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Synthesis and Pharmacological Screening of 5-(4-Aroyl)-aryloxy methyl-2-thio-1,3,4-oxadiazole

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The title compounds were synthesized by the intramolecular cyclisation of thiosemicarbazides generated by the action of hydrazides 3a-d on carbon disulfide in the presence of potassium hydroxide in good yield. All the compounds have been characterized by elemental analysis and spectral data. All the synthesized compounds were screened for their antibacterial, antifungal, anticonvulsant, antiinflammatory and diuretic activities.

Reverse transcriptase (RT) is the key enzyme in the life cycle of human immuno deficiency virus (HIV), the etiologic agent of acquired immuno deficiency syndrome (AIDS)[1]. Recently studies have been performed to determine the effectiveness of a new class of inhibitors known as non-nucleoside inhibitors. Two non-nucleoside inhibitors which are widely studied are nevirapine[2] and benzophenones derivatives[3]. Substituted oxadiazoles have been reported to act as fluorescent whiteners, as herbicides, as fungicides, as hypnotics and as sedatives[4]. These compounds also showed anti-convulsant[5], anti-mitotic activity[6], analgesic, antiinflammatory and diuretic activities[7].

In view of these observations, we are reporting the synthesis and pharmacological screening of 5-(4-aroyl)-aryloxy methyl-2-thio-1,3,4-oxadiazoles 4a-d. The substituted 4-hydroxybenzophenones 1a-d were synthesized by the benzoylation of phenols followed by Fries rearrangement[8,9]. Their structural elucidation was confirmed by IR and ^{1}H NMR data. 4-Hydroxybenzophenones 1a-d on treatment with ethyl bromoacetate in the presence of anhydrous potassium carbonate and dry acetone gave the corresponding aroyl aryloxy esters 2a-d in excellent yield[10]. The esters 2a-d on treatment with hydrazine monohydrate gave acid hydrazides 3a-d with relatively good yield[11]. A mixture of 3a-d, potassium hydroxide and carbon disulphide in absolute alcohol was refluxed for about 2 h[12]. The solvent was removed under reduced pressure and the residue digested with water and acidified with dilute hydrochloric acid. The resulting 2-thio-1,3,4-oxadiazoles 4a-d were collected by filtration, washed with water dried and recrystallised from alcohol.

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MATERIALS AND METHODS

Melting points were determined in an open capillary and are uncorrected. The IR spectra in nujol were recorded on a Shimadzu FT-IR 8300 spectrophotometer. ^{1}H NMR spectra were measured on a Hitachi R-600 (60 MHz) with TMS as internal standard and chemical shift values were expressed in ppm (δ). Mass spectra were obtained on a Finnigan 4021 mass spectrometer.

5-(4-Aroyl) aryloxyacetic esters 2a-d:

A mixture of 4-hydroxybenzophenone 1a (1.98 g, 10 mmol) ethyl bromoacetate (1.65 g, 10 mmol) and potassium carbonate (4 g, 30 mmol) in dry acetone (50 ml) were refluxed for 6 h. To the cooled reaction mixture, water (50 ml) was added and extracted with ether (3x50 ml). The ether layer washed with 5% aqueous sodium hydroxide (3x25 ml) and with water (2x25 ml) and dried over anhydrous sodium sulphate and evaporated. The crude product on

recrystallisation from ethanol gave 2a as white crystalline solid. Similarly compounds 2b-d were prepared (Table 1).

5-(4-Aroyl)-aryloxyacetic acidhydrazides 3a-d:

To an ethanolic solution (25 ml) of ester 2a (2.84 g, 10 mmol), hydrazine monohydrate (0.52 g, 10 mmol) was added and the reaction mixture was kept aside for 1 h. The white crystalline solid separated was filtered, washed with ethanol, dried and recrystallised from hot ethanol to give 3a. Similarly compounds 3b-d were prepared (Table 1).

5-(4-Aroyl)-aryloxy methyl-2-thio-1,3,4-oxadiazoles 4a-d:

To a solution of 3a (1.92 g, 5 mmol) in absolute alcohol (25 ml), carbon disulphide (5 g, 66 mmol) and potassium hydroxide (0.56 g, 10 mmol) were added and refluxed for 2 h. After the completion of the reaction, the solvent was removed under reduced pressure and the residue digested with water (30 ml) and acidified with dilute hydrochloric acid. The resulting 2-thio-1,3,4-oxadiazole 4a was collected by filtration, washed with water (25 ml), dried and recrystallised from alcohol to give 4a. Similarly compounds 4b-d were prepared (Table 1).

Antimicrobial activity:

Antibacterial activity of newly synthesized compounds was determined against *Staphylococcus auereus, Pseudomonas aeruginosa and Escherichia coli* by the cup plate method[13]. The test compounds were dissolved in dimethyl formamide and different aliquots were placed in

TABLE 1. CHARACTERISTIC DATA OF THE SYNTHESISED COMPOUNDS 2a-d, 3a-d AND 4a-d.

Compd.	m.p.°	Yield %	Mol. formula	Analysis Found (Calcd.) %		
				C	H	N
2a	72-3	80	$C_{17}H_{16}O_4$	71.80 (71.83)	5.66 (5.63)	-
2b	56-8	85	$C_{18}H_{18}O_4$	72.46 (72.48)	6.06 (6.04)	-
2c	76-7	75	$C_{17}H_{15}O_4Cl$	64.06 (64.05)	4.68 (4.71)	-
2d	73-4	75	$C_{18}H_{17}O_4Cl$	64.94 (64.96)	5.12 (5.11)	-
3a	170-2	85	$C_{15}H_{14}O_3N_2$	66.64 (66.66)	5.18 (5.19)	10.39 (10.37)
3b	168-70	85	$C_{16}H_{16}O_3N_2$	67.63 (67.61)	5.62 (5.63)	9.85 (9.86)
3c	166-7	85	$C_{15}H_{13}O_3N_2Cl$	59.10 (59.11)	4.29 (4.27)	9.17 (9.19)
3d	162-3	80	$C_{16}H_{15}O_3N_2Cl$	60.30 (60.28)	8.77 (4.71)	4.70 (8.79)
4a	180-2	65	$C_{16}H_{12}O_3N_2S$	61.12 (61.44)	5.18 (5.19)	8.9 (8.96)
4b	190-2	70	$C_{17}H_{14}O_3N_2S$	61.3 (61.2)	4.0 (4.2)	8.5 (8.4)
4c	185-7	68	$C_{16}H_{11}O_3N_2SCl$	55.6 (55.68)	3.19 (3.20)	8.10 (8.12)
4d	192-4	69	$C_{17}H_{13}O_3N_2SCl$	57.00 (57.12)	3.44 (3.64)	7.84 (7.86)

each cup. Incubation was carried out at 37° for 24 h. Chloromycetin was used as the standard drug. The diameter of zone of inhibition was measured for 100 μg/ml concentration.

The antifungal activity of the compounds 4a-d was evaluated against *Aspergillus flavus* and *Fusarium oxysporium* by adopting the usual cup plate technique[13] and using commercial griseofulvin as the standard. The diameter of zone of inhibition was measured for 100 μg/ml concentration. The results of antibacterial and antifungal activities are recorded in Table 2.

Anticonvulsant activity:

The anticonvulsant activity of the substituted oxadiazoles 4a-d was based on maximal electroshock-induced convulsions in rats[14]. Male Swiss rats were procured from Virus Diagnostic Laboratory, Mysore and maintained at Farooqia College of Pharmacy, Mysore, were fed with standard diet, water and *libitum*. All protocols of animal experiments have been approved by the Institutional Animal Ethics Committee (IAEC). Six groups of three rats were selected and to the first group saline (control) injected i.p. and placed corneal electrodes on the cornea then applied the prescribed current. The different stages of convulsions were noted and used as control. To the second group 25 mg/kg of phenytoin sodium (standard) were injected i.p. and after 30 min subjected to electroconvulsions. The same procedure was repeated for remaining four groups using test compounds 4a-d. Various stages of convulsions were recorded at different intervals. The mean value for each group was calculated and compared with control. The results are summarised in Table 3.

Antiinflammatory activity:

Antiinflammatory activity was measured using the carrageenan-induced paw oedema test in rats[15]. Male Swiss rats (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 ibuprofen (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 carrageenan (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 2 h and 4 h 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 h and 4 h was calculated the formula % inhibition = $(1-v_t/v_c)100$, where v_t and v_c are the mean relative changes in the volume of paw oedema in the test and control respectively. The results are summarized in Table 4.

Diuretic activity:

Diuretic activity evaluation is based on the effects of drugs on water and electrolytes excretion in rats[16]. 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 a water load of 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 diuresis and the

TABLE 2. RESULTS OF *IN VITRO* ANTIMICROBIAL ACTIVITY OF THE NEWLY SYNTHESISED COMPOUNDS 4a-d.

Compd.	Diameter of zone of inhibition (mm)*				
	S. aureus	*P. aeruginosa*	*E. coli*	*A. flavus*	*F. oxysporium*
4a	9.2	9.6	10.0	11.5	9.0
4b	10.0	12.0	9.9	9.6	10.8
4c	14.8	12.8	12.5	10.0	15.5
4d	13.8	11.8	14.5	13.	12.6
Chloromycetine	25.0	24.0	22.0	-	-
Griseofulvin	-	-	-	28	25

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

volume of urine collected was measured periodically. Results were expressed as the mean of six samples and were compared to that of standard furosemide (Table 5).

RESULTS AND DISCUSSION

The compound 2a showed IR absorption at 1751 cm^{-1} assigned to ester carbonyl and at 1641 cm^{-1} due to aromatic carbonyl group. ^1H NMR spectrum of 2a showed a triplet centered at δ 1.3 assigned to methyl group, a quartet centered at δ 4.25 assigned to methylene protons of ester, a singlet at δ 4.63 assigned to methylene protons and a broad multiplet at δ 6.8 to 7.8 assigned to aromatic protons. The compound 3a showed IR absorption at 1645 cm^{-1} assigned to aromatic carbonyl, 1668 cm^{-1} assigned to amide carbonyl and a broad absorption at 3100-3400 cm^{-1} due to NH-NH_2. ^1H NMR spectrum showed a broad singlet centered at δ 3.9 for NH_2 and a broad singlet centered at δ 9.2 due to amide NH. Compound 4a exhibited NH, C=N and C=S absorptions at 3300, 1624 and 1134 cm^{-1} respectively and at 1655 cm^{-1} assigned to aromatic carbonyl. ^1H NMR spectrum of 4a showed a singlet at δ 4.0 assigned to OCH_2 , a broad multiplet at δ 6.8-7.8 assigned to aromatic protons and a broad singlet centered at δ 9.9 due to NH proton .The mass spectrum of compound 4a showed the molecular ion peak at m/z 312 consistent with the molecular formula $C_{15}H_{12}O_3N_2S$.

From the antibacterial activity results (Table 2), it was

TABLE 3. RESULTS OF ANTICONVULSANT ACTIVITY OF THE NEWLY SYNTHESISED COMPOUNDS 4a-d.

Compd.	Dose	Time (sec) in various phases of convulsion				Recovery
	mg/kg	Flexion	Extensor	Clonus	Stupor	/ death
4a	25	2.3	4.0	1.5	88	Recovery
4b	25	2.1	3.5	1.4	85	Recovery
4c	25	1.6	3.0	1.25	90	Recovery
4d	25	1.5	2.9	1.20	95	Recovery
Control (Saline)	-	3.0	10.0	2.0	120	Recovery
Standard (Phenyntoin)	25	0.5	1.0	0.5	100	Recovery

No. of animals in each group is 6.

TABLE 4. RESULTS OF ANTIINFLAMMATORY ACTIVITY OF THE NEWLY SYNTHESISED COMPOUNDS 4a-d.

Compd.	Dose mg/kg	Oedema volume(ml) different interval (mean ± S.E.M.)		% Inhibition	
		2h	4h	2h	4h
4a	100	0.22±0.02	0.18±0.02	11.9	22.8
4b	100	0.21±0.17	0.18±0.18	13.4	27.0
4c	100	0.19±0.01	0.16±0.02	23.9	45.6
4d	100	0.20±0.01	0.11±0.02	22.9	42.5
Standard (Ibuprofen)	100	0.17±0.02	0.10±0.02	25.6	49.6
Control (Tween-80)	-	0.28±0.02	0.24±0.02	-	-

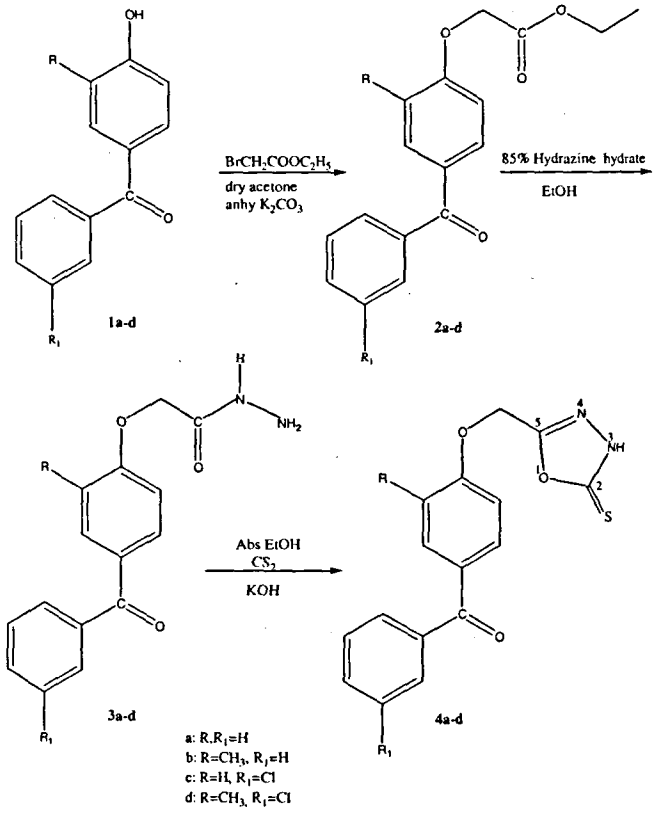
Values are mean±S.E.M., No. of animals in each group is 06.

TABLE 5. RESULTS OF DIURETIC ACTIVITY OF THE NEWLY SYNTHESISED COMPOUNDS 4a-d.

Compd.	Dose mg/kg	Total amount of urine collected (ml)				
		15'	30'	60'	120'	240'
4a	10	0	0	0	3.0	3.5
4b	10	0	0	0	3.5	2.9
4c	10	0	0	0	3.9	3.4
4d	10	0	0	0	3.8	3.3
Standard (Frusemide)	10	0	0	0	4.3	3.6
Control (Water)	25ml/kg	0	0	0	2.3	2.2

No. of animals in each group is 06.

observed that chloro substituted compounds 4c and 4d were more active than methyl substituted 4b and unsubstituted 4a. On comparison of the fungicidal data of the compounds 4a-d (Table 2), 4c and 4d possess better fungicidal activity than the compounds 4a and 4b. This implies that the presence of chloro moiety in 4c and 4d has enhanced the antifungal activity when compare to absence of chloro in 4a and 4b. Amongst the compounds subjected to anticonvulsant activity (Table 3), compounds 4c and 4d were found to possess promising activity compared to that of standard phenyntoin. The antiinflammatory activity (Table 4) also revealed that compounds, 4c (45.6%) and 4d (42.5%) showed significant activity compared to standard ibuprofen (49.6%). The diuretic activity (Table 5) results showed the test compounds produced slight diuresis compared to standard frusemide.



Scheme 1

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