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Synthesis and Pharmacological Evaluation of Substituted-2-triazolo [3,4-b][1,3,4]-thiadiazoles

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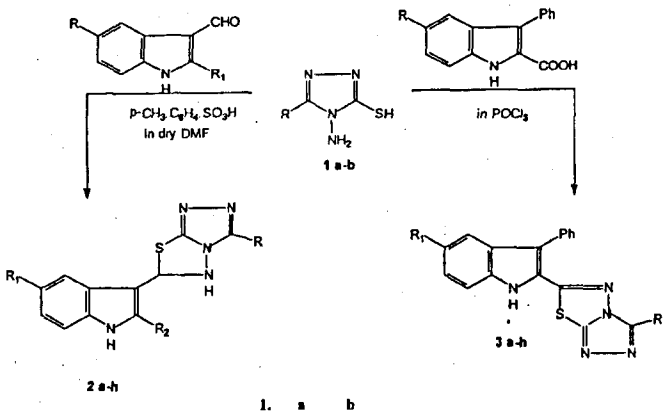
5,6-Dihydro-3-alkyl-6-(5'-substituted-2'-phenylindol-3'-yl)-s-triazolo[3,4-b][1,3,4]thiadiazoles(2a-h) and 3-substituted-6-(5'-substituted-3'phenylindol-2-yl)-s-triazolo[3,4-b][1,3,4]thiadiazoles (3a-h) have been synthesised by the reaction of 5-alkyl-1-amino-2-mercapto[1,3,4] triazoles (1a-b) with 5-substituted-2-phenylindol-3-carboxaldehydes in dry DMF containing p-toluenesulphonic acid as catalyst and 5-substituted-3-phenylindol-2-carboxylic acids in the presence of phosphorousoxychloride respectively. The structures of the newly synthesised compounds were elucidated on the basis of analytical and spectral data.These compounds have been screened for antimicrobial, antiinflammatory and analgesic activities.

Indole and its derivatives possess a wide range of pharmacological[1-4], fungicidal[5], bactericidal[6] and herbicidal activities[7-9]. Symmetrical triazoles and 1,3,4-thiadiazoles exhibit a broad spectrum of biological activities[10-17]. 1,3,4-Thiadiazole ring is associated with a wide spectrum of biological activities by virtue of incorporating toxophoric N=C-S linkage. A triazolothiadiazole system may be viewed as a cyclic analogue of two important components thiasemi-carbazide[18] and biguanide[19] which display diverse biological activities. In view of these observations and in continuation of our work on synthesis of heterocyclic systems[20-23], we thought of synthesising fused heterocyclic systems containing indole, s-triazole and 1,3,4-thiadiazole nucleus and subject these compounds for biological screening.

Compounds, 5-alkyl-1-amino-2-mercapto-[1,3,4]-triazoles (1a-b)[24] and 5-substituted-2-phenylindole-3-carboxaldehydes[25] were condensed in dry dimethylformamide and catalytic amount of p-toluenesulphonic acid to give 5,6-dihydro-3-alkyl-6-(5'-substituted-2'-phenylindol-3'yl)-s-triazolo[3,4-b][1,3,4]thiadiazoles (2a-h). Compounds (1a-b) on reaction



1. a b

R: CH_3 C_2H_5

2.	R	R_1	R_2	3	R	R_1
a.	CH_3	H	Ph	a.	CH_3	Cl
b.	C_2H_5	H	Ph	b.	C_2H_5	Cl
c.	CH_3	CH_3	Ph	c.	CH_3	Br
d.	C_2H_5	CH_3	Ph	d.	C_2H_5	Br
e.	CH_3	Cl	Ph	e.	CH_3	CH_3
f.	C_2H_5	Cl	Ph	f.	C_2H_5	CH_3
g.	CH_3	H	H	g.	CH_3	OCH_3
h.	C_2H_5	H	H	h.	C_2H_5	OCH_3

Scheme - I

*For correspondence

TABLE 1: CHARACTERISATION DATA OF THE SYNTHESISED COMPOUNDS 2a-h AND 3a-h

Compd.	m.p. °	Yield %	M.F.	Analysis Found (Calcd.) %		
				C	H	N
2a	219-220	65	$C_{18}H_{15}N_5S$	64.80 (64.86)	4.42 (4.50)	21.00 (21.02)
2b	259-260	62	$C_{19}H_{17}N_5S$	65.70 (65.71)	4.85 (4.90)	20.12 (20.17)
2c	228-229	70	$C_{19}H_{17}N_5S$	65.73 (65.71)	4.82 (4.90)	20.11 (20.17)
2d	251-252	64	$C_{20}H_{19}N_5S$	66.43 (66.48)	5.21 (5.26)	19.33 (19.39)
2e	249-250	68	$C_{18}H_{14}N_5SCl$	58.70 (58.78)	3.69 (3.81)	19.00 (19.05)
2f	261-262	70	$C_{19}H_{16}N_5SCl$	59.72 (59.76)	4.16 (4.19)	18.31 (18.35)
2g	235-236	65	$C_{12}H_{11}N_5S$	56.00 (56.03)	4.24 (4.28)	27.20 (27.24)
2h	259-260	62	$C_{13}H_{13}N_5S$	57.51 (57.56)	4.72 (4.80)	25.79 (25.83)
3a	264-265	69	$C_{18}H_{12}N_5SCl$	59.01 (59.10)	3.21 (3.28)	19.07 (19.15)
3b	237-238	70	$C_{19}H_{14}N_5SCl$	60.00 (60.08)	3.61 (3.69)	18.38 (18.45)
3c	245-246	64	$C_{18}H_{12}N_5SBr$	52.53 (52.68)	2.78 (2.93)	17.00 (17.07)
3d	223-224	68	$C_{19}H_{14}N_5SBr$	53.73 (53.77)	3.24 (3.30)	16.45 (16.51)
3e	200-201	75	$C_{19}H_{15}N_5S$	66.00 (66.09)	4.26 (4.35)	20.21 (20.29)
3f	183-184	78	$C_{20}H_{17}N_5S$	66.73 (66.85)	4.65 (4.74)	19.42 (19.50)
3g	221-222	66	$C_{19}H_{15}N_5OS$	63.09 (63.16)	4.08 (4.16)	19.27 (19.38)
3h	194-195	70	$C_{20}H_{17}N_5OS$	63.91 (64.00)	4.45 (4.53)	18.61 (18.67)

Recrystallisation was carried out in ethanol for the compounds 2a-b, 3e-g and in Benzene-Ethanol (1:1) for 2c-d, 3a-b and h. IR spectra of all the compounds exhibited characteristic peaks in cm^{-1} for NH (3400-3200), C=N) (1620-1600), C=N (1600-1580) and C-S-C (750-720).

with 5-substituted-3-phenylindol-2-carboxylic acids[26] in the presence of phosphorousoxychloride produced 3-substituted-6-(5'-substituted-3'-phenylindol-2'-yl)-s-triazolo[3,4-b][1,3,4]thiadiazoles (3a-h) (Scheme-1). Newly synthesised compounds were characterised by IR, PMR, mass spectral data and elemental analysis. These

compounds were screened for their antimicrobial, antiinflammatory and analgesic activities.

EXPERIMENTAL

Purity of the compounds was checked by TLC. Melting points were determined in open capillaries and are uncorrected. IR spectra are recorded on a Hitachi 270-50 double beem spectrometer using nujol mull. PMR spectra are recorded on a Bruker AM300, 300MHz spectrometer using TMS as internal reference and instrument 5970 is used for recording the mass spectra.

5,6-Dihydro-3-alkyl-6-(5'-substituted-2'-phenylindol-3'-yl)-s-triazolo[3,4-b][1,3,4]thiadiazoles (2a-h):

A mixture of triazoles (1a-b) (0.005 mol), 5-substituted-2-phenylindol-3-carboxaldehydes (0.005 mol) and p-toluenesulphonic acid (20 mg) in 40 ml dry dimethylformamide was stirred at 80-90° for 8 h. The excess of solvent was removed under reduced pressure. The reaction mixture was cooled and poured onto crushed ice. The solid thus separated was filtered, washed with water, dried and recrystallised from suitable solvents. The PMR spectrum of 2a exhibited signals at δ 2.4 (s, 3H, CH_3), 7.2-8.4 (m 10H, Ar-H) and position-2 of thiadiazole H), 9.8 (s, 1H, indole NH) and 12.4 (s, 1H, thiadiazole H), while its mass spectrum showed peaks at m/z 333 (100%), which is equivalent to its molecular weight, 312 (45%), 256 (7%), 241 (9%), 213 (5%), 239 (2%), 141 (2%) and 115 (4%). Physical and elemental analysis data of the compounds are given in Table 1.

3-Substituted-6-(5'-substituted-3'-phenylindol-2'-yl)-s-triazolo[3,4-b][1,3,4]thiadiazoles (3a-h):

A mixture of triazoles (1a-b) (0.01 mol) and 5-substituted-3-phenylindol-2-carboxylic acids (0.01 mol) in 15 ml phosphorousoxychloride was refluxed for 5 h.

TABLE 2: RESULTS OF *IN VITRO* ANTIMICROBIAL ACTIVITY OF THE COMPOUNDS 2a-h AND 3a-h

Compound	Diameter of zone of inhibition in mm*					
	S. aureus	*B. subtilis*	*E. coil*	*S. species*	*A. niger*	*C. albicans*
2a	12	15	10	17	17	15
2b	15	18	12	20	15	19
2c	16	17	12	19	18	16
2d	18	20	14	20	16	14
2e	20	19	15	21	16	16
2f	22	22	16	25	22	18
2g	14	12	10	20	17	15
2h	17	20	16	20	19	20
3a	20	19	16	22	17	16
3b	22	21	14	23	20	22
3c	23	17	16	21	18	20
3d	24	16	14	25	21	24
3e	15	14	10	20	17	16
3f	17	20	14	22	15	14
3g	12	10	15	18	16	13
3h	15	17	12	20	14	12
Gentamicin	26	27	19	32	-	-
Griseofulvin	-	-	-	-	28	25

*Including diameter of the well-8 mm, Control (DMF) = No activity.

The excess of phosphorousoxychloride was distilled off under reduced pressure. The residue was cooled and poured onto the crushed ice. The solid thus obtained was filtered and treated with dilute sodium hydroxide solution. The precipitate formed was collected by filtration, washed with water, dried and recrystallised from appropriate solvents. The PMR spectrum of 3a displayed signals at δ 2.7 (s, 3H, CH_3), 7.2-7.7 (m, 8H, Ar-H) and 9.45 (s, 1H, indole NH). The mass spectrum showed peaks at m/z 365 and 367 (25%), 299 and 301 (80%) 253 and 255 (100%). 190(85%), 191, (60%) and 163 (42%). Physical and analytical data of the compounds are given in Table 1.

Antimicrobial Activity:

The antimicrobial screening of the synthesised compounds was determined by agar cup-plate method[27] at a concentration of 1000 µg/ml using DMF as solvent. All the compounds were screened *in vitro* for antibacterial activity against two gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* and two gram-negative bacteria, *Escherichia coli* and *Salmonella* species and antifungal activity against *Aspargillus niger* and *Candida albicans*. The plates were incubated at 37° for 24 in case of antibacterial activity where as in case of antifungal activity for 48 h. The control was also maintained with

TABLE 3: RESULTS OF ANTIINFLAMMATORY ACTIVITY OF THE COMPOUNDS 2a-h AND 3a-h

Compd.	Dose (mg/kg)	Oedema volume (ml) different interval (mean±S.E.M.)		% Inhibition	
		2h	4h	2h	4h
2a	100	0.24 (±0.02)	0.17 (±0.02)	11.7	25.8
2b	100	0.24 (±0.02)	0.17 (±0.02)	12.4	28.0
2c	100	0.22 (±0.17)	0.15 (±0.02)	18.2	34.5
2d	100	0.22 (±0.01)	0.15 (±0.02)	18.2	36.5
2e	100	0.20 (±0.00)	0.11 (±0.05)	26.6	52.5
2f	100	0.19 (±0.01)	0.10 (±0.00)	28.5	55.9
3a	100	0.19 (±0.01)	0.10 (±0.00)	28.8	55.1
3b	100	0.19 (±0.01)	0.10 (±0.00)	29.2	56.8
3c	100	0.20 (±0.00)	0.11 (±0.02)	24.00	51.7
3d	100	0.19 (±0.01)	0.10 (±0.02)	27.7	53.8
Standard (Phenylbutazone)	100	0.17 (±0.02)	0.10 (±0.00)	26.3	57.6
Control Tween-80)	—	0.27 (±0.02)	0.23 (±0.02)	—	—

All the values are mean±S.E.M. of 6 samples

TABLE 4: RESULTS OF ANALGESIC ACTIVITY OF THE COMPOUNDS 2a-h AND 3a-h

Compd.	Dose (mg/Kg)	Time taken to remove the tail at different time interval (mean±S.E.M. S)				% Analgesic Activity			
		0 min	30 min	60 min	120 min	0 min	30 min	60 min	120 min
2a	100	3.70 (±0.17)	5.20 (±0.00)	5.50 (±0.28)	4.90 (±0.10)	5.7	57.6	77.4	36.1
2b	100	3.80 (±0.10)	4.20 (±0.00)	5.30 (±0.23)	5.00 (±0.00)	8.6	27.3	71.0	38.9
2c	100	3.60 (±0.00)	4.20 (±0.00)	4.90 (±0.25)	5.20 (±0.00)	2.8	27.3	58.1	44.4
2d	100	3.60 (±0.28)	4.20 (±0.00)	5.00 (±0.00)	5.35 (±0.50)	2.9	27.3	61.3	48.6
2e	100	3.80 (±0.20)	4.40 (±0.20)	5.60 (±0.23)	6.00 (±0.00)	8.6	33.3	80.6	66.7
2f	100	4.20 (±0.00)	5.20 (±0.00)	5.75 (±0.25)	6.10 (±0.00)	20.0	57.6	85.5	69.4
2g	100	3.70 (±0.12)	4.70 (±0.29)	5.00 (±0.39)	4.80 (±0.31)	5.7	42.4	61.3	33.3
2h	100	3.80 (±0.26)	4.70 (±0.17)	5.30 (±0.23)	5.00 (±0.00)	8.6	42.4	71.0	38.8
3a	100	4.40 (±0.20)	5.40 (±0.20)	5.70 (±0.17)	6.10 (±0.00)	25.7	63.6	83.9	69.4
3b	100	4.60 (±0.28)	5.40 (±0.21)	5.90 (±0.10)	6.20 (±0.00)	31.4	63.6	90.3	72.2
3c	100	4.50 (±0.28)	5.30 (±0.30)	5.60 (±0.28)	6.00 (±0.00)	28.6	60.6	80.6	66.7
3d	100	4.50 (±0.28)	5.30 (±0.23)	5.85 (±0.15)	6.15 (±0.10)	28.6	60.6	88.7	70.8
2e	100	3.90 (±0.23)	4.20 (±0.00)	5.90 (±0.10)	5.40 (±0.20)	11.4	27.3	90.3	50.0
3f	100	3.60 (±0.23)	4.50 (±0.28)	5.20 (±0.00)	5.50 (±0.28)	2.9	36.4	67.7	52.8
3g	100	3.80 (±0.10)	4.20 (±0.00)	5.30 (±0.23)	5.00 (±0.00)	8.6	27.3	71.0	38.9
3h	100	4.20 (±0.00)	5.10 (±0.00)	5.55 (±0.26)	5.10 (±0.00)	20.0	54.5	79.0	41.7
Standard (Analgin)	100	4.70 (±0.28)	5.50 (±0.28)	6.60 (±0.00)	6.30 (±0.23)	34.3	66.7	93.5	75.0
Control (Tween-80)	—	3.50 (±0.28)	3.30 (±0.23)	3.10 (±0.00)	3.60 (±0.28)	—	—	—	—

0.1 ml of DMF and zone of inhibition of the growth was measured in mm. The activity was compared with the standard drugs gentamicin (100 μg/ml) and griseofulvin (40 μg/ml) for antibacterial activity and antifungal activity respectively. Each experiment was conducted thrice and the average of three were recorded. The results are presented in Table 2.

Antiinflammatory Activity:

Antiinflammatory activity was measured using the formalin-induced paw odema test in rats[28].

Albino rats of either sex (150-200 g) were divided into control, standard and test groups, each consisting of six rats. A group of rats was treated with Tween-80 (1%) suspension i.p. (control). Another group was administered a dose of 100 mg/kg of suspension of phenylbutazone (standard) p.o. and the third group was treated with 100 mg/kg of the suspension of the test compounds. After 30 min the animals were injected with 0.1 ml of formalin (1% w/v) in the sub plantar region of left hind paw of the rats. The volume of the paw was measured using the mercury displacement technique with the help of a plethysmograph both in control as well as in standard animals including the test animals at 2 and 4h hour after injection. The initial volume of the paw was measured within 30 s of the injection. The per cent inhibition of the inflammation after 2 and 4 h was calculated by using the following formula. % inhibition=$(1-v_t/v_c)$ 100, where vc and vt are the mean relative changes in the volume of paw oedema in the control and test, respectively. The results are summarised in the Table 3.

Analgesic Activity:

Test for the analgesic activity was performed using the tail flick method[28]. Albino mice of either sex (20-25 g) were distributed into control, standard and test groups, each of four mice. The test compounds were treated orally at a dose of 100 mg/kg body weight. The standard group was administered with analgin at a dose of 100 mg/kg body weight, orally. The reaction time was recorded at 0.30, 60 and 120 min after the drug administration. Per cent protection against tail flicking was calculated by using the following formula, % protection = (1-wt/wc) 100, where wc and wt are the means of the tail flicking in the control and test respectively. The results are listed in the Table 4.

RESULTS AND DISCUSSION:

Antibacterial activity evaluation (Table 2) reveals that, compounds 2e, f and 3a-d showed highest activity against *S. aureus*. Highest activity was also observed in the compounds 2d, f, 3a, b and f against *B. subtilis*. Compounds 2b-h and 3a-f displayed good activity against *S. species* and non of the compounds tested showed highest activity against *E. coli*. Remaining compounds exhibited moderate to weak activity against all organisms. Antifungal activity evaluation (Table 2) displayed that compounds 2f, 3b and d showed very good activity against *A. niger* and *C. albicans* respectively. Other compounds exhibited moderate to weak activity against both organisms. Amongst the compounds tested for antifungal activity, compounds possessing halogen showed highest activity.

Amongst the compounds subjected to antiinflammatory screening (Table 3), compounds, 2e (52.5%), f(55.9%), 3a(55.1%), b (56.8%), c(51.7%) and d (53.8%) were found to possess significant compared to that of the standard, phenylbutazone activity. The remaining compounds showed moderate activity (25.8-36.5%). Analgesic activity results (Table 4) revealed that compounds, 2e, f and 3a-f showed significant activity (50-72.2%), whereas other compounds exhibited 33.3-48.6% activity compared to standard (75%). Highest activity was observed with 3b.

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