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Synthesis, Biological and Pharmacological Activities of Some New Derivatives of 5-Aryl-1,3,4-thiadiazolin-2-thiones

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Two different S-(5-aryl-1,3,4-thiadiazol-2-yl)mercapto acetic acids were synthesized from the respective 5-aryl-1,3,4-thiadiazolin-2-thiones on reaction with chloroacetic acid. Each of these acids has been converted *in situ* into acid chloride using thionyl chloride and condensed with three different seecondary amines *viz.*, dicyclohexylamine, morpholine and piperidine. The products obtained in each case were purified and characterized as the respective N,N-disubstituted S-(5-aryl-1,3,4-thiadiazol-2-yl)mercaptoacetamides. Alternatively the *in situ* acid chlorides were allowed to react with 2-methoxyethanol and 2-ethoxyethanol. These products have been purified and characterized as the respective esters, *i.e.*, 2-alkoxyethyl S-(5-aryl-1,3,4-thiadiazol-2-yl)mercaptoacetates. Similarly, the acid chlorides *in situ* have been also allowed to react with two different N-substituted aminoethanols and the products were characterized as the respective 2-N,N-substituted aminoethyl S-(5-aryl-1,3,4-thiadiazole-2-yl)-mercaptoacetates. All the three types of new derivatives of 5-aryl-1,3,4-thiadiazolidin-2-thiones were assayed for their antibacterial, antifungal, antihistaminic and anticholinergic activities by standard methods.

Key Words: 5-Aryl-1,3,4-thiazolidin-2-thiones, Secondary amines, Antimcirobial activity, Anticholinergic activity, Antihistaminic activity.

INTRODUCTION

1,3,4-Thiadiazole system is known for its wide variety of biological and pharmacological actions such as: antimicrobial[1] (mostly antifungal), insecticidal[2], anticholinergic[3], antihypertensive[4], CNS depressant[5], anti-inflammatory[6], hypoglycemic[7], antihelmintic[8], anticancer[9], *etc*. Though the literature presents several fused and bridged macromolecular thiadiazole, it appears that less attention has been made on simple thiadiazole derivatives. Therefore, in this paper, the synthesis of some new functional derivatives of 1,3,4-thiazolidin-2-thiones are reported and screened them for their antibacterial, antifungal, anticholinergic and H_1-antihistaminic activities by standard methods.

EXPERIMENTAL

Melting points were determined in open capillaries using Cintex melting point apparatus and are uncorrected. The IR spectra of the compounds were recorded in Perkin-Elmer infracord-283 spectrophotometer as KBr pellets, ^{1}H NMR spectra as OMEGA-500 MHz spectrometer using TMS as an internal standard and mass spectra on a FINNIGAN MAT-90 in the EI mode and by the direct inlet method.

Benzoic acid (a) and 4-nitrobenzoic acid (b) were converted into their corresponding acid hydrazides by known procedure and the acid hydrazide was reacted with ammonia and carbondisulphide to obtain the corresponding 5-aryl-1,3,4-thiadiazolidin-2-thione (**Ia** and **b**) purified by recrystallization and identified by their literature[10,11] melting points and other data.

Synthesis of S-(5-aryl-1,3,4-thiadiazol-2-yl)mercaptoacetic acids (IIa and b): To a solution of appropriate 5-aryl-1,3,4-thiadiazolin-2-thione (**I**; 0.01 mol) in alcoholic sodium hydroxide (4 %; 10 mL) chloroacetic acid (0.012 mol) was added and heated under reflux for about 4 h. The reaction mixture was cooled, filtered, if necessary and the clear, cold solution was neutralized with dilute hydrochloric acid. The product thus separated was filtered, washed with cold water, dried and purified.

Adopting the above specified procedure the following two different substituted mercepto acetic acids were prepared and characterized:

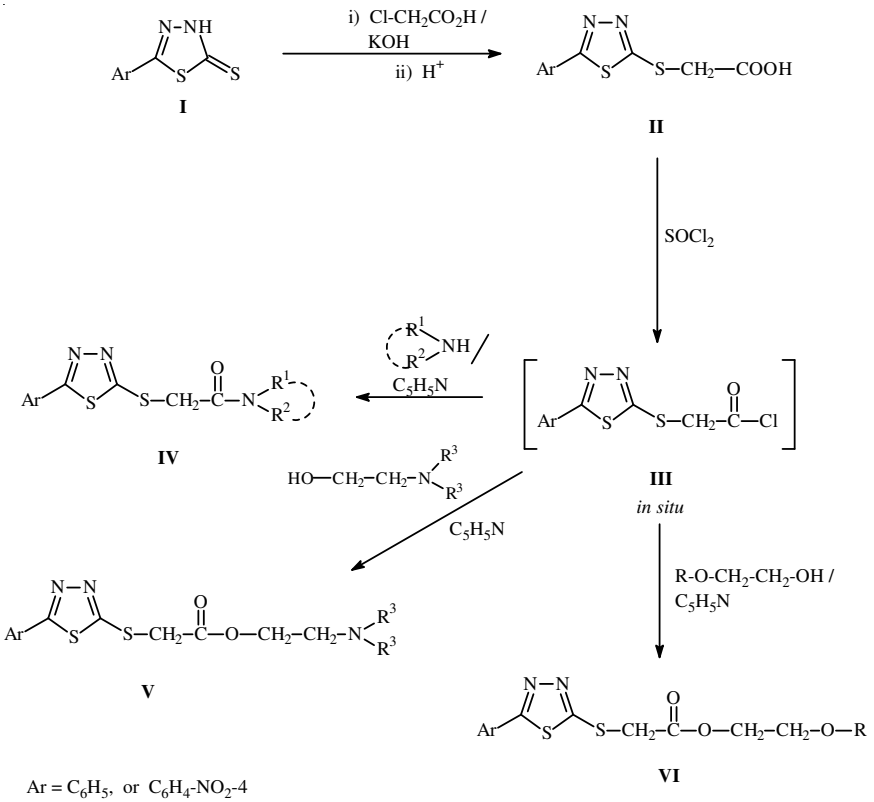
S-(5-Phenyl-1,3,4-thiadiazol-2-yl)mercaptoacetic acid (IIa; Ar = -C_6H_5): Purified by recrystallization from methanol to obtain a colourless crystalline solid, m.p. 154 ºC. Yield : 65 %; [Found: C, 47.55; H, 3.14; N, 11.05; m.f. $C_{10}H_8N_2O_2S_2$ requires: C, 47.61; H, 3.17; N, 11.11, respectively].

S-[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)mercaptoacetic acid (IIb; Ar = 4-NO_2-C_6H_4): It was purified by recrystallization from methanol to yield an yellow crystalline solid m.p. 150 ºC, yield: 68 % [Found: C, 40.20; H, 3.35; N, 14.65; m.f. $C_{10}H_4N_3O_4S_2$ requires: C, 40.26; H, 3.40; N, 14.09].

Synthesis of N,N-disubstituted S-(5-aryl-1,3,4-thiadiazol-2-yl)-mercaptoacetamides (IV): The mercaptoacetic acid (**II**; 0.01 mol) was taken into a dry flask and thionyl chloride (0.015 mol) was added. It was then heated under reflux for 0.5 h on a hot water-bath under anhydrous conditions, using calcium chloride guard tube. After the fumes of excess thionyl chloride were ceased off, an appropriate secondary amine was added (0.012 mol) followed by pyridine (2 mL). The reaction mixture was heated under reflux for further 6 h. It was cooled and poured onto crushed ice (~ 100 g) while stirring with a glass rod. The product separated was filtered under suction, washed thoroughly with small portions of cold water and purified.

Synthesis of 2-dialkylaminoethyl S-(5-aryl-1,3,4-thiadiazol-2-yl)-mercaptoacetates (V): To the acid chloride (*in situ*) prepared as above an appropriate 2-dialkylaminoethanol (0.012 mol) and pyridine (2 mL) were added. The reaction mixture was heated under reflux for 6 h. It was then cooled and poured onto crushed ice (~100 g) while stirring with a glass rod. The product thus separated was filtered under suction, washed thoroughly with small portions of cold water, dried and purified.

Synthesis of 2-alkoxyethyl S-(5-aryl-1,3,4-thiadiazol-2-yl)mercapto-acetates (VI): To the acid chlorides (**II**; 0.010 mol) an appropriate 2-alkoxy-ethanol (0.012 mol) and pyridine (2 mL) were added. The reaction mixture was heated under reflux for 6 h. The reaction mixture was cooled and poured onto crushed ice (~100 g) while stirring. The product obtained was filtered under suction, washed with cold water, dried and purified (**Scheme-I**). The characterization data of compounds **IV**, **V** and **VI** are presented in Table-1.



Scheme-I

TABLE-1
CHARACTERIZATION DATA OF DERIVATIVES OF
5-ARYL-1,3,4-THIADIAZOLIN-2-THIONES

Compd.	Nature of the substituents		m.p. (ºC) / Yield (%)	Elemental analyses (%): Calcd. (Found)		
	Ar	N R^1R^2/R^3(IV)		C	H	N
IVa	C_6H_5	Dicyclohexylamino	121 (70)	63.55 (63.61)	6.90 (6.98)	10.08 (10.12)
IVb	C_6H_5	Morpolino	132 (68)	52.30 (52.33)	4.60 (4.67)	13.00 (13.08)
IVc	C_6H_5	Piperidino	140 (60)	56.39 (56.42)	5.30 (5.32)	13.10 (13.16)
IVd	4-NO_2-C_6H_4	Dicyclohexylamino	242 (80)	57.20 (57.26)	6.00 (6.07)	12.10 (12.14)
IVe	4-NO_2-C_6H_4	Morpolino	130 (78)	45.72 (45.77)	3.78 (3.81)	15.20 (15.25)
IVf	4-NO_2-C_6H_4	Piperidino	188 (70)	49.28 (49.31)	4.34 (4.38)	15.30 (15.34)
Va	C_6H_5	Diethylamino	275 (58)	54.65 (54.70)	5.95 (5.98)	11.92 (11.96)
Vb	C_6H_5	Dimethylamino	256 (60)	51.08 (52.01)	5.25 (5.26)	12.95 (13.00)
Vc	4-NO_2-C_6H_4	Diethylamino	252 (55)	48.30 (48.36)	5.00 (5.03)	13.80 (14.10)
Vd	4-NO_2-C_6H_4	Dimethylamino	220 (65)	45.45 (45.52)	4.30 (4.33)	15.10 (15.17)
VIa	C_6H_5	C_2H_5	172 (82)	51.80 (51.85)	4.90 (4.93)	8.60 (8.64)
VIb	C_6H_5	CH_3	135 (75)	50.28 (50.32)	4.45 (4.51)	9.00 (9.03)
VIc	4-NO_2-C_6H_4	C_2H_5	273 (78)	45.35 (45.40)	4.00 (4.05)	11.25 (11.35)
VId	4-NO_2-C_6H_4	CH_3	170 (80)	43.79 (43.82)	3.60 (3.65)	11.74 (11.79)

*Purification of compounds has been effected by recrystallization from appropriate solvents, *viz.*,

*Characterization data of a representative compound: **IVb**: IR (KBr, ν_{max}, cm^{-1}): 1720 (amide C=O), 1625 (thiadiazole C=N) 1610 (aromatic C=C); ^{1}H NMR ($CDCl_3$, ppm) peaks at 2.92 [t, 4H, $O(CH_2)_3$, morpholine], 3.68 [t, 9H, -CO-$N(CH_2)_2$ morpholine], 3.90 (t, 2H, -S-CH_2-CO) and 6.72-7.88 (m, 5H, aromatic). **Vb**: IR (KBr, ν_{max}, cm^{-1}): 1725 (C=O, ester), 1625 (C=N, heteryl) 1605 (C=C, aromatic). ^{1}H NMR (DMSO-d_6, δ ppm): 3.68 (s, 6H, -NMe_2), 4.10 (t, 2H, -N-CH_2), 4.40 (t, 2H, -CO-O-CH_2), 4.80 (s, 2H, -S-CH_2-CO). **VIa**: IR (KBr, ν_{max}, cm^{-1}): 1720, 1620, 1605 for C=O, C=N and C=C, ^{1}H NMR (DMSO-d_6, δ, ppm): 2.30 (t, 3H, -O-CH_2-CH_3), 2.55 (q, 2H, -O-CH_2-CH_3), 3.85 (t, 2H, -CO-O-CH_2-CH_2-O).

Biological/Pharmacological activities[12,13]: Male albino mice fasted for 24 h, administered all test compounds suspended in sodium CMC intraperitoneally in doses of 50 mg to 500 mg/kg (b.w.). The animals were observed for 48 h from the time of administration of test compounds to record the mortality.

Acute toxicity and gross behavioural studies: All the compounds tested for acute toxicities were also observed for gross behaviour changes continuously for 3 h starting from the administration of the compounds and for 48 h, intermittently and compared with that of control groups of mice.

Antibacterial activity: The antibacterial activity of the test compounds was assayed, systematically against five different strains of bacteria: *B. subtilis, B. mycoides, E. coli, P. aeruginosa* and *P. vulgaris* (two gram positive and three gram-negative) by agar diffusion method[14] benzyl penicillin and streptomycin as standards.

Antifungal activity[14]: All the compounds were also tested for their antifungal activity by the cup-plate method and the fungi employed were for *F. oxysporum* and *C. lunata*. Clotrimazole employed as the standard comparison.

Antihistaminic activity: The test compounds were screened for their antihistaminic activity by using isolated Guinea-pig ileum method[15] at concentration employed as a reference for comparison.

Anticholinergic activity: The anti-cholinergic activity of the compounds were screened by using rat intestine, following the standard procedure.

RESULTS AND DISCUSSION

Acute toxicity for gross behavioural studies: All the test compounds upto an oral dose of 250 mg/kg (bw) were found to be safe. No significant changes were recorded in the gross behavioural studies of the compounds.

Antibacterial activity: It is interesting to note that the present compounds and relatively less effective against both the gram (+)ve bacteria and specificially more effective against the gram (-)ve. Even amongst the three strains of gram (-)ve bacteria, the compounds were significantly potent against *Pseudomonas aeruginosa* than the other two. But, however, their potency was not at all comparable with that of the standard, since the compounds could exhibit antibacterial activity only at considerably higher concentration in comparison to that of the standard (Table-2).

Antifungal activity: Table-3 revealed that new derivatives of thiadiazole are relatively more effective towards *C. lunata* when compared with *F. oxysporum*. Compound **IVd**, **IVe**, **VIa** and **VIb** were found to exhibit reasonable activity. But however, the antifungal activity of these compounds is not comparable with that of the standard employed.

H_1-Antihistaminic activity: As can be observed from Table-4, the compound **VIc**, with a 4-nitrophenyl and ethyl groups is relatively more potent amongst all the present. Compounds with IC_{50}; 508 μg and the second in order being **IVd** with 4-nitrophenyl and a dicyclohexylamino groups with IC_{50}: 570 μg. But, however, their potency is not at all comparable with the standard.

TABLE-2
ANTIBACTERIAL ACTIVITY DATA OF NEW DERIVATIVES OF
5-ARYL-1,3,4-THIADIAZOLIN-2-THIONES

Compd.	Substituents		Zone of inhibition (mm)				
	Ar	N R^1R^2/R^3(IV)	BS	BM	EC	PA	PV
IVa	C_6H_5	Dicyclohexylamino	17	8	10	15	14
IVb	C_6H_5	Morpolino	12	7	12	24	15
IVc	C_6H_5	Piperidino	8	6	13	29	16
IVd	4-NO_2-C_6H_4	Dicyclohexylamino	15	9	15	35	16
IVe	4-NO_2-C_6H_4	Morpolino	15	5	8	36	14
IVf	4-NO_2-C_6H_4	Piperidino	16	7	10	29	17
Va	C_6H_5	Diethylamino	10	6	14	31	11
Vb	C_6H_5	Dimethylamino	9	5	14	30	10
Vc	4-NO_2-C_6H_4	Diethylamino	12	6	16	36	15
Vd	4-NO_2-C_6H_4	Dimethylamino	10	6	8	38	14
VIa	C_6H_5	C_2H_5	10	5	16	31	13
VIb	C_6H_5	CH_3	9	4	15	30	11
VIc	4-NO_2-C_6H_4	C_2H_5	11	5	16	34	10
VId	4-NO_2-C_6H_4	CH_3	12	4	16	35	15
Gram(+)ve	Benzylpenicillin	(Standard)	30	31	–	–	–
Gram(-)ve	Streptomycin	(Standard)	–	–	40	42	43
	Control	0.1 mL DMF	–	–	–	–	–

BS = *B. subtilis*, BM = *B. mycoides*, EC = *E. coli*, PA = *P. auroginosa*, PV = *P. vulgaris*
Test compound 1000 µg/mL concentration; Standard 100 µg/mL concentration.

TABLE-3
ANTIFUNGAL ACTIVITY DATA OF NEW DERIVATIVES OF
5-ARYL-1,3,4-THIADIAZOLIN-2-THIONES

Compd.	Substituents		Zone of inhibition (mm)	
	Ar	N R^1R^2 / R^3	*F. oxysporum*	*C. lunata*
IVa	C_6H_5	Dicyclohexylamino	13	20
IVb	C_6H_5	Morpolino	15	25
IVc	C_6H_5	Piperidino	13	26
IVd	4-NO_2-C_6H_4	Dicyclohexylamino	15	33
IVe	4-NO_2-C_6H_4	Morpolino	13	33
IVf	4-NO_2-C_6H_4	Piperidino	14	24
Va	C_6H_5	Diethylamino	11	29
Vb	C_6H_5	Dimethylamino	19	27
Vc	4-NO_2-C_6H_4	Diethylamino	16	18
Vd	4-NO_2-C_6H_4	Dimethylamino	18	20
VIa	C_6H_5	C_2H_5	17	31
VIb	C_6H_5	CH_3	–	32
VIc	4-NO_2-C_6H_4	C_2H_5	20	28
VId	4-NO_2-C_6H_4	CH_3	–	30
Clotrimazole (standard) (1 mg/mL conc. in DMF)			20	39

TABLE-4
ANTIHISTAMINIC ACTIVITY DATA OF NEW DERIVATIVES OF 5-ARYL-1,3,4-THIADIAZOLIN-2-THIONES

Compd.	Substituents		Dose of which 50 % inhibition is observed IC_{50} (μg)
	Ar	N R^1R^2	
IVa	C_6H_5	Dicyclohexylamino	602.66
IVb	C_6H_5	Morpolino	895.63
IVc	C_6H_5	Piperidino	899.49
IVd	4-NO_2-C_6H_4	Dicyclohexylamino	570.65
IVe	4-NO_2-C_6H_4	Morpolino	905.37
IVf	4-NO_2-C_6H_4	Piperidino	892.85
Va	C_6H_5	Diethylamino	750.05
Vb	C_6H_5	Dimethylamino	764.26
Vc	4-NO_2-C_6H_4	Diethylamino	946.00
Vd	4-NO_2-C_6H_4	Dimethylamino	781.25
VIa	C_6H_5	C_2H_5	777.60
VIb	C_6H_5	CH_3	927.41
VIc	4-NO_2-C_6H_4	C_2H_5	508.13
VId	4-NO_2-C_6H_4	CH_3	774.64
	Avil standard		480.00

Anticholinergic activity: The anticholinergic potency of new derivatives is not comparable with that of the standard atropine employed in the investigation (Table-5). They could exhibit the activity only at quite higher dose. But, amongst all the present compounds, compound **VIb** with a 4-nitrophenyl and ethyl substituent was found to be relatively more potent with an IC_{50} value: 141 μg.

TABLE-5
ANTICHOLINERGIC ACTIVITY DATA OF NEW DERIVATIVES OF 5-ARYL-1,3,4-THIADIAZOLIN-2-THIONES

Compd.	Substituents		Dose of which 50 % inhibition is observed IC_{50} (μg)
	Ar	N R^1R^2	
IVa	C_6H_5	Dicyclohexylamino	142.45
IVb	C_6H_5	Morpolino	166.40
IVc	C_6H_5	Piperidino	142.04
IVd	4-NO_2-C_6H_4	Dicyclohexylamino	135.86
IVe	4-NO_2-C_6H_4	Morpolino	173.61
IVf	4-NO_2-C_6H_4	Piperidino	141.12
Va	C_6H_5	Diethylamino	169.37
Vb	C_6H_5	Dimethylamino	184.85
Vc	4-NO_2-C_6H_4	Diethylamino	161.49
Vd	4-NO_2-C_6H_4	Dimethylamino	127.98
VIa	C_6H_5	C_2H_5	141.75
VIb	C_6H_5	CH_3	129.43
VIc	4-NO_2-C_6H_4	C_2H_5	130.20
VId	4-NO_2-C_6H_4	CH_3	176.71
	Standard	Atropine (1 mg/mL conc.)	30.00

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