possess very good activity against fungi *C. albicans* and *A. niger* at the concentration of 100ug/0.1mL. Compounds 3d and 4f showed moderate activity against all bacterial and fungal tested strains. The results of the screening assay are given in Table 2.

Table 2: Antibacterial and Antifungal activity of Pyrimidine

Comp.	Zone of inhibition in mm at 100 µg/0.1ml			
	S. aureus	*E. coli*	*C. albicans*	*A. niger*
3a	14	13	16	13
3b	15	13	20	16
3c	16	15	21	15
3d	14	12	18	13
3e	13	10	21	22
3f	12	11	15	12
4a	15	13	15	13
4b	17	15	20	16
4c	16	14	20	14
4d	12	12	17	11
4e	12	11	21	22
4f	14	12	16	10
Ciprofloxacin	24	24	-	-
Clotrimazole	-	-	24	24
Control	6	6	6	6

4. CONCLUSIONS

Pyrimidines are an important group of compounds reported to have different biological activities and hence the present studies were undertaken in order to synthesize new derivatives and to investigate them for their antibacterial and antifungal activity. Compounds with methoxy and chlorine substituent exhibited significant antibacterial and antifungal activity when compared with the control. The compounds with nitro and dimethylamino substituents on phenyl groups showed significant activity when compared to standard drug ciprofloxacin and clotrimazole respectively.

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