New Fungitoxic Fused-Ring Synthetics Incorporating Azoles and Azines in Different Combinations

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2-Aryl-3-thioureido-4-thiazolidinones **IIa,b** obtained by addition–condensation of aldehyde thiosemicarbazones **Ia,b** and mercaptoacetic acid underwent chemoselective intramolecular heterocyclizations to 5-aryl-2-mercapto-1,5-dihydrothiazolo[3,4-*b*]-1,2,4-triazoles **IIIa,b**, 2-amino-5-aryl-5-hydrothiazolo-[4,3-*b*]-1,3,4-oxadiazoles **IVa,b**, and the corresponding thiazolo[4,3-*b*]-1,3,4-thiadiazoles **Va,b** with NaOH, I_2/NaOH, and concentrated H_2SO_4, respectively. **III–V** were condensed with HCHO and α-amino acids to yield 7-aryl-2-(carboxyalkyl)thiazolo[3′,4′:2,3]-1,2,4-triazolo[5,4-*b*]-1,3,5-thiadiazines **VIa–d** and 7-aryl-3-(carboxyalkyl)thiazolo[3′,4′:3,2]-1,3,4-thia(oxa)diazolo[4,5-*a*]-1,3,5-triazines **VIIa–d** and **VIIIa–d**. Fungitoxicities of compounds **III–VIII** were evaluated in vitro against *Aspergillus niger* and *Fusarium oxysporium*. Some of the compounds displayed activities comparable with that of the commercial fungicide Dithane M-45. Structure–activity relationships for the screened compounds are discussed.

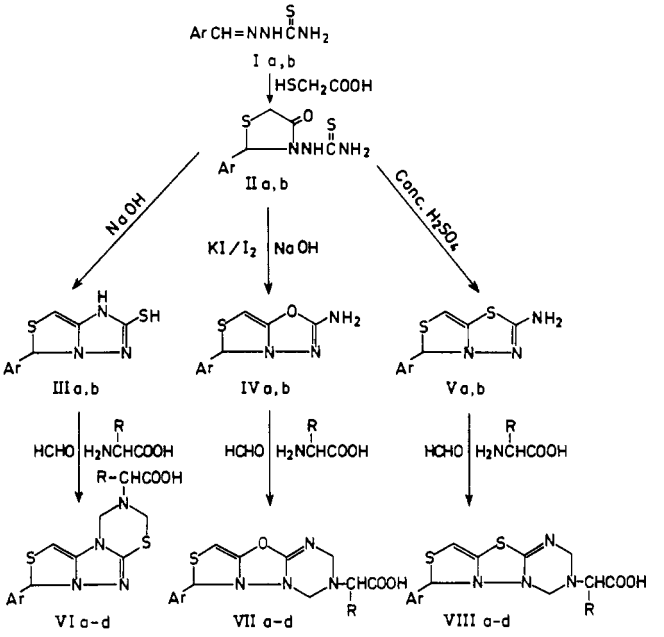
INTRODUCTION

Compounds bearing a thiazole structure as part of a fused-ring system have evoked considerable attention owing to its presence in the fused-ring system of penicillins. Various heterocyclic systems in which a thiazole nucleus is fused with 1,2,4-triazole, 1,3,4-oxa(thia)diazole, 1,3,5-thiadiazine, or 1,3,5-triazine structure have been reported to display significant antifungal activity (Kano et al., 1977; Morishima and Mizuno, 1977; Nippon Soda Co. Ltd., 1981; Paget, 1977; Sahu and Nayak, 1990; Singh et al., 1983, 1991, 1992).

In view of the above papers and our desire to develop new agricultural antifungal agents of high potency, the synthesis of hitherto unknown tricyclic fused-ring systems (**VI–VIII**) incorporating thiazole, 1,2,4-triazole, 1,3,4-oxa-(thia)diazole, 1,3,5-thiadiazine, and 1,3,5-triazine structures in different combinations was undertaken. Compounds **II–V** are also new ones, but these ring systems have already been reported (Singh et al., 1992). Further, all of the tricyclic compounds **VI–VIII** are unnatural α-amino acids; hence, it was thought that these might act as fatal antimetabolites for fungi because it is well-known that change in a single amino acid in a single protein can be fatal to organisms.

Compounds **III–V** have been synthesized from the same precursor **II** by three types of intramolecular chemoselective heterocyclizations as outlined in Scheme 1 (Singh et al., 1992). The requisite compounds **II** were obtained by addition–condensation of mercaptoacetic acid and aldehyde thiosemicarbazones **I** (Singh et al., 1992). All of the compounds gave satisfactory elemental analyses (C, H, and N). The structural assignments of the synthesized compounds were based on their elemental analyses and IR, ^{1}H NMR, and mass spectra (Table 1). Of the tested compounds **III–VIII**, compounds **VIIb–d** displayed in vitro fungitoxicity comparable with that of the commercial fungicide Dithane M-45 [a mixed manganous and zinc salt of *N,N′*-ethylenebis(dithiocarbamic acid)] at 1000 ppm concentration against *Aspergillus niger* and *Fusarium oxysporium* (Table 2).

Scheme 1



I–V : a, Ar = C_6H_5 ; b, Ar = 4-ClC_6H_4

VI–VIII : a, Ar = C_6H_5 ; R=H ; b, Ar = C_6H_5, R=Me ;
c, Ar = 4-ClC_6H_4, R=H ; d, Ar = 4-ClC_6H_4, R=Me

EXPERIMENTAL PROCEDURES

Melting points were determined by open glass capillary method and are uncorrected. IR spectra in KBr were recorded on a Perkin-Elmer 993 infrared spectrophotometer (ν_{max} cm^{-1}). ^{1}H NMR spectra were recorded on a Varian EM-360 (60 MHz) spectrometer in $CDCl_3$ plus DMSO-d_6 using TMS as an internal reference; chemical shifts are expressed in δ values. Mass spectra were recorded on a JEOL D-300 mass spectrometer.

2-Aryl-3-thioureido-4-thiazolidinones (IIa,b). To a well-stirred solution of an aldehyde thiosemicarbazone (0.05 mol) in dry benzene (50 mL) was added mercaptoacetic acid (0.075 mol). The reaction mixture was refluxed for 6 h on a water bath. The clear solution thus obtained was cooled and poured into water. The upper organic layer was separated, washed with aqueous sodium bicarbonate followed by water, and dried with anhydrous Na_2SO_4. Benzene was distilled off under reduced pressure, and the residue was recrystallized from ethanol to obtain the desired product.

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Table 1. Yields, Melting Points, and Spectral Data of Compounds II–VIII

compd	yield, %	mp, °C	IR (KBr) ν_{max},[a] cm^{-1}	^1H NMR ($CDCl_3$ + DMSO-d_6), δ	MS m/z (M^+)
IIa	80	170–172	1695	2.65 (2H, s, SCH_2CO), 6.68 (1H, s, SCHN), 7.27–7.30 (5H, m, ArH), 8.46–9.10 (3H, br s, NHC(=S)NH_2)	253
IIb	84	185–187	1700	2.67 (2H, s, SCH_2CO), 6.32 (1H, s, SCHN), 7.31–7.66 (4H, m, ArH), 8.48–9.16 (3H, br s, NHC(=S)NH_2)	287
IIIa	82	136–137	2570	3.58 (1H, s, SH), 6.24 (1H, s, SCHN), 7.02–7.82 (6H, m, ArH, =CHS)	235
IIIb	80	200–203	2575	3.61 (1H, s, SH), 6.26 (1H, s, SCHN), 7.03–7.88 (5H, m, ArH, =CHS)	269
IVa	76	148	3345, 3270	3.30 (2H, br s, NH_2), 6.27 (1H, s, SCHN), 7.04–7.80 (6H, m, ArH, =CHS)	219
IVb	78	160	3350, 3275	3.34 (2H, br s, NH_2), 6.30 (1H, s, SCHN), 7.06–8.10 (5H, m, ArH, =CHS)	253
Va	78	165	3350, 3365	3.25 (2H, br s, NH_2), 6.23 (1H, s, SCHN), 7.00–7.81 (6H, m, ArH, =CHS)	235
Vb	80	203–205	3355, 3370	3.28 (2H, br s, NH_2), 6.24 (1H, s, SCHN), 7.04–7.86 (5H, m, ArH, =CHS)	269
VIa	75	89–90	1710	4.24–4.65 (6H, m, 3 × CH_2), 6.26 (1H, s, SCHN), ArH, =CHS)	334
VIb	72	79–80	1705	1.52 (3H, d, J = 8, Me), 4.25–4.64 (5H, m, 2 × CH_2, COCH), 6.24 (1H, s, SCHN), 7.04–7.82 (6H, m, ArH, =CHS)	348
VIc	70	202–204	1710	4.25–4.67 (6H, m, 3 × CH_2), 6.29 (1H, s, SCHN), 7.05–7.96 (5H, m, ArH, =CHS)	368
VId	68	190–191	1705	1.54 (3H, d, J = 8, Me), 4.26–4.66 (5H, m, 2 × CH_2, COCH), 6.28 (1H, s, SCHN), 7.03–7.94 (5H, m, ArH, =CHS)	382
VIIa	74	103	1710	4.50–4.66 (6H, m, 3 × CH_2), 6.27 (1H, s, SCHN), 7.05–7.87 (6H, m, ArH, =CHS)	318
VIIb	73	94	1705	1.56 (3H, d, J = 8, Me), 4.54–4.68 (5H, m, 2 × CH_2, COCH), 6.23 (1H, s, SCHN), 7.04–7.88 (6H, m, ArH, =CHS)	332
VIIc	70	148	1710	4.52–4.67 (6H, m, 3 × CH_2), 6.30 (1H, s, SCHN), 7.07–8.10 (5H, m, ArH, =CHS)	352
VIId	71	153–154	1705	1.57 (3H, d, J = 8, Me), 4.56–4.69 (6H, m, 2 × CH_2, COCH), 7.06–8.98 (5H, m, ArH, =CHS)	366
VIIIa	80	90	1710	4.49–4.64 (6H, m, 3 × CH_2), 6.25 (1H, s, SCHN), 7.02–7.84 (6H, m, ArH, =CHS)	334
VIIIb	77	86–87	1705	1.54 (3H, d, J = 8, Me), 4.53–4.66 (5H, m, 2 × CH_2, COCH), 6.22 (1H, s, SCHN), 7.02–7.85 (6H, m, ArH, =CHS)	348
VIIIc	75	192–194	1710	4.50–4.63 (6H, m, 3 × CH_2), 6.29 (1H, s, SCHN), 7.04–7.94 (5H, m, ArH, =CHS)	368
VIIId	74	194–195	1705	1.55 (3H, d, J = 8, Me), 4.54–4.65 (5H, m, 2 × CH_2, COCH), 6.28 (1H, s, SCHN), 7.03–7.92 (5H, m, ArH, =CHS)	382

[a] II, VI, VII, VIII, $\nu_{C=O}$; III, ν_{SH}; IV, V, ν_{NH_2}.

5-Aryl-2-mercapto-1,5-dihydrothiazolo[3,4-*b*]-1,2,4-triazoles (IIIa,b). Compound **II** (0.01 mol) was dissolved in 33 mL of aqueous NaOH (4.4%) and ethanol (20 mL), and the reaction mixture was refluxed for 2 h. The resulting mixture was cooled to 0 °C and poured into water. On acidification with 5 N HCl the desired product was obtained, which was filtered, washed with water, and recrystallized from ethanol.

2-Amino-5-aryl-5-hydrothiazolo[4,3-*b*]-1,3,4-oxadiazoles (IVa,b). To a solution of **II** (0.01 mol) in ethanol (50 mL) was added 15% aqueous solution of NaOH followed by addition of iodine in KI solution (5%) till a permanent tinge of excess of iodine persisted. The reaction mixture was refluxed for 2 h. It was cooled to room temperature and poured into ice-cold water. The desired product obtained on basification with NH_4OH was filtered, washed with CS_2, and recrystallized from ethanol.

2-Amino-5-aryl-5-hydrothiazolo[4,3-*b*]-1,3,4-thiadiazoles (Va,b). These were prepared by treating compounds **IIa,b** (0.01 mol) dropwise with concentrated H_2SO_4 (5 mL) in an ice bath. After 30 min, the reaction mixture was poured into water. The product obtained on neutralization with liquor ammonia was filtered, washed with water, and recrystallized from ethanol.

7-Aryl-2-(carboxymethyl)(or carboxyethyl)thiazolo[3′,4′:2,3]-1,2,4-triazolo[5,4-*b*]-1,3,5-thiadiazines (VIa–d) and 7-Aryl-3-(carboxymethyl)(or carboxyethyl)thiazolo[3′,4′:3,2]-1,3,4-oxa(thia)diazolo[4,5-*a*]-1,3,5-triazines (VIIa–d and VIIIa–d). A solution of **III, IV,** or **V** (0.01 mol) in ethanol was refluxed with HCHO (0.015 mol) and concentrated HCl (0.01 mol) for 30 min. Then, the α-amino acid (glycine or alanine, 0.01 mol) was added, and the reaction mixture was refluxed for another 30 min. Finally, HCHO (0.015 mol) was added and refluxing was again carried on for a further 2.5 h. The desired precipitate was obtained by pouring the reaction mixture on crushed ice. It was filtered, washed with water, dried, and recrystallized from ethanol to obtain the analytical samples of **VI, VII,** or **VIII**, respectively.

Yields, melting points, and spectral data of compounds **II–VIII** are recorded in Table 1.

Antifungal Screening. In vitro antifungal activity of compounds **III–VIII** was evaluated against *A. niger* and *F. oxysporium* by poisoned food technique (Horsfall, 1945) at 1000, 100, and 10 ppm concentrations using Czapek's agar medium as described earlier (Yadav et al., 1989, 1991). A standard commercial fungicide, Dithane M-45, was also tested under similar conditions for comparison. No remarkable morphological change was observed in the developing fungi. The antifungal screening results are summarized in Table 2.

For the most active compounds **VIIb–d** it was ascertained whether these were fungistatic or fungicidal. Thus, following the procedure of Garber and Houston (1959), compounds **VIIb–d** were added separately to Czapek's agar medium in different Petri dishes to maintain the final concentrations at their respective lethal doses (1000, 950, and 800 ppm). The test fungi were inoculated in the center of these Petri dishes and incubated at 28 °C (±1 °C) for 96 h, after which time the percent inhibition of mycelial growth compared with that in control dishes was recorded. Then the fungal disks were taken from the treated and control dishes, washed with sterilized double-distilled water, and reinoculated in fresh Petri dishes containing Czapek's agar medium only. The plates were incubated for 96 h at 28 °C (±1 °C), and the percent inhibition was recorded. The number of replicate assays in each case was three, and six replicate controls were used. It was found that compounds **VIIb–d** caused complete inhibition of mycelial growth of the test fingi in treated as well as reinoculated dishes and hence were fungicidal.

RESULTS AND DISCUSSION

Chemoselectivity in the intramolecular cyclizations of the thiazolidinones **II** to the corresponding **III–V** may be rationalized on the basis of the "hard and soft acids and bases (*HSAB*) principle". In the case of cyclization of **II** with concentrated H_2SO_4, the hard proton protonates the hard carbonyl oxygen, leading to the formation of **IV** via intramolecular nucleophilic attack by the thionic S of the thioureido moiety. The cyclization of **II** to **V** involves the attack of I_2, a soft Lewis acid, on the soft thionic S of the thioureido function, followed by cyclization to **V** via nucleophilic attack by carbonyl oxygen. The reaction of NaOH with the thiazolidinones **II** furnishes N,S–ambident anion, the terminal nitrogen of which attacks the carbonyl carbon to yield the corresponding **III**.

The antifungal screening data (Table 2) indicate that all of the compounds were more active against *A. niger* than against *F. oxysporium*, but the difference was

Table 2. Antifungal Screening Results of Compounds III–VIII

compd	av % inhibition against *A. niger* at 1000 ppm	100 ppm	10 ppm	*F. oxysporium* at 1000 ppm	100 ppm	10 ppm
IIIa	55	36	12	53	35	11
IIIb	59	39	15	57	36	13
IVa	64	45	20	62	42	17
IVb	69	51	26	67	50	24
Va	58	40	15	56	37	13
Vb	62	45	19	60	42	17
VIa	63	41	20	61	38	18
VIb	69	47	24	67	46	21
VIc	90	66	44	88	64	42
VId	94	75	49	92	73	46
VIIa	84	64	40	82	61	39
VIIb	100	74	52	99	72	50
VIIc	100	76	55	100	74	53
VIId	100	80	56	100	78	54
VIIIa	73	52	29	71	50	28
VIIIb	78	55	33	76	53	30
VIIIc	90	71	46	89	69	44
VIIId	94	70	47	92	68	46
Dithane M-45	100	82	67	100	85	68

marginal. Most of the compounds showed significant activity at 1000 ppm against both of the fungal species, but their fungitoxicity decreased markedly on dilution. Of these, compounds **VIIb–d** exhibited fungitoxicity comparable with that of Dithane M-45 at 1000 ppm and inhibited 50–56% growth of both test fungi even at 10 ppm concentration.

The tricyclic fused-ring compounds **VI–VIII** were invariably more fungitoxic than their parent bicyclic fused-ring compounds **III–V**. Presumably, owing to the presence of an α-amino acid moiety, the former act as better antimetabolites for fungi than the latter. Among several combinations of ring-fused heterocycles, the compounds bearing the thiazole, 1,3,4-oxadiazole, and *s*-triazine nuclei fused together were most active.

Further, the presence of a chloro or methyl group in these compounds tends to augment their antifungal action. Presumably, this is due to the lipophilic character of the chloro and methyl groups, which favors the permeation of the compound through lipoid barriers in fungal cell membranes.

In conclusion, the new types of tricyclic ring-fused heterocycles reported herein might be useful for developing efficacious fungicides by optimizing the electronic and lipophilic properties of these compounds.

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