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Research Article

SYNTHESIS AND EVALUATION OF SOME 1, 2, 4-TRIAZOLE DERIVATIVES AS ANTICONVULSANT, ANTI-INFLAMMATORY AND ANTIMICROBIAL AGENTS

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ABSTRACT

A new series of substituted 1,2,4-triazoles as substituted 4,5-diphenyl *4H*-1,2,4-triazole-3-thiols [$3a_1$-a_6 – $3d_1$-d_6] have been synthesized and evaluated for their anticonvulsant, anti-inflammatory, antibacterial, antifungal activities. Structures of the compounds have been characterized by IR, ^{1}H-NMR spectroscopy. All the compounds exhibited potent to moderate anti-inflammatory, anticonvulsant and antimicrobial activities.

Keywords: Substituted 1, 2, 4-triazole derivatives, Anti-inflammatory, Anticonvulsant, Antibacterial, Antifungal activities.

INTRODUCTION

The efficacy of azole derivatives as chemotherapeutic agent is well established and their chemistry has extensively studied. In the past years, the literature enriched with progressive finding about the synthesis and biological activities of various substituted 1,2,4–triazoles possess potent biological activities such as antimicrobial[1,3,4,6] antitubercular[1], antitumour[2] , analgesic[3], diuretic[3], anti-inflammatory[4], anticonvulsant[4], antiviral[5], anti-HIV[6] activities. Hence in the present study, substituted 4,5-diphenyl 4*H*-1,2,4-triazole-3-thiols[$3a_1$-a_6–$3d_1$-d_6] have been synthesized **[of scheme-I]**. The compounds have been screened for their antibacterial, antifungal, anticonvulsant, anti-inflammatory activities.

MATERIALS AND METHODS

Melting points were determined in open capillary tubes and were uncorrected. The chemicals and solvents used are of analytical grade. The purity of the compounds was checked by TLC. IR spectra were recorded (in **KBr**) on Perkin Elmer FT-IR Spectrophotometer, ^{1}H-NMR spectra in $CDCl_3$ on a 300MHz Bruker Ultrashield NMR Spectrometer using TMS as internal standard. (Chemical shifts in δppm). The physical and analytical data of synthesized compounds are given in table **1** and **2** respectively.

Table 1: Physical Data of Compounds ($3a_1$-A_6 to $3d_1$-D_6)

COMPD NO.	R	R_1	MOL.FORMULA	M.P (^{0}C)	YIELD (%)
$3a_1$	2-Cl	4-OCH_3	$C_{15}H_{12}N_3SOCl$	228-230	**79**
$3a_2$	2-Cl	4-CH_3	$C_{15}H_{12}N_3SCl$	214-216	**95**
$3a_3$	2-Cl	4-Cl	$C_{14}H_{19}N_3SCl_2$	134-136	**92**
$3a_4$	2-Cl	2-OCH_3	$C_{15}H_{12}N_3SOCl$	208-210	**87**
$3a_5$	2-Cl	H	$C_{14}H_{10}N_3SC1$	178-180	**83**
$3a_6$	2-Cl	3-Cl	$C_{14}H_9N_3SCl_2$	152-154	**75**
$3b_1$	3-CH_3	4-OCH_3	$C_{16}H_{15}N_3SO$	226-228	**91**
$3b_2$	3-CH_3	2-OCH_3	$C_{16}H_{15}N_3SO$	209-211	**61**
$3b_3$	3-CH_3	4-Cl	$C_{15}H_{12}N_3SCl$	188-190	**96**
$3b_4$	3-CH_3	H	$C_{15}H_{13}N_3S$	175-177	**82**
$3b_5$	3-CH_3	4-CH_3	$C_{16}H_{15}N_3S$	216-218	**73**
$3b_6$	3-CH_3	3-Cl	$C_{15}H_{12}N_3SCl$	232-234	**67**
$3c_1$	4-CH_3	4-OCH_3	$C_{16}H_{15}N_3SO$	222-224	**53**
$3c_2$	4-CH_3	4-CH_3	$C_{16}H_{15}N_3S$	244-246	**54**
$3c_3$	4-CH_3	4-Cl	$C_{15}H_{12}N_3SCl$	144-146	**49**
$3c_4$	4-CH_3	2-OCH_3	$C_{16}H_{15}N_3SO$	218-220	**62**
$3c_5$	4-CH_3	H	$C_{15}H_{13}N_3S$	168-170	**46**
$3c_6$	4-CH_3	3-Cl	$C_{15}H_{12}N_3SCl$	214-216	**69**
$3d_1$	3,4 dimethoxy	4-CH_3	$C_{17}H_{17}N_3SO_2Cl$	200-202	**53**
$3d_2$	"	4-Cl	$C_{16}H_{14}N_3SO_2Cl$	196-198	**74**
$3d_3$	"	2-OCH_3	$C_{17}H_{17}N_3SO_3$	200-202	**52**
$3d_4$	"	4-OCH_3	$C_{17}H_{17}N_3SO_3$	212-214	**48**
$3d_5$	"	H	$C_{16}H_{15}N_3SO_2$	178-180	**64**
$3d_6$	"	3-Cl	$C_{16}H_{14}N_3SClO_2$	186-188	**74**

GENERAL PROCEDURE

Synthesis of substituted benzohydrazides[7] (1a-d)

To a solution of various benzoyl chlorides (0.12mol) in 10ml of Methanol, (0.12 mol) Hydrazine hydrate was added. Then the reaction mixture was refluxed for 6hrs. On cooling, the product formed was filtered, dried in vacuum and recrystalized.

Synthesis of 5-substituted phenyl-1, 3, 4-oxadiazole-2-thiols[8] (2a-d)

A mixture of **(1a-d)** (0.01mol) in ethanol, potassium hydroxide (0.015mol) in ethanol, carbon disulphide (0.015mol) was refluxed for 8hrs. After refluxing the mixture, acidified with dil HCl, resulting solid was collected, washed with distilled water and dried in vacuum and recrystalized.

Synthesis of substituted 4, 5-diphenyl 4*H*-1, 2, 4-triazole-3-thiols[8] ($3a_1$-a_6 to $3d_1$-d_6)

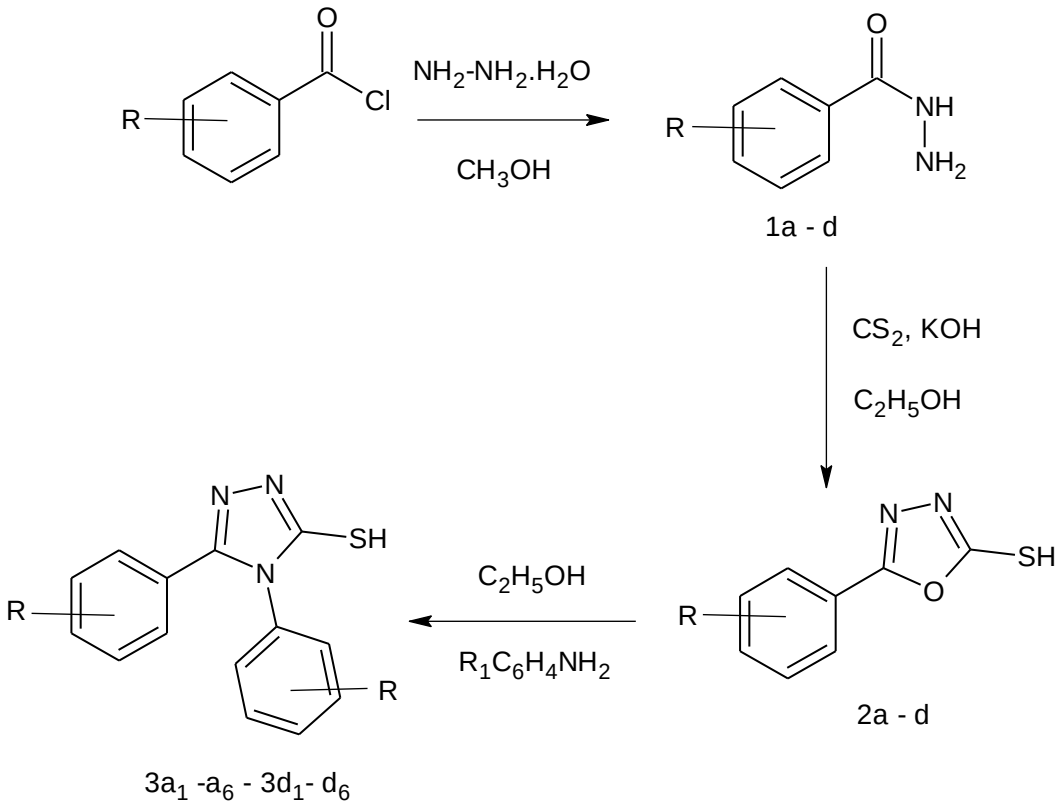
To a solution of substituted 1, 3, 4–oxadiazoles (0.01mol) in ethanol, 0.03mol of substituted anilines were added and refluxed for 6hrs. On

cooling, the product formed was filtered, dried in vacuum and recrystalized.

Table 2: Analytical Data of the Compounds ($3a_1 – A_3$ to $3d_1 – D_3$).

Compd No	IR (KBr) ν (cm^{-1})	^{1}H-NMR ($CDCl_3$) δ ppm
$3a_1$	1601(C=N), 2358(-SH), 3176 (Ar-H), 1457 (C-N)	**7.36 – 7.77 (8H, m, Ar-H),10.00 (1H, SH)**
$3a_2$	1602 (C=N), 2362(-SH), 3175 (Ar-H), 1494 (C=C), 1453 (C-N)	**2.9 – (3H, s, CH_3),7.18 – 7.70 (8H, m, Ar-H), 10.26 (S, 1H, SH)**
$3a_3$	1604 (C=N), 2360 (SH), 1458 (C-N)	**7.30 – 7.68 (8H, m, Ar-H), 10.29 (s, 1H, SH)**
$3b_1$	1637 (C=N), 2370 (SH), 3213 (Ar-H), 1541 (C=N), 1035 (C-O-C)	**2.58 (3H, s, CH_3), 3.06 (3H, s,OCH_3), 7.33 – 7.77 (8H, m-Ar-H), 10.14 (1H, s, SH)**
$3b_2$	1638 (C=N), 2362 (SH), 1540(C-N)	**2. 39 (3H, s, CH_3), 7.25 – 7.67 (8H, m, Ar-H), 9.41 (1H, s, SH)**
$3b_3$	1637 (C=N), 2358 (SH), 3212(Ar-H), 1536 (C-N)	**2.39 (3H, s, CH_3), 7.25-7.67 (8H, m, Ar-H), 9.40 (1H, s, SH)**
$3c_1$	1627(C=N), 2361 (SH), 1505 (C-N), 3204 (Ar-H)	**2.23 (3H, s, CH_3), 3.84 (3H, s, OCH_3), 6.66 – 7.77 (8H, m, Ar-H), 9.16 (1H,s,SH)**
$3c_2$	1628 (C=N), 2361(SH), 3204 (Ar-H), 1500 (C-N)	**2.32 (3H, s, CH_3), 2.42 (3H, s, CH_3), 6.61-7.71 (8H, m, Ar-H), 9.14 (1H,s,SH)**
$3c_3$	1627 (C=N), 2361 (SH), 3201(Ar-H), 1543(C-N)	**2.32(3H,s, CH_3), 6.61-7.77 (8H,m,Ar-H), 9.16 (1H, s, SH)**
$3d_1$	1605 (C=N),2361 (SH), 3180 (Ar-H), 1571(C=C), 517 (C-N), 1020(C-O-C)	**2.33 (3H, s, CH_3), 3.48, 3.94 (3H, s, OCH_3), 7.25 – 7.49 (8H,m, Ar-H) 9.57(1H,s,SH)**
$3d_2$	1572 (C=N), 2360 (SH), 3256(Ar-H), 1572 (C=C),1513 (C-N), 1021 (C-O-C)	**3.48, 3.94 (3H, s, OCH_3),6.83 – 7.49 (8H, m, Ar-H),9.65 (1H, s, SH)**
$3d_3$	1605 (C=N), 2362 (SH), 1572 (C=C), 1518 (C-N), 1022 (C-O-C)	**3.80, 3.92 (3H, s, OCH_3),6.84 – 7.48 (7H, m, Ar-H), 9.50 (1H, s, SH)**

SCHEME- 1



BIOLOGICL ACTIVITY

Anti-inflammatory activity[9]

Carrageenan induced rat paw edema method was employed for evaluating the anti-inflammatory activity of synthesized compounds [$3a_1$-a_3 – $3d_1$-d_3] given intra-peritonially at the dose of 50mg in albino rats using diclofenac sodium as a standard drug. The difference mean paw volumes of control and test compound treated groups were expressed with reference to standard. Values are expressed as ANOVA followed by Newmann's Keul's multiple range tests. Results are present in the table-3.

Anticonvulsant activity[10]

Some of the selected compounds namely [$3a_1$-a_3 – $3d_1$-d_3] were screened for anticonvulsant activity. The principle is being supramaximal electric shock method. Male Albino rats (200-250g) are stimulated through pinna electrodes (150mA, 0.2sec). The animals were divided into groups, six in each. The control groups are served with DMSO and test groups and standard drug groups served with test compounds [$3a_1$-a_3 – $3d_1$-d_3] and phenytoin at a dose of 25mg respectively given intraperitonially. The evaluation started 30min after administration of test compounds. Inhibition of seizure relative to the control was calculated. The data shown on table 4. The values are expressed as ANOVA followed by Newmann's Keul's multiple range tests. All the animal experiment protocols are approved by Institutional Animal Ethics Committee.

Table3 : Anti-Inflammatory Activity Of Compounds ($3a_1$-A_3 To $3d_1$-D_3).

COMPD NO	NORMAL PAW VOLUME	PAW VOLUME AFTER 4HRS (MEAN ± SEM)
Control	0.25 ± 0.007	0.60 ± 0.013
Std	0.24 ± 0.017	0.43 ± 0.014***
$3a_1$	0.35 ± 0.027	0.38 ± 0.022***
$3a_2$	0.24 ± 0.005	0.33 ± 0.016***
$3a_3$	0.28 ± 0.011	0.56 ± 0.010*
$3b_1$	0.29 ± 0.006	0.55 ± 0.012**
$3b_2$	0.26 ± 0.009	0.39 ± 0.009***
$3b_3$	0.26 ± 0.006	0.54 ± 0.010**
$3c_1$	0.28 ± 0.010	0.35 ± 0.006 ***
$3c_2$	0.27 ± 0.001	0.32 ± 0.006 ***
$3c_3$	0.31 ± 0.007	0.54 ± 0.010 **
$3d_1$	0.23 ± 0.001	0.26 ± 0.007***
$3d_2$	0.23 ± 0.009	0.22 ± 0.004***
$3d_3$	0.26 ± 0.026	0.54 ± 0.008 **

Values are expressed as Mean ± SEM, *(P < 0.05), ** (P < 0.01), *(P < 0.001)**

Table 4: Anticonvulsant Activity of the Compounds ($3a_1$-A_3 To $3d_1$-D_3)

COMPD NO	DURATION OF EXTENSION PHASE IN SEC (Mean ± SEM)
Control	**12.83 ± 0.714**
Std	**2.25 ± 0.381**
$3a_1$	**8.08 ± 0.506***
$3a_2$	**10.83 ± 0.980**
$3a_3$	**10.58 ± 0.650**
$3b_1$	**10.5 ± 0.707**
$3b_2$	**11.8 ± 0.971**
$3b_3$	**9.41 ± 1.793**
$3c_1$	**6.33 ± 0.477****
$3c_2$	**10.91 ± 0.0907**
$3c_3$	**4.91 ± 0.472****
$3d_1$	**6.33 ± 0.44****
$3d_2$	**5.0 ± 0.483****
$3d_3$	**6.91 ± 0.768****

Values are expressed as Mean ± SEM; * (P < 0.05), * * (P < 0.01)

Anti-microbial activity[11]

Compounds **[$3a_1$-a_3 – $3d_1$-d_3]** were evaluated for their antibacterial activity[11] at a concentration of 20µg/disc against the bacteria *E.coli as* gram –ve where as *Staphylococcus aureus* as gram +ve using Amikacin as standard, DMSO as control employing disc diffusion method. The zone of inhibition was measured in mm and compared with standard. The same compounds **$3a_1$-a_3 to $3d_1$-d_3** was also tested for antifungal activity against *Candida albicans* using fluconazole are standard. Results are present in table-**5**.

RESULTS AND DISCUSSION

Substituted Benzohydrazides **(1a-d)** were obtained by the reaction of various benzoyl chlorides with hydrazine hydrate in Methanol under reflux. 5-substituted phenyl 1,3,4-oxadiazole-2-thiols **(2a-d)** were prepared by refluxing compounds **(1a-d)** with CS_2 and KOH in ethanol followed by acidification with HCl **(2a-d)** precipitated, which is on treatment with substituted anilines in ethanol afforded substituted 4,5-diphenyl 4*H*-1,2,4-Triazole-3-thiols. **[$3a_1$-a_6 – $3d_1$-d_6] (of scheme-I)** in varying yields.

Table 5: Antimicrobial Activity of the Compounds ($3a_1$-A_3 To $3d_1$-D_3)

COMPD NO	BACTERIAL GROWTH INHIBITION (Diameter in mm S.aureus)	FUNGAL GROWTH INHIBITION (diameter in mm) Candida Albicans
Control	Nil	**Nil**
Std	21	**15**
$3a_1$	12	**11**
$3a_2$	14	**11**
$3a_3$	10	**10**
$3b_1$	12	**10**
$3b_2$	8	**8**
$3b_3$	15	**13**
$3c_1$	10	**11**
$3c_2$	11	**10**
$3c_3$	11	**9**
$3d_1$	8	**10**
$3d_2$	17	**11**
$3d_3$	18	**9**

Standard: Amikacin; Control: DMSO

Out of all the tested compounds **$3a_1$, $3a_2$, $3b_2$, $3c_1$, $3c_2$, $3d_1$, $3d_2$** showed potent anti-inflammatory activity, compounds **$3c_1$, $3c_3$, $3d_1$, $3d_2$, $3d_3$** were found to possess good anticonvulsant activity. All the compounds except **$3b_2$, $3c_3$, $3d_3$** showed significant antifungal activity. Compounds **$3a_1$-a_3, $3b_1$-b_3, $3c_1$-c_3, $3d_2$, $3d_3$** displayed potent antibacterial activity against *S.aureus* and rest of them are least active. All the compounds were inactive against *E.coli*.

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