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Ring Transformation of Michael Adducts of 4-Benzylidene-5-oxazolones and 3-Mercapto-*s*-triazoles to 2,3-Dihydro-4*H*-*s*-triazolo[3,4-*b*][1,3]thiazin-4-ones with Some Antifungal Activity

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Michael addition of 3-mercapto-*s*-triazoles Ia–e to 4-benzylidene-5-oxazolones IIa,b followed by ring transformation yielded a novel class of compounds, the 2,3-dihydro-2,6-diaryl-3-benzamido-4*H*-*s*-triazolo[3,4-*b*][1,3]thiazin-4-ones IVa–j, in one pot. The compounds IVa–j were compared with Dithane M-45, a commercial fungicide, for their antifungal activity against *Aspergillus niger* and *Fusarium oxysporium*, and the results have been correlated with the structural features of the tested compounds.

Among the most widely used antibiotics, cephalosporins essentially contain the 1,3-thiazine nucleus. Likewise, the *s*-triazole ring is also associated with various useful pesticidal activities (Greenfield et al., 1970; Okano and Yasujaga, 1970; Reisser, 1969; Bucchel and Draber, 1971). In view of these facts and with the hope of achieving antifungal compounds of high potency, we have fused the biolabile 1,3-thiazine and *s*-triazole nuclei to probe how this combination could enhance the antifungal action. Further, all of these compounds possess a fluoroaryl moiety, which might be expected to enhance their antifungal activity (Filler and Kobayashi, 1983). The investigation appeared quite interesting as the 2,3-dihydro-4*H*-*s*-triazolo[3,4-*b*][1,3]thiazin-4-ones IVa–j reported here constitute a hitherto unknown class of nitrogen-bridged heterocyclic compounds.

3-Mercapto-*s*-triazoles Ia–e were refluxed with 4-benzylidene-5-oxazolones IIa,b in dioxane for 2 h to furnish

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Table I. Analytical Data for IVa–j

compd IV	yield, %	mp, °C	formula	found (calcd), % C	H	N
a	78	228–230	$C_{24}H_{17}FN_4O_2S$	64.92 (64.86)	3.71 (3.82)	12.82 (12.61)
b	86	133–135	$C_{24}H_{16}ClFN_4O_2S$	60.38 (60.18)	3.42 (3.34)	11.61 (11.70)
c	83	160–161	$C_{26}H_{16}ClFN_4O_2S$	60.26 (60.18)	3.36 (3.34)	11.82 (11.70)
d	81	136–137	$C_{25}H_{19}FN_4O_2S$	65.63 (65.50)	4.34 (4.14)	12.14 (12.22)
e	82	131–132	$C_{25}H_{18}ClFN_4O_3S$	59.12 (58.99)	3.45 (3.53)	11.31 (11.11)
f	80	165–166	$C_{24}H_{17}FN_4O_2S$	65.00 (64.86)	3.92 (3.82)	12.71 (12.61)
g	84	158–160	$C_{24}H_{16}ClFN_4O_2S$	60.29 (60.18)	3.36 (3.34)	11.90 (11.70)
h	82	186–187	$C_{24}H_{16}ClFN_4O_2S$	60.32 (60.18)	3.44 (3.34)	11.77 (11.70)
i	81	174–175	$C_{25}H_{19}FN_4O_2S$	65.70 (65.50)	4.24 (4.14)	12.34 (12.22)
j	85	152–153	$C_{25}H_{18}ClFN_4O_3S$	58.82 (58.99)	3.62 (3.53)	11.22 (11.11)

Table II. Spectral Data of the Compounds IVa–j

compd IV	IR (KBr) $\nu_{C=O}$, cm^{-1} thiazinone	amido	^1H NMR ($CDCl_3$) δ (J, Hz)	MS/M^+, m/z
a	1660	1635	3.94 (1 H, d, J = 5, 2-H), 4.82 (1 H, dd, J = 5, 8, 3-H), 7.24–8.22 (14 H, m, aromatic H), 8.60 (1 H, br s, NH)	444
b	1665	1640	3.98 (1 H, d, J = 5, 2-H), 4.84 (1 H, dd, J = 5, 8, 3-H), 7.30–8.25 (13 H, m, aromatic H), 8.62 (1 H, br s, NH)	478, 480
c	1665	1640	3.96 (1 H, d, J = 5, 2-H), 4.82 (1 H, dd, J = 5, 8, 3-H), 7.30–8.24 (13 H, m, aromatic H), 8.62 (1 H, br s, NH)	478, 480
d	1655	1630	2.42 (3 H, s, CH_3), 3.90 (1 H, d, J = 5, 2-H), 4.80 (1 H, dd, J = 5, 8, 3-H), 7.24–8.22 (13 H, m, aromatic H), 8.60 (1 H, br s, NH)	458
e	1660	1635	3.92 (1 H, d, J = 5, 2-H), 4.80 (1 H, dd, J = 5, 8, 3-H), 5.28 (2 H, s, OCH_2), 7.24–8.20 (13 H, m, aromatic H), 8.58 (1 H, br s, NH)	508, 510
f	1660	1635	3.92 (1 H, d, J = 5, 2-H), 4.82 (1 H, dd, J = 5, 8, 3-H), 7.22–8.20 (14 H, m, aromatic H), 8.60 (1 H, br s, NH)	444
g	1665	1640	3.96 (1 H, d, J = 5, 2-H), 4.84 (1 H, dd, J = 5, 8, 3-H), 7.28–8.25 (13 H, m, aromatic H), 8.62 (1 H, br s, NH)	478, 480
h	1665	1640	3.96 (1 H, d, J = 5, 2-H), 4.82 (1 H, dd, J = 5, 8, 3-H), 7.30–8.22 (13 H, m, aromatic H), 8.62 (1 H, br s, NH)	478, 480
i	1655	1630	(2.40 (3 H, s, CH_3), 3.90 (1 H, d, J = 5, 2-H), 4.80 (1 H, dd, J = 5, 8, 3-H), 7.22–8.20 (13 H, m, aromatic H), 8.58 (1 H, br s, NH)	458
j	1660	1635	3.90 (1 H, d, J = 5, 2-H), 4.80 (1 H, dd, J = 5, 8, H-3), 5.26 (2 H, s, OCH2), 7.22–8.20 (13 H, m, aromatic H), 8.60 (1 H, br s, NH)	508–510

Table III. Antifungal Screening Results of Compounds IIIa and IVa–j

compd	av % inhibn after 96 h against *A. niger* 1000 ppm	100 ppm	10 ppm	*F. oxysporium* 1000 ppm	100 ppm	10 ppm
IIIa	58	28	16	56	35	20
IVa	80	49	39	82	52	40
IVb	98	65	51	99	66	52
IVc	96	63	48	98	63	50
IVd	75	48	30	79	50	35
IVe	87	61	40	83	60	42
IVf	78	44	22	81	45	31
IVg	97	61	46	95	60	45
IVh	89	61	43	88	59	40
IVi	70	40	21	67	42	29
IVj	84	52	35	82	46	32
Dithane M-45[a]	100	81	67	100	85	68

[a] Commercial fungicide.

the novel compounds IVa–j in 78–86% yields. The starting materials, triazoles Ia–e, were prepared by intramolecular cyclization of the corresponding 1-acylthiosemicarbazides with alkali (Hoggarth, 1949), and the 4-benzylidene-5-oxazolones IIa,b were obtained from hippuric acid and *o*- or *p*-fluorobenzaldehyde following the standard procedure (Vogel, 1956).

All the synthesized compounds were well characterized by their elemental analyses and IR, ^1H NMR, and mass spectra (Tables I and II). In the IR spectra of compounds

IVa–j, thiazinone and aminocarbonyl absorptions appeared around 1660 and 1635 cm^{-1}, respectively, instead of the carbonyl band at 1800 cm^{-1} in the starting oxazolones IIa–b. The ^1H NMR spectra of compounds IVa–j, in addition to other signals, revealed a characteristic doublet for the C-2 proton at about δ 4.08 and double doublet for the C-3 proton near δ 5.04.

Of the 10 tested compounds (IVa–j) compounds IVb, c, and g displayed antifungal activity of the order of Dithane M-45 (a commercial fungicide) at 1000 ppm against *Aspergillus niger* and *Fusarium oxysporium* (Table III).

EXPERIMENTAL SECTION

All melting points were determined in open-glass capillaries and are uncorrected. IR spectra in KBr were recorded on a Perkin-Elmer 157 infrared spectrophotometer. ^1H NMR spectra were recorded on a EM-360L (60-MHz) NMR spectrometer in $CDCl_3$ using TMS as an internal reference; chemical shifts are expressed in δ. Mass spectra were recorded on a JEOL JMS-D 300 instrument.

5-Aryl- and 5-[(Aryloxy)methyl]-3-mercapto-*s*-triazoles Ia–e. These were prepared according to the method of Hoggarth (1949), which involves the intramolecular cyclization of the corresponding 1-acylthiosemicarbazides with alkali. Compounds Ia–d agreed well with analytical data already reported in the literature (Hoggarth, 1949; Balse and Mahajanshetti, 1980).

Ie: mp 253–254 °C (EtOH); yield 72%. Anal. Calcd for $C_9H_8ClN_3OS$: C, 44.72; H, 3.31; N, 17.39. Found: C, 44.61; H, 3.33; N, 17.18.

4-(*o*- or *p*-Fluorobenzylidene)-2-phenyl-5-oxazolone (IIa,b). Following the standard procedure (Vogel, 1956) hippuric acid was treated with *o*- or *p*-fluorobenzaldehyde in Ac_2O to furnish II.

IIa: mp 174–175 °C (benzene); yield 75%; IR (KBr) 1800 cm^{-1} ($\nu_{C=O}$). Anal. Calcd for $C_{16}H_{10}FNO_2$: C, 71.91; H, 3.74; N, 5.24. Found: C, 71.80; H, 3.72; N, 5.0.

IIb: mp 151–152 °C (benzene); yield 78%; IR (KBr) 1800 cm^{-1} ($\nu_{C=O}$). Anal. Calcd for $C_{16}H_{10}FNO_2$: C, 71.91; H, 3.74; N, 5.24. Found: C, 72.02; H, 3.61; N, 5.11.

2,3-Dihydro-2,6-diaryl-3-benzamido-4*H*-*s*-triazolo[3,4-*b*][1,3]thiazin-4-ones IVa–j. An equimolar mixture of *s*-triazole I and oxazolone II was dissolved in a minimum amount of dioxane, and the solution was refluxed for 3 h. The reaction mixture was cooled and poured into water. The yellowish precipitate thus obtained was washed with water and recrystallized from benzene to afford light yellow needles of the desired products IVa–j. Analytical data are recorded in Table I. The spectral data are given in Table II.

Antifungal Screening. The pure cultures of the test fungi (*A. niger*, *F. oxysporium*), the pathogenicity of which was already verified, were obtained from the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, Delhi. Agar (bacteriological grade) supplied by Sarabhai M. Chemicals was used as such. Compounds IIIa and IVa–j were screened by the agar plate technique (Horsfall, 1945) using Czapek's agar medium.

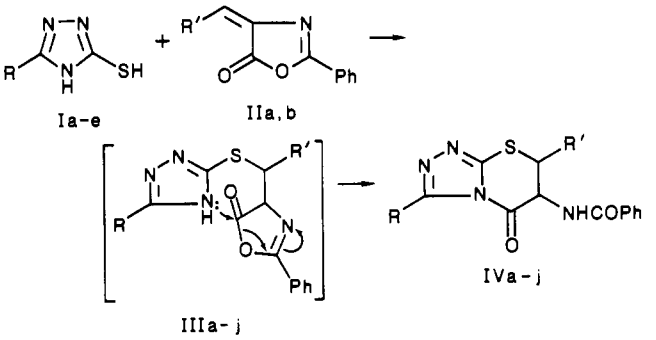
Suspensions of different concentrations of each compound, viz. 10000, 1000, and 100 ppm, were prepared in an acetone–water (20:80, v/v) mixture. One milliliter of each concentration of the test compound was added separately to presterilized Petri plates containing 9 mL of the sterilized Czapek's agar medium to maintain the final concentrations of 1000, 100, and 10 ppm. The compound was thoroughly mixed with the medium by rotating the plates on table top, thus swirling the contents. A fungal disk of 5-mm diameter, cut out with the help of a sterilized cork borer from the periphery of 1-week-old culture of the test fungus already planted on the Czapek's medium, was inoculated in the center of each Petri plate in inverted position to bring the mycelia in direct contact with the medium. Petri plates containing 9 mL of Czapek's medium and 1 mL of acetone–water (20:80, v/v) mixture served as controls. The number of replicate assays in each case was three, whereas six replications of the controls were provided. The plates were incubated at 28 °C (±1 °C) for 96 h. No remarkable morphological change was observed in the developing fungi. After 96 h, four diameters of the fungal colony, intersecting one another at about 45°, were measured with a millimeter scale, and percent inhibition of mycelial growth was calculated by

$$\% \text{ inhibn} = \frac{(C - T) \times 100}{C}$$

where C = average diameter of fungal colony (mm) in control plates and T = average diameter of fungal colony (mm) in treated plates.

Dithane M-45, a standard commercial fungicide, was also tested under similar conditions for comparison. The antifungal activity displayed by the screened compounds is summarized in Table III. The most active compounds IVb, c, and g were also assayed at 5, 30, 50, 70, 400, 700, 1200, and 1400 ppm, besides 1000, 100, and 10 ppm. However, the plotting of probits of percent inhibition against log doses, for the representative compounds IVb, c, and g gave points that could not be fit adequately to linear dose–inhibition curves.

Scheme I[a]



[a] R′: IIa, IIIa–e, IVa–e, 2-FC_6H_4; IIb, IIIf–j, IVf–j, 4-FC_6H_4. R: a and f, C_6H_5; b and g, 2-ClC_6H_4; c and h, 4-ClC_6H_4; d and i, 3-MeC_6H_4, e and j, 4-$ClC_6H_4OCH_2$.

RESULTS AND DISCUSSION

The formation of *s*-triazolothiazinones IVa–j involves the Michael addition of 2-mercapto-*s*-triazoles Ia–e to 4-benzylidene-5-oxazolones IIa,b to afford the Michael adducts IIIa–j, which undergo intramolecular nucleophilic attack of the nitrogen atom of the triazole ring (N-4) at the carbonyl carbon (C-5) of the oxazolone nucleus with the simultaneous cleavage of the oxazolone ring to yield IVa–j (Scheme I). This conclusion was based on the observation that compound IIIa could be isolated in 45% yield when the reaction of Ia and IIa was carried out in dioxane at 40–50 °C for 1 h.

IIIa: mp 163 °C dec; IR 1780 cm^{-1} ($\nu_{C=O}$); ^1H NMR δ 4.02 (1 H, d, J = 5 Hz, SCH), 5.00 (1 H, d, J = 5 Hz, 4-H oxazolone ring), 6.88–8.20 (14 H, m, aromatic H), 9.88 (1 H, br s, NH). Anal. Calcd for $C_{24}H_{17}FN_4O_2S$: C, 64.86; H, 3.82; N, 12.61. Found: C, 64.67; H, 3.80; N, 12.42. Compound IIIa was converted into IVa quantitatively by refluxing in dioxane for 1.5 h.

Results of the antifungal assay are summarized in Table III. All the tested compounds IVa–j displayed significant antifungal activity at 1000 ppm against both the fungal

species, but their activity decreased markedly at lower concentrations (100 and 10 ppm). Compounds IVb, C, and g exhibited antifungal activity of the order of Dithane M-45 (a commercial fungicide) at 1000 ppm and inhibited 45–52% growth of both the test fungi even at 10 ppm. However, compound IIIa was far less active than its successor IVa.

Perusal of the screening data (Table III) clearly indicates that there was significant alteration in the antifungal activity with the change in the relative positions of the substituents on phenyl ring. For example, compounds IV bearing the *o*-fluoro group were more active than the corresponding IV with the *p*-fluoro group. Similarly, the 2-chloro group was more effective than the 4-chloro group.

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Registry No. Ie, 113056-45-4; IIa, 397-60-4; IIb, 449-81-0; IIIa, 113056-46-5; IVa, 113056-47-6; IVb, 113056-48-7; IVc, 113056-49-8; IVd, 113086-35-4; IVe, 113056-50-1; IVf, 113056-51-2; IVg, 113056-52-3; IVh, 113056-53-4; IVi, 113056-54-5; IVj, 113086-36-5.

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Deltamethrin Residues in an Organic Soil under Laboratory Conditions and Its Degradation by a Bacterial Strain[1]

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An organic soil was treated with the insecticide deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl *cis*-(1*R*,3*R*)-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropanecarboxylate] labeled with ^{14}C at the methyl position at a level of 10 mg/kg for a laboratory incubation study. At the end of a 40-month incubation period the extractable and nonextractable (bound) ^{14}C residues amounted to 19.5% and 16.3%, respectively, of the initially added ^{14}C. The bound residues were characterized as the parent compound and its metabolite [3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid]. The microbial population including bacteria, actinomycetes, and fungi decreased considerably from the initial numbers. A bacterial species capable of utilizing deltamethrin as a sole source of carbon was isolated by enrichment from the incubated soil.

In recent years, the synthetic pyrethroid deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl *cis*-(1*R*,3*R*)-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropanecarboxylate] has become of great interest for use in crop protection because it is very effective in controlling a wide range of insects in agriculture at very low application rates (FAO, 1981). The increased use of this chemical for application to vegetable and field crops requires periodic assessment of its residues behavior and fate in soil. Recent studies have indicated a half-life of deltamethrin in mineral soils in the range of 1–8 weeks (Chapman et al., 1981; Miyamoto and Mikami, 1983; Hill, 1983), and the degradation appears to be mainly mediated by soil microorganisms (Kaufman et al., 1981; Chapman et al., 1981). In organic soils, the degradation of deltamethrin was found to be slower under the anaerobic than aerobic conditions (Zhang et al., 1984). A half-life of deltamethrin of 72 days was observed under aerobic conditions in an organic soil (Zhang et al., 1984).

It has been suggested that pyrethroids or their metabolites will not persist for lengthy periods in soil (Miyamoto and Mikami, 1983). Buildup of bound (nonextractable) residues has been observed to take place in the short term in soil (Roberts, 1981), but bound residues are considered to be degraded further (Roberts, 1981; Miyamoto and Mikami, 1983). Previously we reported a steady increase in the formation of bound ^{14}C residues over a 6-month incubation period of an organic soil treated with radiolabeled deltamethrin (Zhang et al., 1984). It was also observed that the bacteria and actinomycetes number increased by a factor of approximately 4, whereas the fungal population remained relatively unaffected (Zhang et al., 1984). The present work extends these investigations to determine the fate of deltamethrin in the organic soil in

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