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Synthesis and Biological Evaluation of 3-(2-Aroyl aryloxy)methyl-5-mercaptc-4*H*-1,2,4-triazole Analogues

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The present investigation was aimed at the synthesis of 3-(2-aroyl aryloxy)methyl-5-mercapto-4*H*-1,2,4-triazole analogues 3a-j, by intramolecular cyclization of (2-aroyl aryloxy)acetates 2a-j with thiosemicarbazide. The title compounds were screened for antibacterial, antifungal, anticonvulsant, diuretic and antiinflammatory activities and some of the compounds were found to be active.

Benzophenone analogues have been found to possess a broad spectrum of biological activities. Literature survey revealed that benzophenone analogues are inhibitors of HIV-1 reverse transcriptase and the growth of HIV-1 in MT-4 cells[1]. These compounds also showed antianaphylactic[2] and anti-inflammatory activity[2,3]. Bakana *et al*[4]. have isolated a benzophenone analogue (garcinol), from the stem bark of *Garcinia huillensis* grown in Zaire and used in Central African traditional medicine and this has been shown to exhibit activity against gram-positive and gram-negative cocci, mycobacteria and fungi. In the past years a number of reports about the synthesis and pharmacological actions of mercapto-4*H*-1,2,4-triazole analogues as herbicides[5], antiviral[6], bacteriostatic[7], antifungal[5], anticonvulsant[8], diuretic[9] and hypoglycemic[10] agents have appeared in the literature. Vanden *et al.*[11] have reported that the major effect of triazoles on fungi is inhibition of sterol 14-α-demethylase, a microsomal cytochrome P450-dependent enzyme system. Triazoles thus impair the biosynthesis of ergosterol for the cytoplasmic membrane and lead to the accumulation of 14-α-methylsterols. These methylsterols may disrupt the close packing of acyl chains of phospholipids impairing the functions of certain membrane bound enzyme systems such as adenosine triphosphatase and enzymes of the electron transport system and thus inhibiting growth of the fungi.

In retrospect of the above observations, this investigation is directed towards synthesis and evaluation of pharmacological activities of 3-(2-aroyl aryloxy)methyl-5-mercapto-4*H*-1,2,4-triazole analogues 3a-j. The synthetic route for the present work is depicted in Scheme 1. The substituted 2-hydroxybenzophenones 1a-j were synthesized by the benzoylation of substituted phenols with the required acid chlorides followed by Fries rearrangement[12,13]. Condensation of 1a-j with ethyl chloroacetate yielded substituted ethyl (2-aroyl aryloxy) acetates 2a-j[14], which on intramolecular cyclization with thiosemicarbazide afforded respective 3-(2-aroyl aryloxy)methyl-5-mercapto-4*H*-1,2,4-triazole analogues 3a-j. Structures of all the products were confirmed by elemental analysis and spectral data.

MATERIALS AND METHODS

Melting points were determined using a Thomas Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded in Nujol on an FT-IR Shimadzu 8300 spectrophotometer, ^{1}H NMR spectra were recorded on a Hitachi R-600 (60 MHz) spectrophotometer in $CDCl_3$ and chemical shift were recorded in parts per million down field from tetramethylsilane. Mass spectra were obtained with a VG70-70H spectrophotometer Elemental analysis results are within 0.4% of the calculated value. All the animal experiments with Swiss rats were carried out at Farooqia College

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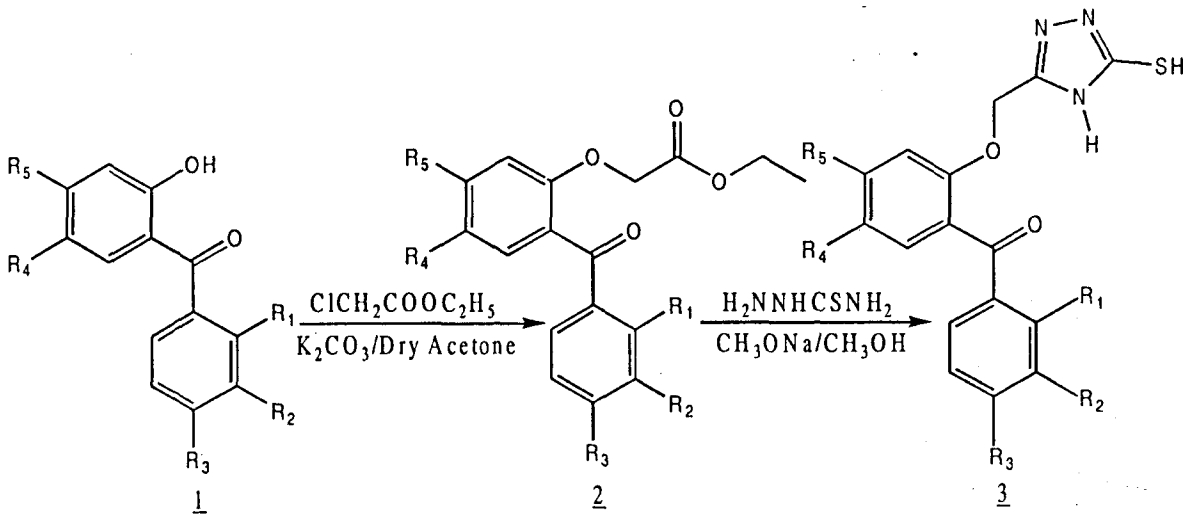

a: $R_1=R_3=R_5=H$, $R_2=Cl$, $R_4=CH_3$ b: $R_1=R_2=R_3=R_5=H$, $R_4=Cl$
c: $R_1=Cl$, $R_2=R_3=R_5=H$, $R_4=CH_3$ d: $R_1=R_2=R_5=H$, $R_3=Cl$, $R_4=CH_3$
e: $R_1=R_2=R_3=H$, $R_4=Cl$, $R_5=CH_3$ f: $R_1=R_2=R_3=R_4=H$, $R_5=Br$
g: $R_1=R_2=R_5=H$, $R_3=OCH_3$, $R_4=CH_3$ h: $R_1=R_3=R_5=H$, $R_2=R_4=Cl$
i: $R_1=R_2=R_3=R_5=H$, $R_4=CH_3$ j: $R_1=R_2=R_5=H$, $R_3=R_4=CH_3$

Scheme 1: Synthetic pathway of the tittle compounds(3a-j).

of Pharmacy, Mysore and permission for conducting these experiments was obtained from institutional Animals Ethics Committee (CPCSEA Regd. No. 443/01/a).

Ethyl (2-aroyl aryloxy)acetates 2a-j:

A mixture of 2-hydroxy-5-methyl-3-chlorobenzophenone 1a (5 g, 0.02 mol), ethyl chloroacetate (2.4 g, 0.02 mol) in dry acetone (50 ml) and anhydrous potassium carbonate (2.8 g, 0.02 mol) was refluxed for 8 h then cooled and the solvent removed under reduced pressure. The residual mass was triturated with cold water to remove potassium carbonate and extracted with ether (3x50 ml) and the ether layer was washed with 10% sodium hydroxide solution (3x30 ml) followed by water (3x30 ml). The organic layer was dried over anhydrous sodium sulphate and evaporated to dryness to get crude solid, which on recrystallization with ethanol gave ethyl [2-(3-chlorobenzoyl)-4-methylphenoxy]acetate 2a as white crystalline solid. Similarly compounds 2b-j were prepared.

3-(2-aroyl aryloxy)methyl-5-mercapto -4*H*-1,2,4-triazoles (3a-j):

A mixture of 2a (5 g, 0.015 mole), thiosemicarbazide (1.1 g, 0.012 mole) and a methanolic sodium methoxide solution (0.3 g of sodium and 15 ml methanol) refluxed for 8 h then cooled and the solvent removed under reduced pressure. The residual mass was triturated with cold water and extracted with ether (3x20 ml). The ether layer was washed with 10% sodium carbonate solution (3x15 ml) followed by water (3x20 ml) and then dried over anhydrous sodium sulphate and evaporated to dryness to get crude solid, which on recrystallization with benzene gave 3a as white solid. Similarly compounds 3b-j were prepared.

Evaluation of antimicrobial activity:

The compounds 3a-j were screened for *in vitro* antimicrobial activity using the cup plate method[15]. Pure cultures of the test microorganism were procured from the cultures maintained at Farooqia College of Pharmacy, Mysore. The activity was carried out against three pathogenic bacteria, *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli* and three fungal cultures, *Penicillium nigricans, Aspergillus fumigatus* and *Fusarium solani.* The standard drugs used were norfloxacin and griseofulvin. The compounds were tested at a concentration of 100 μg/ml in dimethyl formamide. The zone of inhibition was compared with the standard drug

TABLE 1: CHARACTERISTIC DATA OF THE SYNTHESIZED COMPOUNDS 2a-j AND 3a-j.

Compd.	Yield	m.p.°	Mol.	Analysis Found (Calcd.) %					
	%		formula	C	H	Br	Cl	N	S
2a	81	60-62	$C_{18}H_{17}ClO_4$	64.96	5.08		10.67		
				(64.92)	(5.11)	-	(10.66)	-	-
2b	79	65-67	$C_{17}H_{15}ClO_4$	64.02	4.67		11.11		
				(64.05)	(4.70)	-	(11.14)	-	-
2c	75	62-65	$C_{18}H_{17}ClO_4$	64.94	5.09		10.64		
				(64.92)	(5.11)	-	(10.66)	-	-
2d	72	58-60	$C_{18}H_{17}ClO_4$	64.95	5.10		10.63		
				(64.92)	(5.11)	-	(10.66)	-	-
2e	77	57-59	$C_{18}H_{17}ClO_4$	64.96	5.08		10.67		
				(64.92)	(5.11)	-	(10.66)	-	-
2f	70	69-71	$C_{17}H_{15}BrO_4$	56.17	4.10	22.0	-	-	-
				(56.15)	(4.13)	(22.03)	-	-	-
2g	72	58-60	$C_{19}H_{20}O_5$	69.49	6.05				
				(69.51)	(6.09)	-	-	-	-
2h	74	72-74	$C_{17}H_{14}Cl_2O_4$	57.77	3.94		20.10		
				(57.79)	(3.96)	-	(20.11)	-	-
2i	79	60-63	$C_{18}H_{18}O_4$	72.44	6.02				
				(72.48)	(6.04)	-	-	-	-
2j	79	57-59	$C_{19}H_{20}O_4$	73.04	6.44				
				(73.07)	(6.41)	-	-	-	-
3a	73	120-23	$C_{17}H_{14}ClN_3O_2S$	56.71	3.88		9.85	11.66	8.92
				(56.74)	(3.89)	-	(9.87)	(11.68)	(8.9)
3b	70	122-25	$C_{16}H_{12}ClN_3O_2S$	66.55	3.45		10.29	12.12	9.24
				(55.57)	(3.47)	-	(10.27)	(12.15)	(9.26)
3c	71	125-27	$C_{17}H_{14}ClN_3O_2S$	56.72	3.87		9.84	11.65	8.93
				(56.74)	(3.89)	-	(9.87)	(11.68)	(8.9)
3d	72	127-29	$C_{17}H_{14}ClN_3O_2S$	56.75	3.86		9.88	11.65	8.91
				(56.74)	(3.89)	-	(9.87)	(11.68)	(8.9)
3e	69	117-19	$C_{17}H_{14}ClN_3O_2S$	56.73	3.87		9.84	11.69	8.93
				(56.74)	(3.89)	-	(9.87)	(11.68)	(8.9)
3f	65	115-17	$C_{16}H_{12}BrN_3O_2S$	49.21	3.05	20.50		10.74	8.22
				(49.23)	(3.07)	(20.51)	-	(10.76)	(8.20)
3g	67	119-21	$C_{18}H_{17}N_3O_3S$	60.82	4.76			11.82	9.03
				(60.84)	(4.78)	-	-	(11.83)	(9.01)
3h	71	122-24	$C_{16}H_{11}Cl_2N_3O_2S$	50.52	2.90		18.66	11.03	8.44
				(50.54)	(2.92)	-	(18.65)	(11.05)	(8.43)
3i	72	126-28	$C_{17}H_{15}N_3O_2S$	62.42	4.62			12.94	9.82
				(62.4)	(4.6)	-	-	(12.92)	(9.8)
3j	69	121-23	$C_{18}H_{17}N_3O_2S$	63.71	5.02			12.32	9.43
				(63.7)	(5.0)	-	-	(12.3)	(9.4)

Compounds 2a-j were recrystallized from ethanol and 3a-j from benzene.

TABLE 2: *IN VITRO* ANTIMICROBIAL ACTIVITY OF COMPOUNDS 3a-j

Compd.	Diameter of zone of inhibition in mm*					
	Antibacterial			Antifungal		
	B. cereus	*S. aureus*	*E. coli*	*P. nigricans*	*A. fumigatus*	*F. solani*
3a	14	16	18	20	14	16
3b	28	20	29	29	27	12
3c	-	10	-	10	-	11
3d	25	19	27	28	25	14
3e	23	17	22	22	20	11
3f	14	16	18	10	12	21
3g	10	-	21	-	15	28
3h	27	20	26	28	27	18
3i	12	-	14	19	10	15
3j	-	12	13	18	-	20
Norfloxacin	24	22	26	-	-	-
Griseofulvin	-	-	-	27	25	26

*Size of the inhibiton zone by disk diffusion method control (DMF) = No activity. Both test compounds and standards were tested at 100 μg/ml conc.

after 24 h of incubation at 37° for antibacterial activity and 72 h at 25° for antifungal activity. Antimicrobial activity screening results are summarized in Table 2.

Anticonvulsant activity:

The anticonvulsant activity was carried out based on electroshock-induced convulsions in rats[16]. Male Swiss rats were procured from Virus Diagnostic Laboratory, Mysore and maintained at Farooqia College of Pharmacy, Mysore, were fed with standard diet, water *ad libitum*. Six groups of three rats each were selected and to the first group (control) saline was injected i.p. Corneal electrodes were placed on these rats and the prescribed current was applied. Different stages of convulsions produced were noted and these served as control. To the second group of rats, 25 mg/kg of phenytoin sodium (standard) was injected i.p. and after 30 min they were subjected to electroconvulsions. The same procedure was repeated for the remaining four groups using test compounds 3a-j. Various stages of convulsions were recorded at different intervals. The mean value for each group was calculated and compared with control. The results are summarized in Table 3.

Diuretic activity:

The diuretic activity is based on the effect of drugs on water and electrolytes excretion in rats[17]. The animals were marked, weighed and divided into six groups. To the first group, a water load of 25 ml/kg (control) p.o. was administered orally. To the second group frusemide (standard) was given i.p. along with the water load 25 ml/kg. To the remaining four groups suspension of test compounds (100 mg/kg) was administered i.p. along with water. The animals were observed for diuretic activity, their urine samples were collected at periodic intervals and the volume of these samples was measured. Mean values of these reading were calculated and compared with that of the standard group. The results obtained have been summarized in Table 4.

Antiinflammatory activity:

Antiinflammatory activity of compounds 3a-j was evaluated using carrageenan-induced rat hind paw oedema method[18]. Swiss rats of either sex weighing between 150-200 g were divided into control, standard and test groups, each consisting of six rats. The first group of rats was treated with Tween-80 (1%) suspension (control), second group was

TABLE 3: ANTICONVULSANT ACTIVITY OF COMPOUNDS 3a-j

Compd.	Dose	Time (sec) in various phases of convulsion				Recovery/
	mg/kg	Flexion	Extensor	Clonus	Stupor	death
3a	25	1.5	2.2	1.3	90	Recovery
3b	25	1.2	2.0	1.1	95	Recovery
3c	25	1.9	2.5	1.8	85	Recovery
3d	25	1.3	2.1	1.2	93	Recovery
3e	25	1.5	2.4	1.5	96	Recovery
3f	25	1.8	2.5	1.9	88	Recovery
3g	25	2.1	3.0	2.0	82	Recovery
3h	25	1.4	2.2	1.3	95	Recovery
3i	25	2.3	3.5	1.6	80	Recovery
3j	25	2.5	4.0	1.5	82	Recovery
Control (saline)	-	3.1	10.0	2.2	120	Recovery
Standard (Phenyntoin)	25	0.5	1.0	0.5	100	Recovery

No. of animals in each group: 06.

administered with a dose of 100 mg/kg of suspension of phenylbutazone (standard) intraperitoneally 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 carrageenan (1% w/v) in to the sub planter region of left hind paw of the rats. The volume of the paw was measured using mercury displacement technique with the help of a plethysmograph both in control and in animals treated with standard and test compounds at 2 and 4 h after injection of carrageenan. The initial volume of the paw was measured within 30 s of the injection. The percent inhibition of the inflammation after 2 and 4 h was calculated by using the formula, % inhibition=$(1- v_t/v_c)100$, where v_c and v_t are the mean relative changes in the volume of paw oedema in the control and test respectively. The results are summarized in Table 5.

RESULTS AND DISCUSSION

The compound 2a showed IR absorptions at 1670 and 1735 cm^{-1} assigned to (aromatic, C=O) and (ester, C=O) group, respectively. ^1H NMR spectrum of 2a showed a triplet centered at δ 1.2 assigned to methyl group of ester, a singlet at δ 2.3 assigned to aromatic methyl group, a quartet centered at δ 4.2 assigned to methylene protons of ester, a singlet at δ 4.45 assigned to methylene protons and a broad multiplet at δ 7.2-7.6 assigned to aromatic protons. The mass spectrum of 2a showed the molecular ion peak at m/z 332 consistent with the molecular formula $C_{18}H_{17}ClO_4$. The compound 3a showed IR absorptions at 1610, 1640 and 2500-2550 cm^{-1} assigned to (C=N), (C=O) and (S-H) groups, respectively. ^1H NMR spectrum of 3a showed a singlet at δ 2.3 assigned to aromatic methyl group, a singlet at δ 4.46 due to methylene protons, a broad multiplet at δ 7.2-7.7 assigned to aromatic protons and a singlet at δ 10.2 due to thiol proton. The mass spectrum of 3a showed the molecular ion peak at m/z 359 consistent with the molecular formula $C_{17}H_{14}ClN_3O_2S$.

Antimicrobial activity screening results are qualitative in nature (Table 2). The antibacterial screening results have shown that the chloro substituted compounds 3a, 3b, 3d, 3e and 3h exhibit, in general, growth inhibitory activity more relevant than that of the reference compounds. It is worth noting that compounds 3b, 3d and 3h, with a chloro group

TABLE 4: DIURETIC ACTIVITY OF COMPOUNDS 3a-j

Compd.	Dose	Total amount of urine collected (ml)				
	mg/kg	15'	30'	60'	120'	240'
3a	10	0	0	0	4.0	3.3
3b	10	0	0	0	4.1	3.3
3c	10	0	0	0	3.9	3.1
3d	10	0	0	0	4.2	3.3
3e	10	0	0	0	3.5	2.9
3f	10	0	0	0	3.8	3.0
3g	10	0	0	0	3.5	2.9
3h	10	0	0	0	4.3	3.5
3i	10	0	0	0	3.2	2.8
3j	10	0	0	0	3.0	2.8
Control (Water)	25 ml/kg	0	0	0	2.5	2.3
Standard (Frusimide)	10	0	0	0	4.5	3.7

No. of animals in each group is 6.

on the para position, showed growth inhibitory activity higher than norfloxacin against *Bacillus cereus* and *Escherichia coli* but lower activity against *Staphylococcus aureus*, while compound 3c with chloro group at ortho position is inactive against both the strains. This is an example, which shows how the biological properties are influenced by even minor structural modifications. Compounds 3f (with bromo group), 3g (with methoxy and methyl group), 3i and 3j (with methyl group) showed weak to moderate activity against the strains. Even in case of antifungal activity chloro substituted compounds showed growth inhibitory activity more relevant than that of the reference drug. Compounds 3b, 3d and 3h showed higher growth inhibitory activity than griseofulvin against *Aspergillus Fumigatus* and *penicillium nigricans*. Against the other strain these compounds showed weak activity. Compound 3g with methoxy and methyl groups showed higher growth inhibitory activity than standard drug against *Fusarium solani* and weak against the other two strains. Against all the strains compound 3a, 3c, 3e, 3f, 3i and 3j showed weak to moderate activity. In general these compounds are found to possess more antifungal than antibacterial activity. Amongst the compounds subjected to anticonvulsant activity (Table 3), compounds 3a, 3b, 3d, 3e and 3h with chloro group were found to possess promising activity compared to that of standard phenyntoin. The diuretic activity (Table 4) results showed the test compounds produced slight diuresis compared to standard frusemide. Amongst the compounds subjected to antiinflammatory screening (Table 5), compounds with chloro group 3a, 3b, 3c, 3d, 3e and 3h were found to possess significant activity compared to that of the standard phenylbutazone. Compounds with methyl group 3i and 3j show moderate activity and compound with methoxy group 3g show weak activity compared to that of the standard.

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TABLE 5: ANTIINFLAMMATORY ACTIVITY OF COMPOUNDS 3a-j

Compd.	Dose mg/Kg	Oedema volume (ml) different intervals*		% Inhibition	
		2 h	4 h	2 h	4 h
3a	100	0.20 (±0.02)	0.13 (±0.01)	31.4	48.5
3b	100	0.19 (±0.01)	0.12 (±0.00)	34.5	52.4
3c	100	0.20 (±0.03)	0.13 (±0.02)	31.0	48.2
3d	100	0.19 (±0.01)	0.12 (±0.00)	34.3	52.2
3e	100	0.20 (±0.02)	0.13 (±0.01)	31.3	48.1
3f	100	0.21 (±0.17)	0.14 (±0.02)	27.8	43.6
3g	100	0.26 (±0.03)	0.16 (±0.02)	10.3	36.7
3h	100	0.19 (±0.01)	0.12 (±0.00)	34.1	52.1
3i	100	0.24 (±0.03)	0.15 (±0.02)	17.4	39.4
3j	100	0.25 (±0.15)	0.15 (±0.02)	13.9	38.5
Standard Phenylbutazone	100	0.18 (±0.02)	0.12 (±0.00)	36.1	49.5
Control Tween-80	100	0.29 (±0.02)	0.25 (±0.02)	-	-

*Each value is a mean±S.E.M., No. of animals in each group: 06.

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