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SYNTHESIS OF SOME NEW 3-SUBSTITUTED – 4*H*-1, 2, 4-TRIAZOLES AND THEIR EVALUATION FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Some new derivatives of 3-substituted -4*H*-1, 2, 4- triazoles **4 (a-e)** were synthesized through the reaction of 4-alkyl diazo substituted 4*H*-1, 2,4- triazoles- 3-thiole with different aliphatic and aromatic amines to yield the titled compounds. Structure of new compounds were verified on the basis of spectral (UV, IR, ^{1}H-NMR, MS) data and elemental (C, H, N, S) analysis. Antimicrobial activity of all the triazole derivatives were evaluated *in vitro* against the bacteria *S. aureus, B. subtilis, P. aeruginosa, E. coli* and fungi *A. fumigatus* and *C. albicans*. Most of the compounds showed potent and significant results compared to standard ciprofloxacin and fluconazole.

Key words: 4*H*-1, 2, 4-triazole, Antibacterial, Antifungal.

INTRODUCTION

Increasing bacterial resistance towards the existing antibacterial agents became a major reason for introduction of new and potential drugs to combat various bacterial infections. Triazole derivatives (1*H*- 1,2,4 and 4*H*- 1,2,4) showed very promising biological activities such as anticonvulsant[1-2], antifungal[3-5], anticancer[6-9], anti-inflammatory[10-12], antibacterial[13-16], etc. Several compounds (Fig. 1) containing 1, 2, 4-triazole rings are well known as drugs. For example, fluconazole is used as an antimicrobial drug[17], while vorozole, letrozole and anastrozole are non-steroidal drugs used for the treatment of cancer[18] and loreclezole is used as an anticonvulsant.[19] The imidazole derivative i.e. dacarbazine, which contains amino-diazo chain exhibits antineoplastic and antibacterial activity. On the basis of above observations, it was planned to prepare 4*H* – 1, 2, 4 – triazole derivatives substituted

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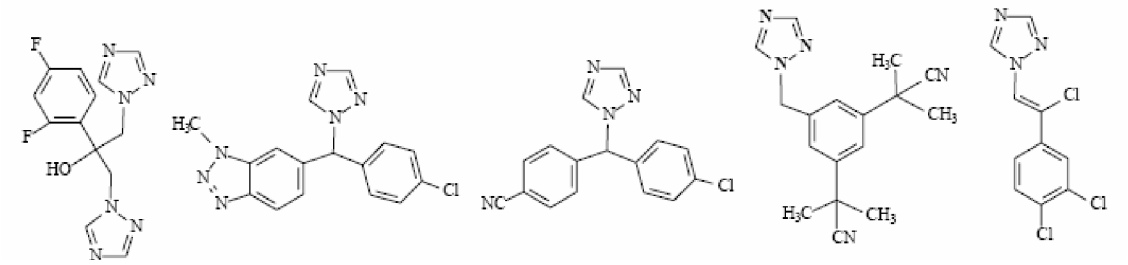
at position 1 by amino-diazo chain. The presence of this chain on triazole moiety was assumed to possess very potent antimicrobial, analgesic and anti-inflammatory activity.



Fluconazole **Vorozole** **Letrozole** **Anastrozole** **Loreclezole**

Fig. 1

EXPERIMENTAL

Materials and methods

The melting points of synthesized compounds were recorded by open capillary method and are uncorrected. The purity and homogeneity of the synthesized compounds was routinely ascertained by TLC using silica gel-G. All the chemicals were purchased from the local suppliers and were purified by established methods. The absorption maxima of the synthesized compounds were recorded in methanol/ethanol (analytical grade, 1 mg/100 mL). The methanolic/ethanol solutions of the synthesized compounds were scanned on Shimadzu UV 1700 spectrophotometer, Kyoto, Japan; in the region 200-400 nm. The IR spectra were recorded in KBr disc on FTIR Shimadzu 8400 S Japan. The ^{1}H NMR spectra were recorded on Bruker Avance II 400 NMR spectrometer using *d*6-DMSO as solvent and TMS as an internal standard. The mass spectra were recorded on Waters Q –Toff-micro spectrometer. The elemental analyses were carried out on elemental analyzer Vario H- III. The cup plate method was used to evaluate antibacterial (using ciprofloxacin as standard drug) and antifungal (using fluconazole as standard drug) activity of synthesized compounds.

Compounds **3 (a-b)** were synthesized by established methods and were obtained as per the reported yield. The compounds **3 (a-b)** were diazotized as per the standard procedure. The corresponding solutions of diazotized product in 10% NaOH were prepared and cooled by addition of crushed ice. To the cooled preparations, cold solution of different amines in. 10% aq. NaOH were added and were stirred vigorously to obtain the titled compound **4 (a-e)**. All the synthesized compounds were recrystallized with rectified spirit. Good results (highest yields) were obtained, when the diazotized compound and amines

were taken in equimolar concentrations. The physical constants of all the synthesized compounds are given in Table 1.

Table 1: Physical constants of the synthesized derivatives 4 (a-e)

Compd.	R_1	R_2	R_3	Mol. For. [Wt.]	Y [%]	M.P. (^0C)	Rf	C. % cal. [fnd.]	H. % cal. [fnd.]	N. % cal. [fnd.]	S. % cal. [fnd.]
4a	C_6H_5	H	C_6H_5	$C_{14}H_{12}N_6S$ [296]	69.42	208-11	0.56	56.75 [56.74]	4.05 [4.03]	28.73 [28.72]	10.81 [10.83]
4b	C_6H_5	H	C_6H_4-NO_2-*p*	$C_{14}H_{11}N_7SO_2$ [341]	28.98	274 - 76	0.72	49.26 [49.24]	3.22 [3.24]	28.73 [28.72]	9.38 [9.36]
4c	C_6H_5	H	C_6H_4-CH_3-*o*	$C_{15}H_{14}N_6S$ [310]	23.99	253-55	0.61	58.06 [58.07]	4.57 [4.50]	27.09 [27.08]	10.32 [10.34]
4d	C_6H_5	H	C_6H_4-OCH_3-*p*	$C_{15}H_{14}N_6SO$ [326]	19.15	284-86	0.74	55.21 [55.23]	4.29 [4.27]	25.76 [25.72]	9.83 [9.69]
4e	C_6H_5	C_6H_5	C_6H_5	$C_{20}H_{16}N_6S$ [372]	57.34	277-79	0.48	64.57 [64.54]	4.30 [4.31]	22.58 [22.56]	8.60 [8.64]

% cal. = % calculated, and % fnd. = % found; Y = yield

The characterization data of all the synthesized compounds are as follows:

4a : 3-Phenyl-4-[3-phenyl-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol; λ_{max} 342 nm (methanol); IR (KBr, ν max, cm^{-1}): 3438.84 (-NH), 3290.33 (-CH, Ar), 1639.38 (-N=N), 1161.07 (-C=S, triazole), 642.25,669.25 (out of plane, Ar, C-H,), ^{1}H NMR (DMSO-*d*6, δ ppm): 4.52 (s, 1H, NH), 6.99-7.48 (m, 10H, Ar-ring), 11.60 (s, 1H,SH), MS: m/z 296 [M^+]

4b : 3-Phenyl-4-[3-(4-nitrophenyl)-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol; λ_{max} 310 nm (methanol); IR (KBr, ν max, cm^{-1}): 3465.84 (-NH), 2918.10 (-CH, Ar), 1589.23 (-N=N), 1164.92 (-C=S, triazole), 754.12 (out of plane, Ar, C-H); ^{1}H NMR (DMSO-*d*6, δ ppm): 4.54 (s, 1H, NH), 6.96-7.36 (m, 9H, Ar-ring), 11.61 (s, 1H,SH), MS: m/z 341 [M^+]

4c : 3-Phenyl-4-[3-(2-methyl phenyl)-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol; λ_{max} 328 nm (ethanol); IR (KBr, ν max, cm^{-1}): 3363.62 (-NH), 3060.82 (-CH, Ar), 1596.95 (-N=N), 1157.21 (-C=S, triazole), 750.26,761.83 (out of plane, Ar, C-H); ^{1}H NMR (DMSO-*d*6, δ ppm): 2.06 (s, 3H, CH_3), 4.31 (S,1H, NH), 6.74-7.12 (m, 9H, Ar-ring), 10.98 (s, 1H,SH]), MS: m/z 310 [M^+]

4d : 3-Phenyl-4-[3-(4-methoxy phenyl)-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol; λ_{max} 366 nm (methanol); IR (KBr, ν max, cm^{-1}): 3421.48 (-NH), 2921.96 (-CH, Ar), 1602.74 (-N=N), 1176.5 (-C=S, triazole), 669.25 (out of plane, Ar, C-H); ^{1}H NMR (DMSO-*d*6, δ ppm): 2.16 (s, 3H, OCH_3), 4.07 (S,1H, NH), 6.70-6.99 (m, 9H, Ar-ring), MS: m/z 326 [M^+]

4e : 3-Phenyl-4-[3,3-diphenyl-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol; λ_{max} 356 nm (ethanol); IR (KBr, ν max, cm^{-1}): 3382.91 (-NH), 3101.32 (-CH, Ar), 1596.95 (-N=N), 1172.64 (-C=S, triazole), 690.47,744.47 (out of plane, Ar, C-H); ^{1}H NMR (DMSO-*d*6, δ ppm): 6.77-7.14, (m, 15H, Ar-ring), 10.86 (S,1H, SH), MS: m/z 372 [M^+]

Antibacterial screening[23,24]

All the synthesized compounds **4 (a-e)** were tested for *in vitro* antibacterial activity against two Gram + ve (S. *aureus and B. subtilis) and* two Gram - ve *(P. aeruginosa and E. coli)* bacteria at a concentration of 100 µg/ mL using cup – plate method . Ciprofloxacin was used as standard reference compound *at a concentration of* 100 µg/ mL. Dimethyl formamide (DMF) was used as a solvent. Zone of inhibition (ZOI) and % inhibition of all the derivatives are given in Table 2.

Table 2: **Results of *in vitro* antibacterial activity of the synthesized compounds 4(a-e)**

Compd.	Gram + ve bacteria				Gram – ve bacteria			
	S. aureus		*B. subtilis*		*E. coli*		*P. aeruginosa*	
	ZOI mm)	**% Inhibition**	**ZOI (mm)**	**% Inhibition**	**ZOI (mm)**	**% Inhibition**	**ZOI (mm)**	**% Inhibition**
4a	12	50	09	37.5	11	45.83	10	40
4b	11	45.83	11	45.83	13	54.16	12	50
4c	17	**70.83**	12	50.0	17	**70.83**	17	68
4d	09	37.5	10	41.66	12	50.0	11	44
4e	21	**87.5**	10	41.66	20	**83.33**	10	40
Std. Ciprof-loxacin]	24	100	24	100	24	100	25	100

ZOI = Zone of inhibition

Antifungal screening[25]

All the synthesized derivatives were tested for antifungal activity against two fungi, *A. fumigatus* and *C. albicans* at a concentration of 100 µg/mL using cup – plate method. Fluconazole was used as standard reference compound at a concentration *of* 100 µg/mL. Zone of inhibition and % inhibition of all the compounds are given in Table 3.

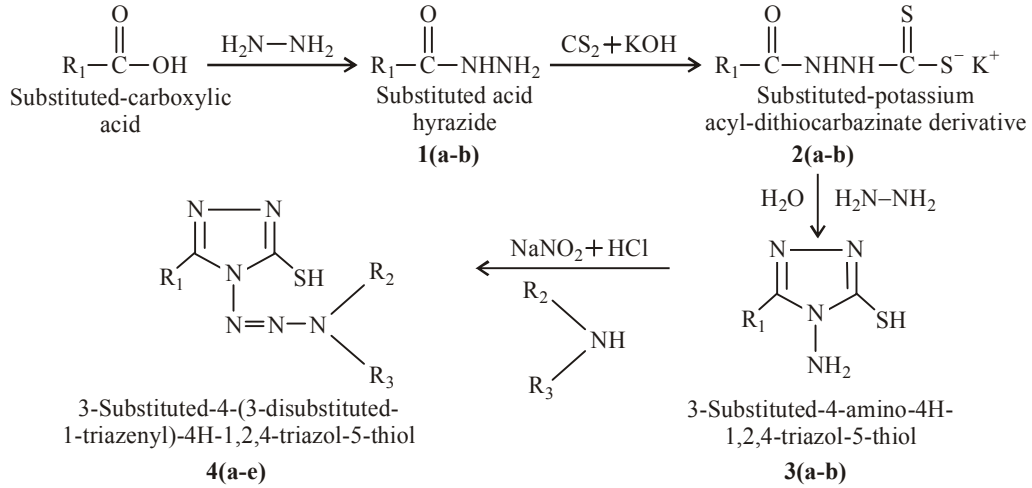
Table 3: Results of *in vitro* antifungal activity of the synthesized compounds 4(a-e)

Compd.	*A. fumigatus*		*C. albicans*	
	Zone of Inhibition (mm)	**% Inhibition**	**Zone of Inhibition (mm)**	**% Inhibition**
4a	01	25	12	48
4b	02	50	10	40
4c	02	50	15	60
4d	03	**75**	13	52
4e	04	**100**	21	**84**
Std. (Fluconazole)	04	100	25	100

RESULTS AND DISCUSSION

The physical constants and characterization data of all the synthesized compounds reveals their successful synthesis. At concentration of 100 µg/mL, all the synthesized compounds **4 (a-e)** exhibited antimicrobial activities against tested pathogens to different strength. Among the tested compounds, the compound ***4c*** exhibited almost similar activity (70.83%) against the bacteria *S.aureus* and *E.coli* where as compound ***4e*** showed remarkable activity against these bacteria (87.5% and 83.33%). It is attributed to the presence of electron releasing groups in these derivatives. The synthesized compounds exhibit mild to excellent activity against the fungi *A.* fumigatus and *C. albicans*. Against *A. fumigatus*, compound **4d** showed very potent activity (75%). The compound **4e** was found to be comparable in activity to fluconazole against *A.* fumigatus and have excellent inhibition (84%) against C. *albicans*. From these findings, it can be concluded that the titled compounds (substituted either with phenyl ring or phenyl rings substituted with strong electron releasing groups like $-OCH_3$, $-CH_3$) showed higher antimicrobial activity.

Synthetic scheme



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