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Synthesis and Pharmacological Activity of 4-Aryl-thieno-[2,3-d]-pyridazines

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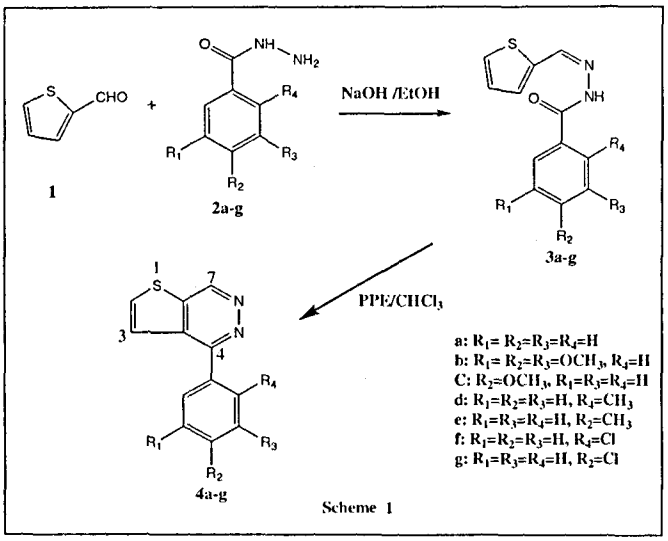
A facile synthesis of 4-aryl-thieno-[2,3-d]-pyridazines (4a-g) have been achieved by cyclisation of 1-aroylhydrazones (3a-g) using polyphosphate ester. All the synthesized compounds have been characterized by elemental analysis and spectral data. In addition, they were screened for antibacterial, antifungal, anticonvulsant and antiinflammatory activities.

Many pyridazine and related analogues were found to possess valuable properties such as antiangiogenic[1], anticancer[2], antineuroinflammatory[3] and anticonvulsant[4] activities. Besides they exhibit antiviral activity against the replication of human immunodeficiency virus[5], inhibit human picornaviruses[6], protein kinase[7] and acyl coenzyme A cholesterol acyltransferase[8]. Previously 7-aryl-thieno[2,3-d]-pyridazines were synthesized by a different route to study the pentobarbital sleep in mice[9].

Based on these findings it was considered valuable to incorporate thiophene ring in pyridizine framework like 4a-g, which might enhance the biological activity. Herein we are reporting the conversion of 1-aroyl-hydrazones 3a-g to the corresponding pyridazines (4a-g) using polyphosphate ester (PPE) in good yield.

The synthetic route is depicted in Scheme 1. The starting material thiophene-2-aldehyde (1) was prepared according to literature by formylation of thiophene with phosphoryl chloride and dimethyl formamide in 1,2-dichloroethane[10]. Thiophene-2-yl-aroylhydrazones 3a-g were prepared by the condensation of thiophene-2-aldehyde (1) with acidhydrazides 2a-g in sodium hydroxide and ethanol. Subsequently, stirring a mixture of hydrazone 3a-g and PPE[11] in chloroform afforded 4-phenyl-thieno[2,3-d]-pyridazine 4a-g in moderate yield.



Scheme 1

MATERIALS AND METHODS

Melting points were determined with Thomas Hoover capillary melting point apparatus. IR spectra were recorded in Nujol on FT IR Shimadzu 8300 spectrometer and ^{1}H NMR spectra were recorded at 300 MHz and chemical shift were recorded in arts per million downfield from tetramethylsilane. Elementary analysis results are within 0.4% of the calculated value. TLC was performed on preactivated (110°) silica gel plates using ethylacetate:chloroform (7:1) as eluent and the plates were visualized with UV light. All the animal experiments with Swiss rats were carried out at Farooqia College of Pharmacy, Mysore and permission for conducting these experi-

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TABLE 1: CHARACTERISTIC DATA OF THE SYNTHESIZED COMPOUNDS 3a-g AND 4a-g

Compd.	Yield%	mp(°)	Mol. formula	Analysis Found (Calcd.) %				
				C	H	Cl	N	S
3a	72	168-70	$C_{12}H_{10}N_2OS$	62.64 (62.60)	4.38 (4.35)	-	12.20 (12.17)	13.95 (13.91)
3b	66	160-62	$C_{15}H_{16}N_2O_4S$	56.28 (56.25)	5.04 (5.05)	-	8.78 (8.75)	10.03 (10.05)
3c	70	163-65	$C_{13}H_{12}N_2O_2S$	60.02 (60.02)	4.62 (4.6)	-	10.75 (10.76)	12.32 (12.3)
3d	71	170-72	$C_{13}H_{12}N_2OS$	63.9 (63.91)	4.92 (4.95)	-	11.45 (11.47)	13.15 (13.12)
3e	73	176-78	$C_{13}H_{12}N_2OS$	63.93 (63.91)	4.9 (4.95)		11.42 (11.47)	13.11 (13.12)
3f	62	180-82	$C_{12}H_9ClN_2OS$	54.45 (54.44)	3.40 (3.43)	13.36 (13.39)	10.55 (10.58)	12.13 (12.11)
3g	65	188-90	$C_{12}H_9ClN_2OS$	54.41 (54.44)	3.44 (3.43)	13.35 (13.39)	10.56 (10.58)	12.10 (12.11)
4a	60	150-51	$C_{12}H_8N_2S$	67.95 (67.92)	3.80 (3.78)	-	13.22 (13.20)	15.12 (15.09)
4b	64	155-57	$C_{15}H_{14}N_2O_3S$	59.62 (59.60)	4.65 (4.63)	-	9.30 (9.27)	10.62 (10.60)
4c	65	145-47	$C_{13}H_{10}N_2OS$	64.45 (64.46)	4.12 (4.14)	-	11.55 (12.57)	13.22 (13.23)
4d	67	135-37	$C_{13}H_{10}N_2S$	69.05 (69.00)	4.42 (4.45)	-	12.35 (12.38)	14.15 (14.17)
4e	65	129-31	$C_{13}H_{10}N_2S$	69.02 (69.00)	4.46 (4.45)	-	12.36 (12.38)	14.18 (14.17)
4f	69	163-65	$C_{12}H_7ClN_2S$	58.45 (58.42)	2.88 (2.86)	14.35 (14.37)	11.33 (11.35)	13.04 (13.00)
4g	62	168-70	$C_{12}H_7ClN_2S$	58.44 (58.42)	2.84 (2.86)	14.38 (14.37)	11.36 (11.35)	13.05 (13.00)

Compounds 3a-g were recrystallized from methanol and 4a-g from benzene.

ments was obtained from Institutional Animals Ethics Committee (CPCSEA Regd. No. 443/01/a).

Thiophene-2-yl-aroyl-hydrazones 3a-g (Table 1):

To a solution of thiophene-2-aldehyde (1, 8.97 g, 0.08 mol) and phenyl acid hydrazide (2a, 11 g, 0.08 mol) [prepared from benzoic acid according to the procedure described by Kudryashova *et al.*[12]] in dry ethanol (100 ml), sodium hydroxide pellets (0.4 g, 0.01 mol) were added and the mixture refluxed for 4 h. The solvent was distilled off at reduced pressure, the yellow gummy mass obtained was washed with water and the crude product was recrystal-

lized from ethanol to give 3a as pale yellow solid. Similarly compounds 3b-g were prepared.

4-Aryl-thieno[2,3-d]-pyridazines 4a-g (Table 1):

A mixture of 3a (6 g, 0.016 mol) and freshly prepared solution of PPE [prepared by refluxing a mixture of phosphorous pentoxide (50 g, 0.357 mol), dry diethyl ether (50 ml) and chloroform (150 ml) until the solution become clear][11] was refluxed for 7 h. The cooled reaction mixture (5-10°) was poured into ice (200 g) and basified with 10% ammonium hydroxide and stirred for 15 min. The organic layer separated was washed with 5% sodium hydroxide solution (3x30 ml) followed by water (3x30 ml), dried over anhydrous sodium sulphate and evaporated the solvent to get solid which on recrystallisation with benzene gave pale yellow crystalline solid 4a. Similarly compounds 4b-g were prepared.

Evaluation of antimicrobial activity:

The compounds 4a-g were screened for *in vitro* antimicrobial activity using the cup plate method[13]. 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 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.

TABLE 2: *IN VITRO* ANTIMICROBIAL ACTIVITY OF COMPOUNDS 4A-G

Compd.	Diameter of zone of inhibition (mm*)					
	Antibacterial			Antifungal		
	B. cereus	*S. aureus*	*E. coli*	*P. nigricans*	*A. fumigatus*	*F. solani*
4a	-	12	13	21	-	20
4b	27	20	26	28	27	18
4c	09	14	27	25	24	11
4d	-	25	19	15	-	28
4e	-	23	20	17	27	-
4f	26	25	29	29	26	19
4g	19	20	28	28	27	16
Norfloxacin	24	22	26	-	-	-
Griseofulvin	-	-	-	27	25	26

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

Anticonvulsant activity:

The anticonvulsant activity was carried out based on 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 and water was provided *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 4a-g. 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.

TABLE 3: ANTICONVULSANT ACTIVITY OF COMPOUNDS 4a-g

Compound	Dose mg/kg	Time (sec) in various phases of convulsion				Recovery/ death
		Flexion	Extensor	Clonus	Stupor	
4a	25	2.3	3.5	1.6	80	Recovery
4b	25	1.5	2.4	1.5	96	Recovery
4c	25	1.5	2.2	1.3	90	Recovery
4d	25	2.5	4.0	1.5	81	Recovery
4e	25	1.3	2.0	1.2	85	Recovery
4f	25	1.6	2.6	1.6	98	Recovery
4g	25	1.4	2.2	1.5	96	Recovery
Control (saline)	-	3.1	10.0	2.2	120	Recovery
Standard (Phenytoin)	25	0.5	1.0	0.5	100	Recovery

Number of animals in each group is six

Antiinflammatory activity:

Antiinflammatory activity of compounds 4a-g was evaluated using carrageenan-induced rat hind paw oedema method[15]. 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 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

TABLE 4: ANTIINFLAMMATORY ACTIVITY OF COMPOUNDS 4a-g

Compound	Dose mg/Kg	Oedema volume (ml) different intervals*		% Inhibition	
		2 h	4 h	2 h	4 h
4a	100	0.25 (±0.15)	0.15 (±0.02)	13.9	38.5
4b	100	0.19 (±0.01)	0.12 (±0.00)	34.1	52.1
4c	100	0.21 (±0.17)	0.14 (±0.02)	27.8	43.6
4d	100	0.26 (±0.15)	0.18 (±0.02)	10.3	28.0
4e	100	0.26 (±0.17)	0.19 (±0.02)	10.3	24.0
4f	100	0.18 (±0.01)	0.12 (±0.00)	37.9	52.1
4g	100	0.18 (±0.01)	0.12 (±0.00)	37.9	52.1
Standard Phenylbutazone	100	0.18 (±0.02)	0.13 (±0.02)	36.1	49.5
Control Tween-80	100	0.29 (±0.02)	0.25 (±0.02)	-	-

*Each value is a mean±SEM, Number of animals in each group is six.

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=$(1-v_t/v_c)\times100$, 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 4.

RESULTS AND DISCUSSION

The structure of synthesized compounds was elucidated by IR, NMR and microanalyses. The IR spectra of 3a-g showed stretching absorption bands at 3318 and 1695 cm^{-1} assigned to N-H and carbonyl group of amide, respectively. The disappearance of N-H and carbonyl group of amide stretching bands of 3a-g and observation of strong C=N and C=C stretching bands at 1620 and 1590 cm^{-1} are evidences for ring closure of 3a-g to 4a-g. In ^1H NMR spectra, all protons of 3a-g and 4a-g were seen according to the expected chemical shift and integral values. The CH_3, OCH_3, aromatic protons and NH protons of 3a-g were observed at δ 2.1-2.3, 3.85-3.9, 6.8-7.5 and 8.4-8.5, respectively. Similarly compounds 4a-g showed the peaks for CH_3, OCH_3 and aromatic protons at δ 2.2-2.3, 3.87-3.92 and 6.85-7.6, respectively. Further, compounds 4a-g showed broad singlet at δ 9.2-9.6 due to C_7-H and this down field absorption is due to the presence of electronegative adjacent nitrogen atom which is in agreement with earlier observation[16].

Antimicrobial activity screening results are qualitative in nature (Table 2). The antibacterial screening results have shown that methoxy substituted compounds 4b and 4c, and chloro substituted compounds 4f and 4g exhibit, growth inhibitory activity more relevant than that of the reference compound. It is worth noting that compound 4f with chloro group at ortho position showed growth inhibitory activity higher than norfloxacin against all the three strains. On the other hand compound 4g with chloro group at para position showed higher activity against only *Escherichia coli*. Compound 4b with three methoxy groups showed growth inhibitory activity higher than norfloxacin against *Bacillus cereus* and *Escherichia coli* but lower activity against *Staphylococcus aureus*. On the other hand compound 4c with a methoxy group at para position exhibited more activity against *Escherichia coli*, moderate against *Staphylococcus aureus* and weak against *Bacillus cereus*. Compounds 4d and 4e with methyl group on ortho and para position respectively, exhibited more activity against *Staphylococcus aureus*, moderate against *Escherichia coli* and no activity against *Bacillus cereus*. Compound 4a with out any substituent, is inactive against *Bacillus cereus* but moderately active against *Staphylococcus aureus* and *Escherichia coli*. Even in case of antifungal activity methoxy and chloro substituted compounds showed growth inhibitory activity more relevant than that of the reference drug. Compounds 4b, 4f and 4g showed higher growth inhibitory activity than griseofulvin against *Aspergillus Fumigatus* and *penicillium nigricans*. Chloro substituted compounds 4f and 4g showed higher growth inhibitory activity than griseofulvin against *penicillium nigricans* and *Aspergillus Fumigatus* and moderate against *Fusarium solani*. Against the two strains *penicillium nigricans* and *Fusarium solani* compound 4a, showed activity but at the same it showed no activity against *Aspergillus Fumigatus*. Compounds 4d and 4e showed more activity against *Fusarium solani and Aspergillus Fumigatus* respectively and moderate activity against *penicillium nigricans*. In general these compounds are found to possess more antifungal than antibacterial activity. Amongst the compounds subjected to anticonvulsant activity (Table 3), compounds 4b and 4c with methoxy group and 4f and 4g with chloro group were found to possess promising activity compared to that of standard phenytoin. Amongst the compounds subjected to antiinflammatory screening (Table 4), compounds with methoxy group 4b and 4f and 4g with chloro group were found to possess significant activity compared to that of the standard phenylbutazone. Further, compounds 4d and 4e with methyl group and compound 4a without substituent, showed weak activity compared to that of the standard.

In conclusion, the present work provides a useful method for the preparation of 4-aryl-thieno[2,3-d]-pyridazines with moderate yields, as well as easily accessible starting materials. Further compounds with methoxy and chloro group have shown more antibacterial, antifungal, anticonvulsant and anti-inflammatory activities.

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