Synthesis and *in vitro* Antimicrobial and Antifungal Properties of Some Novel 1,3,4-Thiadiazole and *s*-Triazolo[3,4-*b*][1,3,4]thiadiazole Derivatives

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A novel series of 2-arylthiocarbamoylhydrazino-5-phenyl-1,3,4-thiadiazoles III-VII was synthesized and cyclodesulfurized into the corresponding 3-arylamino-6-phenyl-*s*-triazolo[3,4-*b*][1,3,4]thiadiazoles VIII-XII with DCCD. Some of the products were found to be only moderately active against *Staphylococcus aureus* and *Candida albicans*.

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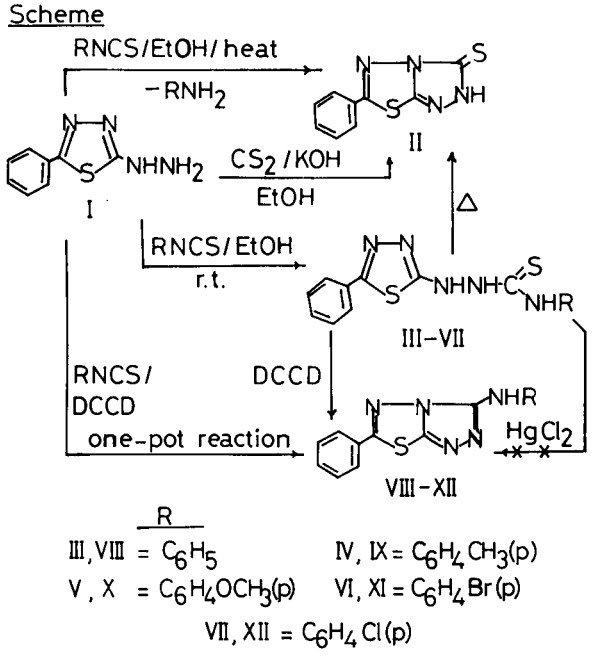
During the past years, considerable evidence has been accumulated to demonstrate the efficacy of 1,3,4-thiadiazole in inducing antibacterial [1-5], antifungal [6-8], amaebicidal [9], antiviral [10], insecticidal [11], CNS depressant [12] and antitumor [13] activities when properly substituted in the 2- and 5-positions or fused to an *s*-triazolo ring systems [8,14]. To further assess the potential of such a class of compounds as antimicrobial and antifungal agents, a novel series of 2-arylthiocarbamoylhydrazino-5-phenyl-1,3,4-thiadiazoles III-VII was synthesized and cyclodesulfurized into the corresponding 3-arylamino-6-phenyl-*s*-triazolo[3,4-*b*][1,3,4]thiadiazole derivatives VIII-XII. The rationale employed in the design of compounds III-VII has been contingent upon the enhancement of biological activity due to the presence of the various bactericidal N^4-substituted thiosemicarbazide moieties. As compared with the activity of compounds VIII-XII, this would reveal the structural requirements, in respect to the flexibility or rigidity of the thiosemicarbazide chains, in such an intercorrelated series of 1,3,4-thiadiazole derivatives.

The designed compounds were synthesized as depicted in Scheme I. 2-Hydrazino-5-phenyl-1,3,4-thiadiazole (I) [15] was allowed to react with the selected arylisothiocyanate in ethanol at room temperature to give the required 2-arylthiocarbamoylhydrazino-5-phenyl-1,3,4-thiadiazoles III-VII in good yields (Table I). The products were found to be sensitive to heat, as has been previously reported for other heat-sensitive thioureas [16,17], and underwent cyclization into 6-phenyl-*s*-triazolo[3,4-*b*][1,3,4]thiadiazole-3(2*H*)-thione (II) when heated during the preparation stages or crystallized from various solvents at elevated temperature. The thione II was identified with an authentic sample prepared by reacting 2-hydrazino-5-phenyl-1,3,4-thiadiazole (I) with carbon disulfide and potassium hydroxide in boiling ethanol as reported [15].

The thiosemicarbazide III-VII were then subjected to cyclodesulfurization with dicyclohexylcarbodiimide (DCCD) in accordance with the procedure recently developed in our laboratory [18] and applied for the synthesis of variety of simple [19,20] and fused heterocyclic systems

Scheme



[21-23]. The reactions smoothly proceeded with 1.5 molar equivalent of DCCD in a boiling mixture of ethanol and benzene and gave the required 3-arylamino-6-phenyl-*s*-triazolo[3,4-*b*][1,3,4]thiadiazoles VIII-XII in high yields (Table II). The treatment of a mixture of equimolar amounts of the hydrazinothiadiazole (I) and *p*-tolyl, *p*-bromophenyl or *p*-chlorophenylisothiocyanate with 1.5 molar equivalent of DCCD, according to the one-pot cyclodesulfurization reaction conditions [18], produced the corresponding *s*-triazolo[3,4-*b*][1,3,4]thiadiazole derivatives in 51, 41 and 47% yield respectively. In contrast, all attempts to cyclodesulfurize 2-(*p*-bromophenyl)thiocarbamoylhydrazino-5-phenyl-1,3,4-thiadiazole (VI) into compound XI with mercuric(II) chloride [24-26] were fruitless.

The structure of the products was confirmed by elemental analyses and ir spectra (experimental section). In the ^{1}H-nmr spectra, three exchangeable singlets at 7.0-8.0, 9.72-10.1 and 10.1-10.93 ppm were respectively identified for N^4-, N^2- and N^1-protons of the thiosemicarbazide

Table I

Synthesized 2-Arylthiocarbamoylhydrazino-5-phenyl-1,3,4-thiadiazoles III-VII

Compound No.	Mp °C	Yield (%)	Molecular formula	Analysis, Calcd./Found % C	H	N	Inhibition Zone (mm) in P.G. [a] S	E	C	in DMF S	E	C
III	160-161	65	$C_{15}H_{13}N_5S_2$	55.04	4.00	21.40	16	—	12	20	18	22
				55.20	4.10	21.60						
IV	175-177	63	$C_{16}H_{15}N_5S_2$	56.30	4.43	20.52	16	—	12	15	16	20
				56.00	4.30	20.30						
V	172-174	75	$C_{16}H_{15}N_5OS_2$	53.78	4.23	19.60	16	—	13	15	17	19
				53.70	4.40	19.80						
VI	179-181	86	$C_{15}H_{12}BrN_5S_2$	44.33	2.95	17.24	18	—	13	16	16	19
				44.10	3.10	17.40						
VII	180-181	78	$C_{15}H_{12}ClN_5S_2$	49.79	3.31	19.36	19	—	13	16	16	22
				49.50	3.30	19.30						
							10	—	10	11	17	17 [b]

[a] Abbreviations: P.G. = propylene glycol, S = *Staphylococcus aureus*, E = *Escherichia coli*, C = *Candida albicans*. [b] Values for control.

Table II

Synthesized 3-Arylamino-6-phenyl-*s*-triazolo[3,4-*b*][1,3,4]thiadiazoles VIII-XII

Compound No.	Mp [a] °C	Yield (%)	Molecular formula	Analysis, Calcd./Found % C	H	N	Inhibition Zone (mm) in P.G. [b] S	E	C	in DMF S	E	C
VIII	205-207	82	$C_{15}H_{11}N_5S$	61.43	3.78	23.88	16	—	13	15	16	22
				61.70	4.10	24.10						
IX	282-283	63	$C_{16}H_{13}N_5S$	62.53	4.26	22.79	14	—	13	11	17	21
				62.50	4.40	22.80						
X	254-256	95	$C_{16}H_{13}N_5OS$	59.44	4.05	21.66	12	—	12	12	17	20
				59.60	4.00	21.80						
XI	329-330	66	$C_{15}H_{10}BrN_5S$	48.38	2.68	18.80	11	—	14	11	18	20
				48.70	2.90	19.10						
XII	319-320	74	$C_{15}H_{10}ClN_5S$	54.96	3.05	21.37	16	—	15	12	18	22
				55.00	3.40	21.50						
							10	—	10	11	17	17 [b]

[a] Compounds VIII and X were crystallized from benzene while compounds IX, XI and XII from chloroform-acetone mixture. [b] Abbreviations and control as in footnote 1, Table I.

moieties of compounds III-VII. The identification of the N-H protons of compounds VIII-XII could not be achieved due to insolubility in the common deuterated solvents. The products were *in vitro* evaluated for antibacterial properties against *Staphylococcus aureus* (NCTC 4163) and *Escherichia coli* (NCTC 5933) and antifungal activity against *Candida albicans* (NCTC 2708) using the agar diffusion method [27]. The results (Table I and II) indicated that the *p*-bromophenyl- (VI) and the *p*-chlorophenylthiosemicarbazide (VII) derivatives were the most active as compared with the remainder of the thiosemicarbazides, and the 3-(*p*-chlorophenyl)amino-6-phenyl-*s*-triazolo[3,4-*b*]-[1,3,4]thiadiazole (XII) which displayed only a moderate activity against *Staphylococcus aureus*. In the antifungal tests, the 3-*p*-bromophenylamino, XI, and 3-*p*-chlorophenylamino, XII, derivatives were found to be only moderately active relative to the remainder of the products which had almost no significant activities. None of the products showed any activity against *Escherichia coli* when tested as solutions in propylene glycol or dimethylformamide (Table I and II). The bacteriostatic and bactericidal potencies of compounds III, VI-VIII and XII, as determined by the serial dilution method [27], indicated that the 2-*p*-chlorophenyl)thiocarbamoylhydrazino-5-phenyl-1,3,4-thiadiazole (VII) possessed one fifth the antifungal activity of Nystatin while the other products tested were without significant activity.

EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. The ir spectra were measured as Nujol mulls on Beckman 4210 Spectrophotometer. The ^{1}H-nmr were recorded on Varian A60 (EM 360L) NMR Spectrophotometer using TMS as an internal reference.

2-Arylthiocarbamoylhydrazino-5-phenyl-1,3,4-thiadiazoles (III-VII). General Procedure.

A solution of 2-hydrazino-5-phenyl-1,3,4-thiadiazole (I) [15] (0.5 g, 2.6 mmoles) in hot ethanol (20 ml) was treated with the appropriate isothiocyanate derivatives (2.6 mmoles) and the mixture was left overnight at room temperature. The deposited crystals were filtered, washed with ethanol and dried. The yields, physical constants and analytical data of the products III-VII are listed in Table I; ir: ν 3290-3180, 3170-3050, 3100-3020 (NH), 1600-1515 (C=N and C=C), 1515-1500, 1465-1405, 1125-1115 and 970-920 cm^{-1} (NCS amide I, II, III and IV respectively).

6-Phenyl-*s*-triazolo[3,4-*b*][1,3,4]thiadiazole-2*H*-3-thione (II).

A mixture of equimolar amounts of 2-hydrazino-5-phenyl-1,3,4-thiadiazole (I) (0.5 g, 2.6 mmoles) and the arylisothiocyanate derivative (2.6 mmoles) in ethanol (25 ml) was heated under reflux for 1 hour. The final mixture was concentrated and the product deposited after cooling was crystallized from ethanol to give compound II as white shiny scales, mp 261-262°. Mixed melting points with a sample of II prepared from the hydrazine I, carbon disulfide and potassium hydroxide, as reported [15], showed no depression. The ir and ^{1}H-nmr spectra of compound II obtained from both sources were superimposable; ir: ν 3020 (NH), 1535 (C=N and C=C), 1505, 1465, 1005 and 940 cm^{-1} (NCS); ^{1}H-nmr (deuteriochloroform + DMSO-d_6): δ 7.59-7.80 and 7.91-8.15 (2m, 5, Ar-H) and 14.21 ppm (s, broad, 1, NH, exchangeable).

3-Arylamino-6-phenyl-*s*-triazolo[3,4-*b*][1,3,4]thiadiazoles (VIII-XII) through Cyclodesulfurization of the Thiosemicarbazides (III-VII) with DCCD. General Procedure.

A mixture of the thiosemicarbazide derivative III-VII (0.3 g) and 1.5 molar equivalent of DCCD in benzene (40 ml) was stirred at room temperature for 2 hours. Ethanol (10 ml) was added and the mixture was heated under reflux for 5 hours, concentrated and left overnight at room temperature. The deposited crystals were filtered and crystallized from the proper solvents. The yields, physical constants and analytical data of the products VIII-XII are given in Table II; ir: ν 3080-3010 (NH), 1635-1600 (C=N), 1600-1560 and 1565-1540 cm^{-1} (C=C).

One-pot Preparation of 3-*p*-Toluidino-6-phenyl-*s*-triazolo[3,4-*b*][1,3,4]-thiadiazole (IX).

A mixture of the hydrazine I (0.25 g, 1.3 mmoles) and the equivalent amount of *p*-tolylisothiocyanate in hot ethanol (25 ml) was stirred at room temperature for 30 minutes. DCCD (0.4 g, (1.94 mmoles) in benzene (15 ml) was added and the mixture was stirred at room temperature for 2 hours and then heatd under reflux for 6 hours, concentrated and left at room temperature overnight. The product was filtered and crystallized from a large volume of chloroform-acetone mixture to give compound IX, 51% yield, mp 282-283°. Similarly, compounds XI and XII were prepared in 41 and 47% yield respectively. Mixed melting point with the samples prepared from the above method showed no depression. The ir spectra obtained from both sources were superimposable.

Cyclodesulfurization of 2-*p*-Bromophenylthiocarbamoylhydrazino-5-phenyl-1,3,4-thiadiazole (VI) with Mercuric Chloride.

A mixture of compound VI (0.25 g, 0.61 mmoles) and three molar equivalents of mercuric chloride in chloroform (50 ml) was stirred while being heated under reflux for 72 hours. Chloroform was evaporated and after treating the residue to remove mercuric-contents [24], no cyclized product (XI) was obtained.

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