Synthesis of New Peptidyl Imidazodithi(and -thiadi)azoles as Potential Fungicides

Lal Dhar S. Yadav* and Supriya Shukla

Department of Chemistry, University of Allahabad, Allahabad 211 002, India

(4-Oxo-3-phenyl-2-thioxoimidazolidin-5-yl) *N*-aryldithiocarbamates **IVa,b** obtained by the reaction of 5-bromo-3-phenyl-2-thiohydantoin (**II**) and ammonium *N*-aryldithiocarbamates **IIIa,b** underwent chemoselective intramolecular heterocyclizations with iodine and $SOCl_2$ to yield 2-(arylimino)-6-phenyl-5-thioxoperhydroimidazo[1,5-*d*][1,3,4]dithiazole-7-thiones **Va,b** and 3,6-diaryl-2,5-dithioxoperhydroimidazo[5,1-*b*][1,3,4]thiadiazol-7-ones **VIa,b**, respectively. Compounds **Va,b** and **VIa,b** were converted into the corresponding 2- and 3-peptidyl derivatives **IXa–d** and **Xa–d**. Representative compounds **IXa,b** and **Xa,b** on dethio-oxygenation furnished the corresponding diones **XIa,b** and triones **XIIa,b**. Fungitoxicities of compounds **IV–VII** and **IX–XII** were evaluated in vitro against *Alternaria solani* and *Fusarium oxysporum*. Some of the compounds displayed activities comparable with that of the commercial fungicide Dithane M-45. Structure–activity relationships for the tested compounds are discussed.

Keywords: *Imidazodithi- and -thiadiazoles; peptidyl heterocycles; fungicides*

INTRODUCTION

Imidazoles have played an important role among a wide variety of nitrogen heterocycles that have been used for developing useful agrochemicals and pharmacological agents. For example, the most used fungicides for controlling a wide variety of fungal diseases include imidazole derivatives glyodin, climbazol, and imazalil and benzimidazole systemic fungicides, benlate, carbendazim, and furidazol. The antifungal compound resulting from the autoxidation of nabam has been shown to be 5,6-dihydroimidazo[2,1-*c*][1,2,4]dithiazole-3-thione (Beer et al., 1979).

Further, the application of peptides as carriers for toxic agents into cells has attracted considerable attention (Ames et al., 1973). A variety of microorganisms, including fungi, are known to have peptide transport systems which translocate di- and oligopeptides against a concentration gradient. Thus, peptides acting as carriers can deliver toxic agents into the cell, leading to high intracellular concentration which ultimately causes cell death (Fickel and Gilvarg, 1973; Payne, 1980).

In view of the above facts and with the hope of achieving efficacious fungicides possessing increased permeability into the fungal cell, a convenient synthesis of hitherto unreported title compounds **IX–XII** incorporating biolabile imidazole, 1,3,4-dithi(and -thiadi)azoles, and peptidyl moieties was devised.

The synthetic route to compounds **IXa–d** and **Xa–d** along with their dethianated products **XIa,b** and **XIIa,b** is outlined in Schemes 1 and 2. Dithiocarbamates **IVa,b** obtained by the reaction of ammonium *N*-aryldithiocarbamates **IIIa,b** and 5-bromo-3-phenyl-2-thiohydantoin (**II**) underwent chemoselective intramolecular heterocyclizations with iodine to yield 2-(arylimino)-6-phenyl-5-thioxoperhydroimidazo[1,5-*d*][1,3,4]thiadiazole-7-thiones **Va,b** and with thionyl chloride to yield 3,6-diaryl-2,5-dithioxoperhydroimidazo[5,1-*b*]-[1,3,4]thiadiazin-7-ones **VIa,b**. Compounds **Va,b** furnished their acid chlorides **VIIa,b** on treatment with thionyl chloride. The acid chlorides **VIIa,b** and **VIa,b**

Table 1. Analytical Data of Newly Prepared Candidate Fungicides IV–VII and IX–XII

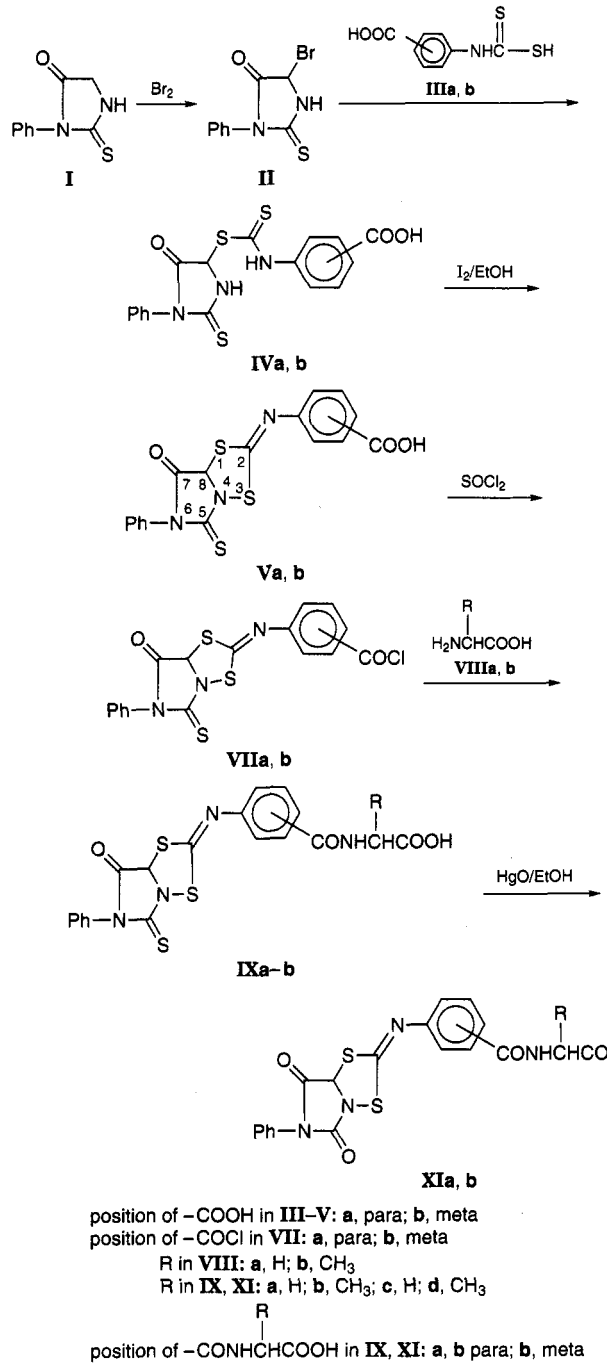
compd	yield (%)	mp (°C)	mol formula	found (calcd) (%) C	H	N
IVa	80	180–181	$C_{17}H_{13}N_3O_3S_3$	50.81 (50.62)	3.06 (3.23)	10.28 (10.42)
IVb	71	185–186	$C_{17}H_{13}N_3O_3S_3$	50.60 (50.62)	3.08 (3.23)	10.30 (10.42)
Va	76	190–191	$C_{17}H_{11}N_3O_3S_3$	50.66 (50.87)	2.56 (2.74)	10.29 (10.47)
Vb	73	192–193	$C_{17}H_{11}N_3O_3S_3$	50.69 (50.87)	2.79 (2.74)	10.39 (10.47)
VIa	68	200–203	$C_{17}H_{10}N_3O_2S_3Cl$	47.75 (47.97)	2.20 (2.39)	10.09 (10.02)
VIb	62	195–198	$C_{17}H_{10}N_3O_2S_3Cl$	47.88 (47.97)	2.30 (2.39)	10.11 (10.02)
VIIa	79	198–199	$C_{17}H_{10}N_3O_2S_3Cl$	47.76 (47.97)	2.23 (2.39)	9.99 (10.02)
VIIb	80	200–202	$C_{17}H_{10}N_3O_2S_3Cl$	47.92 (47.97)	2.29 (2.39)	10.00 (10.02)
IXa	78	292–295[a]	$C_{19}H_{14}N_4O_4S_3$	53.25 (53.14)	3.07 (3.15)	9.36 (9.46)
IXb	75	296–299[a]	$C_{20}H_{16}N_4O_4S_3$	52.22 (52.40)	3.56 (3.49)	9.02 (9.17)
IXc	76	>300	$C_{19}H_{14}N_4O_4S_3$	53.11 (53.14)	3.00 (3.15)	9.31 (9.46)
IXd	72	>300	$C_{20}H_{16}N_4O_4S_3$	52.24 (52.40)	3.29 (3.49)	9.01 (9.17)
Xa	75	290–293[a]	$C_{19}H_{14}N_4O_4S_3$	51.60 (51.52)	3.01 (3.17)	12.51 (12.67)
Xb	71	288–290[a]	$C_{20}H_{16}N_4O_4S_3$	52.55 (52.63)	3.60 (3.51)	12.11 (12.28)
Xc	73	281–283[a]	$C_{19}H_{14}N_4O_4S_3$	51.30 (51.52)	3.02 (3.17)	12.55 (12.67)
Xd	72	288–291[a]	$C_{20}H_{16}N_4O_4S_3$	52.01 (52.40)	3.30 (3.49)	9.23 (9.17)
XIa	79	223–225[a]	$C_{19}H_{14}N_4O_5S_2$	51.38 (51.52)	3.01 (3.17)	12.48 (12.67)
XIb	78	228–231[a]	$C_{20}H_{16}N_4O_5S_2$	52.53 (52.63)	3.70 (3.51)	12.18 (12.28)
XIIa	72	220–222[a]	$C_{19}H_{14}N_4O_6S$	53.41 (53.52	3.19 3.29	13.01 13.15)
XIIb	70	225–227[a]	$C_{20}H_{16}N_4O_6S$	54.70 (54.92)	3.51 (3.66)	12.65 (12.81)

[a] Melts with decomposition.

* Author to whom correspondence should be addressed.

0021-8561/95/1443-2526$09.00/0

Scheme 1



position of –COOH in **III–V**: **a**, para; **b**, meta
position of –COCl in **VII**: **a**, para; **b**, meta
R in **VIII**: **a**, H; **b**, CH_3
R in **IX**, **XI**: **a**, H; **b**, CH_3; **c**, H; **d**, CH_3

position of –CONHCHCOOH in **IX**, **XI**: **a**, **b** para; **b**, meta

reacted with α-amino acids (glycine and DL-alanine) to yield their 2- and 3-peptidyl derivatives **IXa–d** and **Xa–d**, respectively. The representative compounds **IXa,b** and **Xa,b** on dethio-oxygenation with mercuric oxide furnished their 2,7-dione and 2,5,7-trione analogues **XIa,b** and **XIIa,b**, respectively.

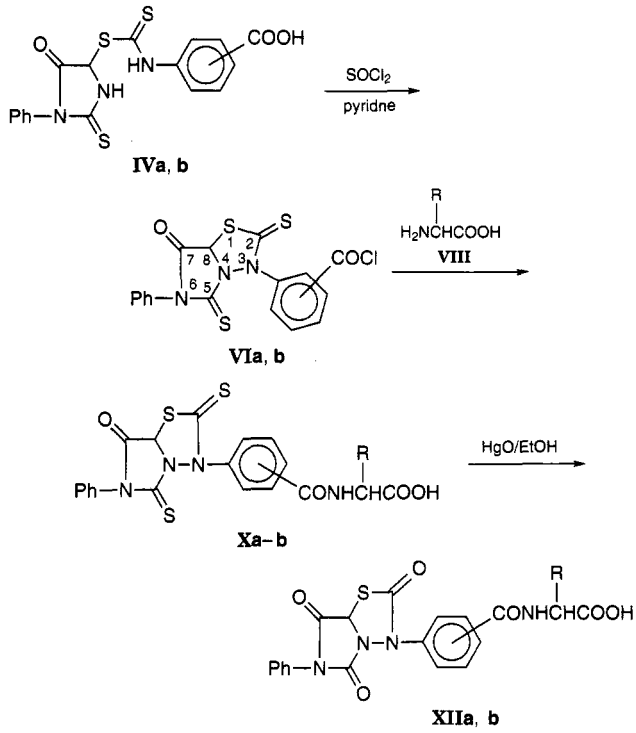
EXPERIMENTAL PROCEDURES

Melting points were determined by an open-glass capillary method and are uncorrected. IR spectra in KBr were recorded on a Perkin-Elmer 577 infrared spectrophotometer (ν_{max}, cm^{-1}). ^1H NMR spectra were recorded on a Varian EM-360 (60 MHz) spectrometer in TMS as internal reference; chemical shifts are expressed as δ values.

3-Phenyl-2-thiohydantoin (**I**) (Beilstein, 1954), its 5-bromo derivative **II**, and ammonium *N*-aryldithiocarbamates **IIIa,b** were prepared by known procedures (Vogel, 1956).

(4-Oxo-3-phenyl-2-thioxoimidazolidin-5-yl) *N*-Aryldithiocarbamates IVa,b. A mixture of **II** (0.05 mol),

Scheme 2



position of –COOH in **IV**: **a**, para; **b**, meta
position of –COCl in **VI**: **a**, para; **b**, meta
R in **VIII**: **a**, H; **b**, CH_3
R in **X**, **XII**: **a**, H; **b**, CH_3; **c**, H; **d**, CH_3

position of –CONHCHCOOH in **X**, **XII**: **a**, **b** para; **c**, **d**, meta

ammonium *N*-aryldithiocarbamate **III** (0.05 mol), and anhydrous sodium acetate was refluxed in absolute ethanol (150–175 mL) for 2 h. The reaction mixture was concentrated to about half of its volume, cooled, and poured into water. The desired product thus precipitated was washed with water and recrystallized from ethanol.

2-(Arylimino)-6-phenyl-6-thioxoperhydroimidazo[1,5-*d*][1,3,4]dithiazol-7-ones Va,b. Compounds **Va,b** (0.02 mol) in ethanol (50 mL) were treated with a saturated solution of iodine in ethanol:water (80:20 v/v at 30 °C) until decolorization of the iodine was no longer observed. On addition of NH_4OH to the reaction mixture, the products **V** precipitated and then recrystallized from ethanol as light yellow needles.

The compounds **Va,b** were converted into their acid chlorides **VIIa,b** by following the standard procedure (Vogel, 1978).

3,6-Diaryl-2,5-dithioxoperhydroimidazo[5,1-*b*][1,3,4]-thiadiazol-7-ones VIa,b. A solution of dithiocarbamates **IV** (0.02 mol) and thionyl chloride (0.05 mol) in pyridine (50 mL) was refluxed for 8 h. Pyridine was evaporated under reduced pressure, and the residue was washed with water and recrystallized from ethanol to furnish an analytical sample of **VI**.

2-Peptidyl-6-phenyl-5-thioxoperhydroimidazo[1,5-*d*]-[1,3,4]dithiazol-7-ones IXa–d and 3-Petidyl-6-phenyl-2,5-dithioxoperhydroimidazo[5,1-*d*][1,3,4]thiadiazol-7-ones Xa–d. Glycine (or DL-alanine) (0.005 mol) was dissolved in 10% aqueous NaOH solution (3.8 mL). To this solution was added an equimolar amount of **VII** (or **VI**) slowly with stirring. After the reaction mixture was allowed to stand for 15 min at room temperature, crushed ice was added, and the reaction mixture was acidified with concd HCl. The desired product was precipitated out and was recrystallized twice from ethanol as light brown needles.

Conversion of IXa,b and Xa,b into Their 5,7-Dione and 2,5,7-Trione Analogues XIa,b and XIIa,b, Respectively. It was performed by oxidative dethianation of **IXa,b** and **Xa,b** using HgO in ethanol (Silberg and Cosma, 1959). Thus, **IX** (0.005 mol) and HgO (0.011 mol) were refluxed in ethanol for 11 h. The precipitated HgS was filtered off, and the filtrate

Table 2. IR and ^{1}H NMR Spectral Data of Newly Prepared Candidate Fungicides IV–VII and IX–XII

compd	IR (KBr) ν_{max} (cm^{-1})	^{1}H NMR (DMSO-d_6) δ (J, Hz)
IVa	1701 (C=O)	5.48 (1H, s, SCH), 6.84–7.56 (9H, m, ArH), 8.24–8.89 (2H, br s, 2 × NH)
IVb	1705 (C=O)	5.50 (1H, s, SCH), 6.86–7.57 (9H, m, ArH), 8.26–9.00 (2H, br s, 2 × NH)
Va	1715 (C=O) 1680 (C=N)	5.51 (1H, s, SCH), 6.87–7.58 (9H, m, ArH)
Vb	1710 (C=O) 1675 (C=N)	5.53 (1H, s, SCH), 6.89–7.59 (9H, m, ArH)
VIa	1715 (C=O)	5.50 (1H, s, SCH), 6.85–7.58 (9H, m, ArH)
VIb	1710 (C=O)	5.52 (1H, s, SCH), 6.88–7.60 (9H, m, ArH)
VIIa	1710 (C=O) 1685 (C=N)	5.55 (1H, s, SCH), 6.87–7.59 (9H, m, ArH)
VIIb	1705 (C=O) 1680 (C=N)	5.54 (1H, s, SCH), 6.89–7.60 (9H, m, ArH)
IXa	1715 (C=O) 1680 (C=N)	4.32 (2H, s, CH_2), 5.52 (1H, s, SCH), 6.86–7.56 9H, m, ArH), 8.60 (1H, br s,NH)
IXb	1710 (C=O) 1675 (C=N)	1.54 (3H, d, J = 8, Me), 4.56 (1H, q, J = 8, Me CH), 5.50 (1H, s, SCH), 6.87–7.58 (9H, m, ArH), 8.58 (1H, br s, NH)
IXc	1710 (C=O) 1680 (C=N)	4.30 (2H, s, CH_2), 5.50 (1H, s, SCH), 6.84–7.53 (9H, m, ArH), 8.60 (1H, br s, NH)
IXd	1705 (C=O) 1675 (C=N)	1.52 (3H, d, J = 8, Me), 4.55 (1H, q, J = 8, MeCH), 5.51 (1H, s, SCH), 6.85–7.54 (9H, m, ArH), 8.62 (1H, br s, NH)
Xa	1715 (C=O)	4.34 (2H, s, CH_2), 4.58 (1H, s, SCH), 6.89–7.58 (9H, m, ArH), 8.59 (1H, br s, NH)
Xb	1710 (C=O)	1.55 (3H, d, J = 8, Me), 4.58 (1H, q, J = 8, MeCH), 5.53 (1H, s, SCH), 6.87–7.56 (9H, m, ArH), 8.60 (1H, br s, NH)
Xc	1715 (C=O)	4.33 (2H, s, CH_2), 4.57 (1H, s, SCH), 6.86–7.54 (9H, m, ArH), 8.61 (1H, br s, NH)
Xd	1710 (C=O)	1.54 (3H, d, J = 8, Me), 4.56 (1H, q, J = 8, MeCH), 5.52 (1H, s, SCH), 6.84–7.53 (9H, m, ArH), 8.60 (1H, br s, NH)
XIa	1720 (C=O) 1680 (C=N)	4.34 (2H, s, CH_2), 5.51 (1H, s, SCH), 6.88–7.57 (9H, m, ArH), 8.63 (1H, br s, NH)
XIb	1715 (C=O) 1765 (C=N)	1.53 (3H, d, J = 8, Me), 4.56 (1H, q, J = 8, MeCH), 5.53 (1H, s, SCH), 6.85–7.53 (9H, m, ArH), 8.64 (1H, br s, NH)
XIIa	1720 (C=O)	4.34 (2H, s, CH_2), (5.50, 1H, s, SCH), 6.86–7.55 (9H, m, ArH), 8.56 (1H, br s, NH)
XIIb	1715 (C=O)	1.52 (3H, d, J = 8, Me), 4.54 (1H, q, J = 8, MeCH), 5.51 (1H, s, SCH), 6.85–7.53 (9H, m, ArH), 8.57 (1H, br s, NH)

was concentrated and cooled to furnish **XI**, which was recrystallized from ethanol as yellow needles. **XII** was similarly prepared from **X** and recrystallized from ethanol.

Yields, melting points, molecular formulas, and elemental analyses of compounds **IV–VII** and **IX–XII** are recorded in Table 1 and spectral data in Table 2.

ANTIFUNGAL SCREENING

In vitro antifungal activity of compounds **IV–VII** and **IX–XII** was evaluated against *Alternaria solani* and *Fusarium oxysporum* 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. As indicated by microscopic analysis, there was no remarkable morphological change in the developing fungi except the mycelial growth or the lack of it. The antifungal screening results are summarized in Table 3.

Table 3. Antifungal Screening Results of Newly Prepared Candidate Fungicides IV–VII and IX–XII

	av % inhibition after 96 h against					
	A. solani at			*F. oxysporum* at		
compd	1000 ppm	100 ppm	10 ppm	1000 ppm	100 ppm	10 ppm
IVa	50	40	29	52	50	30
IVb	49	38	26	50	46	29
Va	57	48	30	59	53	32
Vb	53	44	28	54	47	28
VIa	68	53	35	68	55	38
VIb	63	50	31	66	52	34
VIIa	65	53	33	66	51	36
VIIb	59	48	30	62	59	30
IXa	80	65	40	83	63	49
IXb	79	60	45	81	60	45
IXc	85	64	50	80	66	50
IXd	84	61	48	80	65	46
Xa	95	71	51	93	69	47
Xb	92	68	50	89	65	46
Xc	100	78	55	100	72	56
Xd	100	74	52	99	69	54
XIa	75	60	45	79	60	48
XIb	72	56	42	78	59	44
XIIa	76	61	42	80	63	46
XIIb	74	57	41	78	60	45
Dithane M-45	100	80	65	100	83	69

For the most active compounds **Xc,d** it was ascertained whether these were fungistatic or fungicidal. Thus, following the procedures of Garber and Houston (1959), compounds **Xc,d** were added separately to Czapek's agar medium in different petri dishes to maintain the final concentrations (850 and 900 ppm) at their respective lethal doses (LD100). 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 **Xc,d** caused complete inhibition of mycelial growth of the test fungi in treated as well as reinoculated dishes and hence were fungicidal. Microscopic analysis revealed that there was no difference between fungistatic and fungicidal morphology.

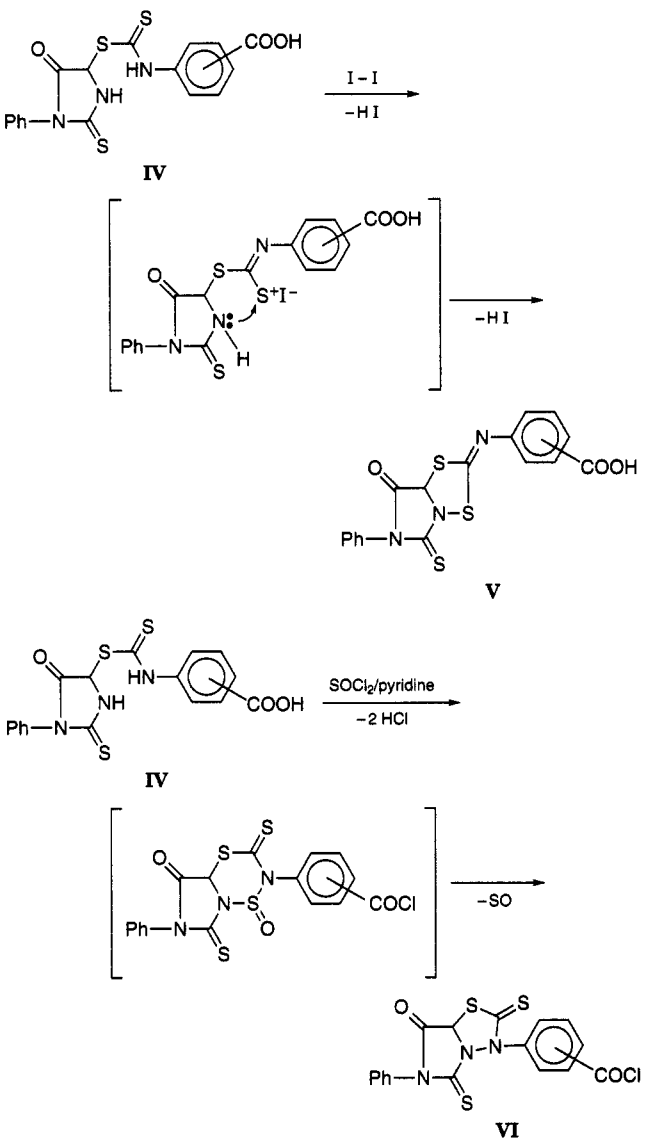
RESULTS AND DISCUSSION

The mechanism which appears to hold for the transformation of **IV** to **V** and **IV** to **VI** is outlined in Scheme 3. The N–N bond formation through the extrusion of SO from the cyclic intermediate is supported by the literature precedent (Barluenga et al., 1979).

The isomeric compounds **VI** and **VII** clearly differ in their IR spectra; **VII** exhibited a strong band attributable to $\nu_{C=N}$ around 1680 cm^{-1}, whereas compounds **VI** were devoid of this band. The ^{1}H NMR spectra of compounds **IX–XII** exhibited a broad singlet at δ 8.60 due to the CONH proton.

The representative compounds **IXa,b** and **Xa,b** were converted into their 5,7-dione and 2,5,7-trione analogues **XIa,b** and **XIIa,b**, respectively, by treatment with HgO. This conversion, involving oxidative dethianation of the exocyclic sulfur, provides chemical evidence for the

Scheme 3



assigned structure of the isomeric **VI** and **VII**, as their dethianated products **XI** and **XII** are not isomeric.

Results of the antifungal assay are summarized in Table 3. All the tested compounds displayed significant fungitoxicity at 1000 ppm against both fungal species. Compounds **Xc** and **Xd** exhibited fungitoxicity equivalent to that of Dithane M-45 at 1000 ppm concentration against both the test fungi and inhibited 52–56% growth of both fungal species even at 10 ppm. Compounds bearing a 1,3,4-thiadiazole or 1,3,4-dithiazole nucleus were found to be more active than their parent compounds. It was noted that 2,7-dione and 2,5,7-trione analogues were less potent than their precursors bearing both the >C=S and >C=O functions. This supports earlier observations that the combination of >C=O and >C=S functions sometimes works better than either alone and that the replacement of the carbonyl oxygen by sulphur enhances the fungicidal activity markedly (Rao and Mittra, 1977). It is noteworthy that the peptidyl derivatives **IX–XII** were invariably far more potent than their nonpeptidyl analogues **V–VII**. In general, the introduction of the peptide linkage at the meta position was more effective than that at the *para* position.

ACKNOWLEDGMENT

We sincerely thank Prof. H. P. Tiwari, Head of the Chemistry Department, Allahabad University, for providing laboratory facilities and RSIC, Lucknow, for recording elemental analyses and spectra. S.S. sincerely thanks UGC, New Delhi, for the award of a project assistantship.

LITERATURE CITED

Ames, B. N.; Ames, G. F.; Young, J. D.; Isuchiya, D.; Lecocq, J. Illicit transport: the oligopeptide permease. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 456–458.

Barluenga, J.; Lopez-Ortiz, J. F.; Gotor, V. Formation of a novel N–N bond through SO extrusion. Regioselective synthesis of 1,2,6-thiadiazine S-oxides and pyrazoles. *J. Chem. Soc., Chem. Commun.* **1979**, 891.

Beer, R. J. S.; Holmes, N. H.; Naylor, A. Novel heteropentalenes. Synthesis of the nabam oxidation product and related compounds. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2909–2913.

Beilstein Handbook of Organic Chemistry; Springer-Verlag: Berlin, 1954; E II 24, H24, 138.

Fickel, T. E.; Gilvarg, C. Transport of impermeant substances in *Escherichia coli* by way of oligopeptide permease. *Nature (London)* **1973**, *241*, 161–163.

Garber, R. H.; Houston, B. R. An inhibitor of *Verticillium alboatrum* in cotton seed. *Phytopathology* **1959**, *49*, 449–450.

Horsfall, J. G. Quantitative bioassay of fungicides in the laboratory. *Bot. Rev.* **1945**, *11*, 357–397.

Payne, J. W. Transport and utilization of peptides by bacteria. In *Microorganisms and Nitrogen Sources*; Payne, J. W., Ed.; Wiley: Chichester, U.K., 1980; Chapter 2.1, pp 211–256.

Rao, S.; Mittra, A. S. Synthesis and fungicidal activity of some 2,4-disubstituted thiazoles. *Indian J. Chem.* **1977**, *15B*, 1062–1063.

Silberg, A.; Cosma, N. On some additions to senevols. II. The addition of some arylhydrazides to phenylsenevol and the behaviour of the Products thus obtained. *Acad. Repub. Pop. Rom. Fil. Cluj. Stud. Cerect. Chim.* **1959**, *10*, 151.

Vogel, A. I. *A Text Book of Practical Organic Chemistry Including Qualitative Organic Analysis*, 3rd ed.; Longmans: London, 1956; p 643.

Vogel, A. I. *Vogel's Textbook of Practical Organic Chemistry*, 4th ed.; Longmans: Harlow, England, 1978; p 498.

Yadav, L. D. S.; Misra, A. R.; Singh, H. Synthesis of new 7*H*-1,3,4-thiadiazolo[3,2-a][1,3,5]triazine-7-thiones as potential fungicides. *Pestic. Sci.* **1989**, *25*, 219–225.

Yadav, L. D. S.; Tripathi, R. K.; Dwivedi, R.; Singh, H. Syntheses of new 1,3,4-oxadiazolo[3,2-d]-1,3,4-dithiazines and 1,3,4-oxadiazolo[3,2-d]thiadiazines with fungicidal action. *J. Agric. Food. Chem.* **1991**, *39*, 1863–1865.

Received for review October 3, 1994. Accepted June 12, 1995.[⊗]

JF940554J

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1995.