Efficacy of Organophosphorus Derivatives against Fungal Pathogens of Sugarcane

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Several novel oraganophosphorus derivatives have been prepared by the reactions of *O,O*-diethyl chlorophosphate/thiophosphate with three important series of heterocyclic compounds, viz., bis(mercaptotriazoles), bis(mercaptooxadiazoles), and bis(mercaptothiadiazoles). The derivatives have been characterized on the basis of analyses and spectral (IR, ^{1}H NMR, and ^{31}P NMR) data. The fungicidal activity of these derivatives against *Colletotrichum falcatum*, *Fusarium oxysporum*, and *Curvularia pallescens* have been evaluated. The screening results have been correlated with the structural features of the tested compounds. The greater potency has been observed with dithiophosphates compared to phosphates, with organophosphorus derivatives containing bis(mercaptotriazole) rings compared to other heterocyclic rings, and with long spacers between the rings.

Keywords: *Synthesis; organophosphorus derivatives; fungal pathogens; sugarcane*

Sugarcane is known to have fungal, bacterial, viral, and phytoplasma pathogens that are responsible for considerable economic losses (Ricaud et al., 1989). Among fungi, *Colletotrichum falcatum* (causing red rot), *Fusarium oxysporum* and *Fusarium moniliforme* (causing wilt disease), and *Curvularia pallescens* (causing leaf spot) are responsible for enormous losses to the sugar industry (Agnihotri, 1990; Rao et al., 1991). A number of synthetic organic compounds, viz., dithiocarbamates, carbamates, organochlorine, organomercurial, thiocarbamates, chalcones, and hydrazides, are now known to be useful in the control of various fungal diseases in plants (Ricaud et al., 1984; Gruzdyaev et al., 1983; Gunther, 1983; Rao et al., 1994). However, studies on organic derivatives of phosphorus as fungicides and herbicides are meager. Studies on organophosphorus derivatives could constitute a new and promising field of application in the Indian national economy (Grapov and Mel'nikov, 1973). One of the useful properties of phosphorus compounds is their relatively low stability and rapid metabolic breakdown in plants, in animal organisms, in soil, and in other components of the environment with the formation of products that are safe for human beings and domestic animals. Another important feature of these compounds is the high selectivity of their action (Fest and Schmidt, 1982; Westheimer, 1992; Chaturvedi et al., 1994, 1995a,b). The discovery of the mechanism of action of organophosphorus compounds made it possible to develop the fundamental principles of the directed synthesis of new substances and to establish the cause of their selective action on an organism. It was realized that on the basis of suitable logic, organic molecules incorporating phosphorus may be designed such that they may be less dangerous in use without losing their values as effective pesticides.

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The present study was therefore undertaken to evaluate the antifungal efficacy of some newly synthesized organophosphorus compounds against various important fungal pathogens of sugarcane.

RESULTS AND DISCUSSION

Three series of organophosphorus derivatives have been synthesized, which are given in Scheme 1.

The analytical data and physical properties of all organophosphorus derivatives are given in Table 1. The methods used for the preparation and isolation of these compounds give materials of good purity as supported by their analyses and TLC. The spectral (IR, ^{1}H NMR, and ^{31}P NMR) data are given in Table 2.

Antifungal Activity. Results of the antifungal assay of the starting materials [bis(mercaptotriazoles), bis(mercaptothiadiazoles), and bis(mercaptooxadiazoles)] and their corresponding organophosphorus derivatives are summarized in Tables 3 and 4, respectively. Thirty newly synthesized organophosphorus compounds containing bis(mercaptotriazoles), bis(mercaptooxadiazoles), and bis(mercaptothiadiazoles) were screened for their antifungal properties against *Co. falcatum*, *F. oxysporum*, and *Cu. pallescens* (all parasitic on sugarcane). The antifungal activity of organophosphorus derivatives was found to be greater than that of the corresponding starting materials. *O,O*-Diethyl thiophosphate derivatives containing bis(mercaptotriazoles) exhibit absolute inhibition against all test fungi at 1000 ppm concentration. The compound containing 1,2-bis(5-mercapto-1,3,4-triazole-2-yl)ethane (**Ig**) was proved 100% antifungal against *Co. falcatum* even at 100 ppm concentration. These compounds showed superiority over the commercial fungicides Bavistin, Blitox 50, Topsin M, and Dithane M-45 during the present study. The compounds were up to 4 times more active than the tested commercial fungicides (Table 5), which are being used in sugarcane fungal disease management. Other derivatives displayed different levels of mycelial

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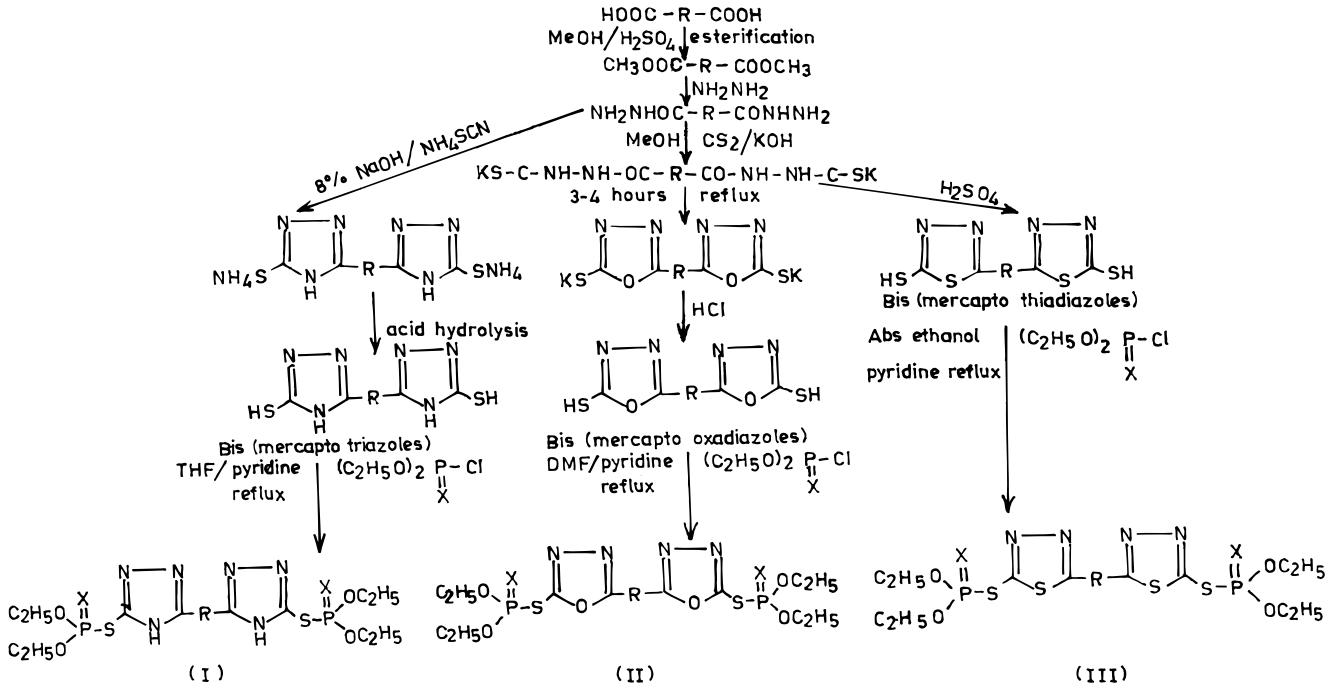
Scheme 1. Synthetic Routes of Organophosphorus Derivatives[a]



[a] **Ia**, **IIa**, and **IIIa**, X = O, R = nil; **Ib**, **IIb**, and **IIIb**, X = O, R = $(CH_2)_2$; **Ic**, **IIc**, and **IIIc**, X = O, R = $(CH_2)_4$; **Id**, **IId**, and **IIId**, X = O, R = $(CHOH)_2$; **Ie**, **IIe**, and **IIIe**, X = O, R = (C_6H_4); **If**, **IIf**, and **IIIf**, X = S, R = nil; **Ig**, **IIg**, and **IIIg**, X = S, R = $(CH_2)_2$; **Ih**, **IIh**, and **IIIh**, X = S, R = $(CH_2)_4$; **Ii**, **IIi**, **IIIi**, X = S, R = $(CHOH)_2$; **Ij**, **IIj**, and **IIIj**, X = S, R = (C_6H_4).

Table 1. Analytical Data for Organophosphorus Compounds

compd	yield (%)	color	mp (°C)	found (calcd) (%) C	H	N	S
Ia	50	white	320	30.3 (30.5)	4.5 (4.7)	17.7 (17.8)	13.5 (13.6)
Ib	46	brown	282	33.3 (33.6)	5.1 (5.2)	16.7 (16.8)	12.7 (12.8)
Ic	52	brown	280	36.2 (36.4)	5.6 (5.7)	15.8 (15.9)	12.0 (12.1)
Id	48	white	290	31.4 (31.6)	4.7 (4.9)	15.7 (15.8)	12.0 (12.0)
Ie	65	white	275	39.3 (39.4)	4.6 (4.7)	12.2 (12.3)	11.5 (11.7)
If	70	brown	235	28.4 (28.6)	4.2 (4.4)	16.6 (16.7)	25.2 (25.4)
Ig	61	brown	225	31.5 (31.6)	4.8 (4.9)	15.7 (15.8)	24.1 (24.1)
Ih	64	brown	185	34.3 (34.3)	5.2 (5.4)	15.0 (15.0)	22.7 (22.7)
Ii	60	brown	135	29.7 (29.8)	4.4 (4.6)	14.7 (14.9)	22.6 (22.7)
Ij	68	brown	210	37.1 (37.2)	4.4 (4.5)	14.4 (14.5)	22.0 (22.1)
IIa	60	white	290	30.2 (30.3)	4.1 (4.2)	11.7 (11.8)	13.3 (13.5)
IIb	68	brown	250	33.4 (33.5)	4.7 (4.8)	11.1 (11.1)	12.8 (12.8)
IIc	62	brown	220	36.1 (31.2)	5.2 (5.3)	10.5 (10.6)	12.1 (12.1)
IId	58	brown	210	31.4 (31.5)	4.4 (4.5)	10.4 (10.5)	11.7 (11.9)
IIe	68	white	280	39.1 (39.3)	4.3 (4.4)	10.1 (10.2)	11.6 (11.6)
IIf	68	white	200	28.3 (28.5)	3.9 (3.9)	11.0 (11.1)	25.2 (25.3)
IIe	70	brown	232	31.3 (31.4)	4.5 (4.4)	10.3 (10.4)	23.9 (24.0)
IIh	60	yellow	240	34.1 (34.2)	5.0 (5.0)	9.8 (9.9)	22.8 (22.8)
IIi	62	yellow	265	29.5 (29.7)	4.1 (4.2)	9.7 (9.9)	22.5 (22.6)
IIj	70	white	225	37.0 (37.1)	4.0 (4.0)	9.5 (9.6)	21.9 (21.9)
IIIa	59	white	280	28.3 (28.5)	3.9 (4.0)	11.0 (11.1)	25.2 (25.3)
IIIb	62	white	255	31.4 (31.4)	4.4 (4.5)	10.2 (10.4)	23.9 (24.0)
IIIc	57	brown	260	34.1 (34.2)	4.9 (4.9)	9.8 (9.9)	22.6 (22.8)
IIId	60	brown	265	29.6 (29.7)	4.1 (4.2)	9.9 (9.9)	22.5 (22.6)
IIIe	70	white	290	37.0 (37.1)	4.0 (4.1)	9.4 (9.6)	21.9 (21.9)
IIIf	65	yellow	170	26.7 (26.8)	3.6 (3.7)	10.4 (10.4)	35.7 (35.7)
IIIg	68	brown	215	29.5 (29.7)	4.2 (4.3)	9.7 (9.9)	35.9 (35.9)
IIIh	60	white	200	32.1 (32.3)	4.5 (4.7)	9.4 (9.4)	32.3 (32.3)
IIIi	56	white	230	28.0 (28.1)	4.0 (4.0)	9.2 (9.4)	32.0 (32.1)
IIIj	72	white	172	35.0 (35.2)	3.8 (3.9)	9.0 (9.1)	31.3 (31.3)

inhibition of the test fungi at different concentration. The minimum fungal inhibition property was recorded with *O*,*O*-diethyl phosphate derivative containing bis(mercaptooxadiazole) derived from tartaric acid (**IId**).

Screening Data Conclusions. (1) There was significant alteration in the antifungal activity with the change in the nature of the heterocyclic ring. For any particular species of fungus, organophosphorus derivatives containing bis(mercaptotriazoles) are found to be more effective as compared to derivatives containing bis(mercaptothiadiazoles), which in turn show better activity than the derivatives containing bis(mercaptooxadiazoles). (2) For any particular species of fungus, thiophosphate derivatives show better activity than phosphate derivatives. This indicates that the presence of sulfur directly attached to phosphorus imparts better

Table 2. Spectral Data of Compounds

	IR (cm^{-1})			^1H NMR (δ, ppm)			^{31}P
compd	ν(C=N)	ν(P−S−C)	ν(P=X)	NH	CH_2	CH_3	NMR
Ia	1580 s	620 m	1180 m	9.50 s	3.25 q	2.50 t	−65 s
Ib	1570 s	600 m	1220 m	9.40 s	2.90 q	2.25 t	−70 s
Ic	1565 s	590 s	1200 m	9.45 s	2.90 q	2.30 t	−52 s
Id	1560 s	625 s	1175 m	9.48 s	3.40 q	2.50 t	−68 s
Ie	1575 s	600 m	1190 m	9.40 s	3.25 q	2.35 t	−60 s
If	1578 s	600 m	700 s	9.50 s	3.30 q	2.40 t	−90 s
Ig	1580 s	610 s	690 s	9.40 s	2.95 q	2.32 t	−95 s
Ih	1570 s	600 m	700 m	9.45 s	2.90 q	2.28 t	−80 s
Ii	1555 s	595 s	700 m	9.48 s	3.10 q	2.35 t	−100 s
Ij	1560 s	600 s	685 m	9.52 s	3.35 q	2.45 t	−85 s
IIa	1550 s	615 s	1200 m		3.10 q	2.30 t	−60 s
IIb	1555 s	610 s	1210 m		3.15 q	2.28 t	−52 s
IIc	1560 s	620 s	1220 m		3.25 q	2.30 t	−65 s
IId	1560 s	590 s	1200 m		2.95 q	2.28 t	−70 s
IIe	1570 s	610 s	1185 m		3.00 q	2.35 t	−75 s
IIf	1572 s	600 s	700 m		3.25 q	2.48 t	−100 s
IIg	1570 s	590 s	685 m		3.40 q	2.42 t	−82 s
IIh	1560 s	600 s	690 s		3.35 q	2.40 t	−105 s
IIi	1580 s	610 s	690 s		3.00 q	2.28 t	−95 s
IIj	1565 s	615 s	700 s		3.25 q	2.28 t	−95 s
IIIa	1550 s	620 m	1220 m		3.40 q	2.45 t	−55 s
IIIb	1560 s	600 s	1200 m		3.38 q	2.45 t	−58 s
IIIc	1550 s	610 m	1215 m		3.38 q	2.45 t	−60 s
IIId	1580 s	615 m	1190 m		3.28 q	2.35 t	−65 s
IIIe	1570 s	620 m	1200 m		3.32 q	2.38 t	−50 s
IIIf	1565 s	620 m	700 m		3.00 q	2.58 t	−100 s
IIIg	1560 s	600 m	710 m		3.10 q	2.60 t	−98 s
IIIh	1550 s	600 m	680 s		3.35 q	2.65 t	−95 s
IIIi	1570 s	600 s	685 s		3.18 q	2.50 t	−80 s
IIIj	1575 s	610 m	700 m		3.20 q	2.62 t	−85 s

Table 3. Antifungal Activity of Bis(mercaptotriazoles), Bis(mercaptothiadiazoles), and Bis(mercaptooxadiazoles)

	% mycelial inhibition								
	Co. falcatum			*F. oxysporum*			*Cu. pallescens*		
compd	10 ppm[a]	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm
(A) bis(mercaptotriazoles)									
(a) R = nil	11.5	28.6	60.7	10.2	36.5	54.2	9.8	22.6	62.8
(b) R = $(CH_2)_2$	15.4	29.8	59.2	14.2	45.8	56.7	10.1	28.7	66.9
(c) R = $(CH_2)_4$	18.4	32.8	61.5	14.2	40.7	65.2	18.1	38.6	67.2
(d) R = $(CHOH)_2$	8.1	18.2	52.3	9.1	25.6	49.1	6.2	20.8	50.3
(e) R = C_6H_4	19.5	39.6	62.8	19.2	48.1	56.2	22.1	42.8	67.2
(B) bis(mercaptooxadiazoles)									
(a) R = nil	8.3	22.8	48.7	7.5	30.7	39.2	5.1	20.8	60.2
(b) R = $(CH_2)_2$	11.1	27.1	55.2	10.5	42.8	52.6	7.2	24.1	66.2
(c) R = $(CH_2)_4$	16.3	30.1	60.2	10.6	40.2	59.1	11.2	29.8	64.2
(d) R = $(CHOH)_2$	4.2	12.4	39.2	2.6	9.8	30.6	4.2	12.6	35.2
(e) R = C_6H_4	16.6	32.2	60.3	14.2	35.8	50.2	17.2	38.8	60.1
(C) bis(mercaptothiadiazoles)									
(a) R = nil	8.7	24.6	52.1	8.1	31.8	52.1	5.6	21.2	62.1
(b) R = $(CH_2)_2$	13.6	28.1	56.6	12.9	44.6	53.8	9.1	26.1	66.2
(c) R = $(CH_2)_4$	18.2	33.8	61.0	12.6	40.6	61.6	12.1	30.7	65.8
(d) R = $(CHOH)_2$	7.1	16.2	48.1	6.1	19.1	40.6	5.1	20.6	48.6
(e) R = C_6H_4	18.6	34.8	62.0	16.8	41.0	56.9	20.6	40.6	66.6

[a] Compound dose.

inhibition against fungi. (3) As the number of $-CH_2-$ groups (R) between two heterocyclic rings increases, activity also increases.

EXPERIMENTAL PROCEDURES

The reactions of *O,O*-diethyl chlorophosphate/thiophosphate were carried out under inert atmosphere and anhydrous conditions. Special precautions were taken to exclude moisture from the apparatus and chemicals as the starting materials (*O,O*-diethyl chlorophosphate or *O,O*-diethyl chlorothiophosphate) and reactions were susceptible to hydrolysis. Glass apparatus with interchangeable joints were used throughout the work. The solvents were purified and dried using the method described in the literature (Vogel, 1956). *O,O*-Diethyl chlorophosphate and *O,O*-diethyl chlorothiophosphate were prepared according to the reported method (Chaturvedi, 1993). Bis(mercaptotriazoles), bis(mercaptooxadiazoles), and bis(mercaptothiadiazoles) were prepared as described (Chaturvedi, 1993). All reactions were carried out in the hood. A hood is a specially constructed workplace that has, at the least, a powered vent to suck noxious fumes outside. The details of analyses and physical measurements were the same as reported eariler (Chaturvedi et al., 1994, 1995a,b).

For antifungal activity, all of the compounds were tested against all of the test fungi by the food poison technique (Grover and Moore, 1962) at three concentrations (10, 100, and 1000 ppm). For this, the desired amount of chemical was dissolved in 0.5 cm^3 of solvent and mixed with the culture medium, on the basis of the the volume of medium in each Petri plate (80 mm diameter). Oatmeal−agar medium (Johnston and Booth, 1983) was used for all test fungi. In

Table 4. Antifungal Activity of Newly Synthesized Organophosphorus Compounds against Sugarcane Fungal Pathogens

	% mycelial inhibition								
	Co. falcatum			*F. oxysporum*			*Cu. pallescens*		
compd	10 ppm[a]	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm
Ia	17.2	41.7	75.5	14.2	50.6	68.7	12.3	41.4	85.2
Ib	26.8	46.7	78.6	18.5	55.2	70.5	14.2	44.8	86.4
Ic	30.2	50.5	80.4	22.8	59.5	78.6	22.5	50.6	88.2
Id	14.2	35.5	68.6	13.6	40.2	58.5	10.1	32.6	68.2
Ie	30.2	50.5	80.5	26.8	62.8	75.6	30.2	50.2	85.6
If	48.5	72.8	100.0[b]	40.5	58.6	100.0[b]	38.4	60.2	100.0[b]
Ig	48.9	100.0[b]	100.0[b]	40.8	70.2	100.0[b]	42.8	68.8	100.0[b]
Ih	52.5	86.6	100.0[b]	48.2	75.6	100.0[b]	50.2	72.4	100.0[b]
Ii	28.4	50.5	100.0[b]	25.5	51.8	100.0[b]	20.8	48.8	100.0[b]
Ij	38.4	60.0	100.0[b]	35.4	67.0	100.0[b]	38.1	55.6	100.0[b]
IIa	14.6	35.6	65.2	11.6	46.0	58.6	9.7	37.6	80.5
IIb	18.7	38.6	70.4	15.8	50.0	62.6	10.5	40.2	82.5
IIc	24.8	42.8	75.4	16.2	54.8	70.5	16.2	44.3	84.1
IId	9.6	25.0	55.2	3.0	15.0	42.8	8.4	20.6	50.7
IIe	26.5	46.2	82.8	22.5	56.2	70.8	26.2	50.6	80.2
IIf	40.2	56.7	88.5	30.6	50.8	72.3	20.8	42.5	82.2
IIg	41.8	64.8	90.8	31.8	60.7	78.6	29.5	58.5	86.3
IIh	46.2	75.2	92.6	40.8	65.6	81.6	42.4	65.5	90.2
IIi	20.0	30.8	58.1	14.2	30.0	48.6	12.6	30.5	56.0
IIj	28.5	46.2	65.0	28.8	50.2	62.1	20.3	40.3	67.4
IIIa	15.6	38.2	68.5	12.8	48.2	60.8	10.2	39.8	82.8
IIIb	21.9	40.2	75.4	16.6	53.8	67.6	12.8	42.6	84.3
IIIc	28.5	45.6	78.2	18.4	56.2	72.8	20.6	46.8	85.2
IIId	11.2	30.6	60.5	10.2	30.8	50.6	9.8	28.2	60.5
IIIe	30.8	48.5	78.6	26.4	54.2	74.6	28.5	45.8	82.8
IIIf	44.6	68.5	90.2	32.8	54.8	86.8	30.2	55.8	90.5
IIIg	45.2	78.2	94.2	32.9	66.6	80.5	38.9	60.6	90.8
IIIh	48.8	80.4	96.6	45.6	72.8	84.6	46.2	68.5	95.6
IIIi	26.4	42.7	66.5	22.6	44.0	56.2	18.6	38.5	62.8
IIIj	30.2	52.0	72.8	31.2	59.6	70.5	29.2	49.6	74.1

[a] Compound dose. [b] Complete mycelial inhibition.

Table 5. Efficacy of Organophosphorus Derivatives Compared with Synthetic Fungicides against Sugarcane Pathogens

		MIC (ppm) against		
common name of fungicide/chemical	trade name	*Co. falcatum*	*F. oxysporum*	*Cu. pallescens*
carbendazim	Bavistin	4000	3000	4000
copper oxychloride	Blitox 50	2000	2000	2000
mancozeb	Dithane M-45	4000	3000	4000
thiophanate methyl	Topsin M	4000	4000	4000
Ig		1000	1000	1000
Ih		1000	1000	1000
Ii		1000	1000	1000
Ij		1000	1000	1000

controls, the same amount of medium containing the requisite amount of solvent was poured in place of text chemicals. A mycelial disk (5 mm diameter) obtained from the periphery of 2-week-old cultures was taken and transferred to the center of each Petri plate. Plates were incubated for 7 days at 28 ± 2 °C. Each treatment was repeated three times, and the inhibition was recorded relative to percent mycelial inhibition calculated using the formula

$$[(dC - dT)/dC] \times 100$$

where dC is the average diameter of the mycelial colony of the control and dT is the average diameter of the mycelial colony of the treatment.

The minimum inhibitory concentration (MIC) of all of the *O*,*O*-diethyl thiophosphate derivatives containing bis(mercaptotriazoles) was determined by poison food technique. Four concentrations, 500, 1000, 2000, and 3000 ppm of each test compound with respect to the culture medium, were prepared. The fungistatic/fungicidal natures of the active chemicals were determined in three replicates against the test fungi following the procedure of Garbour and Houston (1959). This was done by observing if revival of growth of the inhibited mycelial disks occurred following transfer to a chemical-free medium.

Reactions of *O*,*O*-Diethyl Chlorophosphate/Thiophosphate with Bis(mercaptotriazoles). A mixture of *O*,*O*-diethyl chlorophosphate/thiophosphate (0.02 mol) and the appropriate bis(mercaptotriazole) (0.01 mol) was refluxed in THF (~30 cm^3) in the presence of pyridine (1 cm^3) for 30−60 h. The reaction mixture was cooled and poured in ice. The product, thus obtained, was crystallized from alcohol.

Reactions of *O*,*O*-Diethyl Chlorophosphate/Thiophosphate with Bis(mercaptooxadiazoles). *O*,*O*-Diethyl chlorophosphate/thiophosphate (0.02 mol) was added to a solution of the appropriate bis(mercaptooxadiazole) (0.01 mol) in DMF (~30 cm^3) in the presence of pyridine (1 cm^3), and the mixture was refluxed for 35−72 h. The reaction mixture was then cooled and poured in ice. The product, thus obtained, was recrystallized from THF/hexane (1:1).

Reactions of *O*,*O*-Diethyl Chlorophosphate/Thiophosphate with Bis(mercaptothiadiazoles). The appropriate bis(mercaptothiadiazole) (0.01 mol) was dissolved in absolute ethanol (~30 cm^3 containing 1 cm^3 of pyridine), and to this was added *O*,*O*-diethyl chlorophosphate/thiophosphate (0.02 mol). The mixture was refluxed for ~35−70 h under inert atmosphere. The reaction mixture was cooled and poured in ice. The solid product, thus obtained, was recrystallized from acetone.

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