Hetero Diels–Alder Synthesis and Fungitoxicity of New 1,3,4-Thiadiazolo[3,2-*a*]-*s*-triazine-5(*H*)-thiones

Lal Dhar S. Yadav,* Sangeeta Sharma, and Anjum Vaish

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

Hetero Diels–Alder synthesis involving conjugated azomethines, 5-(arylideneamino)-2-mercapto-1,3,4-thiadiazoles **IIa–c**, as dienes (azadienes) and aryl isothiocyanates as dienophiles affords 2,6,7-trisubstituted 6,7-dihydro-1,3,4-thiadiazolo[3,2-*a*]-*s*-triazine-5(*H*)-thiones **IIIa–f**. The azomethines **IIa–c** on ethylation followed by hetero Diels–Alder reaction furnish 6,7-diaryl-2-(ethylthio)-6,7-dihydro-1,3,4-thiadiazolo[3,2-*a*]-*s*-triazine-5(*H*)-thiones **IVa–f**. Fungitoxicities of the compounds **II–IV** were evaluated in vitro aginst *Aspergillus niger* and *Fusarium oxysporum*. 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

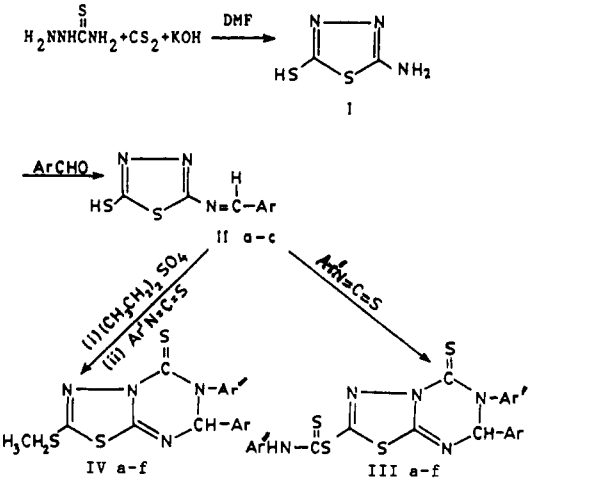
A large number of dithiocarbamates have been investigated for their fungitoxicity (Spencer, 1963; Thorn and Ludwig, 1962), and many of these, viz. maneb, zineb, Dithane M-45, ziram, thiram, and vapam, have attained major recognition as agricultural fungicides. Compounds in which the dithiocarbamate grouping constitutes part of a heterocyclic framework have been relatively less studied, although some of these compounds are known to display useful pesticidal properties. For example, rhodanines are inherently toxic to microorganisms, especially to fungi and bacteria (Brown et al., 1961), and mylone is used as soil sterilant for controlling soil fungi, nematodes, weeds, and insects. Similarly, many *s*-triazine derivatives, viz. simazine, atrazine, prometryn, and anilazine, have attained significance in agriculture as herbicides and fungicides (Kearney and Kaufman, 1975; Lukens, 1971).

Prompted by the above observations and appreciable fungitoxicity exhibited by some 1,3,4-thiadiazolo[3,2-*a*]-*s*-triazine derivatives bearing no dithiocarbamate grouping (Yadav et al., 1989), we have synthesized the title compounds incorporating the dithiocarbamate moiety, especially as a component of a fused-ring heterocyclic system, to probe how far this combination could work for antifungal efficacy. The investigation was quite interesting because compounds **III** and **IV** are hitherto unreported and accessible through a facile hetero Diels–Alder synthesis using compounds **II**, which are also new ones.

The reaction sequence leading to the formation of **III** and **IV** is outlined in Scheme 1. In the synthesis of compounds **III** and **IV**, aryl isothiocyanates act as dienophiles and the conjugated azomethines **IIa–c** as dienes (azadienes). This reaction is an interesting example of hetero Diels–Alder synthesis as reported earlier (Arbuzov and Zobova, 1966; Weinreb and Levin, 1979; Yadav et al., 1988). The required 5-amino-2-mercapto-1,3,4-thiadiazole (**I**) was prepared by addition–cyclocondensation of thiosemicarbazide and carbon disulfide (Guha, 1922). Condensation of compound **I** with aromatic aldehydes afforded the required azomethines **IIa–c**.

The structural assignments of the synthesized compounds were based on their elemental analyses (Table 1) and IR, ^{1}H NMR, and mass spectra (Table 2). Of the tested compounds **IIa–c** and **IIIa–f**, the compounds **IVb**, **IVe**, and **IVf** displayed in vitro fungicidal activity 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 oxysporum*.

Scheme 1



IIa, a, Ar=C_6H_5; b, Ar = p-ClC_6H_4; c, Ar = m-$NO_2C_6H_4$

III,IV a, Ar=C_6H_5, Ar′=C_6H_5; b, Ar = p-ClC_6H_4; Ar′=C_6H_5
c, Ar=m-$NO_2C_6H_4$, Ar′=C_6H_5; d, Ar=C_6H_5, Ar′=o-MeC_6H_4;
e, Ar=p-ClC_6H_4; Ar′=o-MeC_6H_4; f, Ar=m-$NO_2C_6H_4$;
Ar′=o-MeC_6H_4

EXPERIMENTAL PROCEDURES

Melting points were determined by open glass capillary method and are uncorrected. IR spectra in KBr were recorded on a Perkin-Elmer 577 infrared spectrophotometer (ν_{max}, cm^{-1}). ^{1}H NMR spectra were recorded on a Perkin-Elmer R-32 (90-MHz) spectrometer in $CDCl_3$ plus DMSO-d_6 (1:1 by volume) using TMS as internal reference; chemical shifts are expressed in δ. Mass spectra were recorded on a JEOL 300 mass spectrometer. Carbon disulfide is flammable and toxic, and aryl isothiocyanates are corrosive and toxic; hence, they should be handled with care.

5-(Arylideneamino)-2-mercapto-1,3,4-thiadiazoles (IIa–c). A mixture of 5-amino-2-mercapto-1,3,4-thiadiazole (0.02 mol) and appropriate aldehyde (0.02 mol) in absolute ethanol (75 mL) was refluxed for 5 h, and the solution was filtered while hot. The filtrate was reduced to half of its volume and cooled to furnish crystals of the desired product. Yields, melting points, molecular

* Author to whom correspondence should be addressed.

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Table 1. Analytical Data of Compounds II–IV

compd.	yield, %	MP, °C	mol formula	found (calcd), % C	H	N
IIa	81	152–154	$C_9H_7N_3S_2$	48.62 (48.86	3.09 3.18	19.01 18.99)
IIb	83	204–205	$C_9H_6ClN_3S_2$	40.61 (40.75	2.30 2.28	15.72 15.84)
IIc	78	115–117	$C_9H_6N_4O_2S_2$	40.38 (40.59	2.15 2.27	21.29 21.04)
IIIa	82	236–238	$C_{23}H_{17}N_5S_4$	55.76 (56.09	3.41 3.45	14.43 14.22)
IIIb	83	193	$C_{23}H_{16}ClN_5S_4$	52.79 (52.50	3.15 3.06	13.50 13.31)
IIIc	78	215–216	$C_{23}H_{16}N_6O_2S_4$	51.21 (51.47	2.84 3.00	15.47 15.66)
IIId	73	235	$C_{25}H_{21}N_5S_4$	57.52 (57.77	4.02 4.07	13.30 13.47)
IIIe	71	207–208	$C_{25}H_{20}ClN_5S_4$	54.40 (54.18	3.65 3.63	12.58 12.63)
IIIf	68	218–220	$C_{25}H_{20}N_6O_2S_4$	53.02 (53.16	3.48 3.56	14.68 14.88)
IVa	80	190–191	$C_{18}H_{16}N_4S_3$	56.08 (56.21	4.20 4.19	14.38 14.57)
IVb	81	201–202	$C_{18}H_{15}ClN_4S_3$	51.08 (51.10	3.79 3.57	13.08 13.24)
IVc	78	186–187	$C_{18}H_{15}N_5O_2S_3$	49.38 (49.18	3.27 3.44	15.79 15.93)
IVd	76	211–213	$C_{19}H_{16}N_4S_3$	57.09 (57.25	4.73 4.55	14.28 14.05)
IVe	75	235	$C_{19}H_{17}ClN_4S_3$	52.87 (52.69	10.10 9.95	12.78 12.94)
IVf	72	222–224	$C_{19}H_{17}N_5O_2S_3$	51.19 (51.44	3.92 3.86	15.67 15.79)

formulas, and elemental analyses of compounds **IIa–c** are recorded in Table 1; spectral data are given in Table 2.

6,7-Diaryl-6,7-dihydro-5-thioxo-1,3,4-thiadiazolo[3,2*a*]-*s*-triazin-2-yl *N*-Aryldithiocarbamates (IIIa–f). 5-(Arylideneamino)-2-mercapto-1,3,4-thiadiazole (0.02 mol) and aryl isothiocyanate (0.04 mol) were refluxed in toluene for 5 h. Then the solvent was evaporated, the residue was washed with ethanol, and the product thus obtained was recrystallized from ethanol to give light yellow crystals of the analytical sample. Yields, melting points, molecular formulas, and elemental analyses of compounds **IIIa–f** are recorded in Table 1 and spectral data in Table 2.

6,7-Diaryl-2-(ethylthio)-6,7-dihydro-1,3,4-thiadiazolo[3,2-*a*]-*s*-triazine-5(*H*)-thiones (IVa–f). A reaction mixture containing **II** (0.02 mol), diethyl sulfate (0.022 mol), and NaOH (0.02 mol) was refluxed in ethanol (75 mL) for 1 h and then evaporated to dryness. To the residue were added aryl isothiocyanate (0.02 mol) and toluene (80 mL), and the mixture was refluxed for 6 h. The solvent was distilled off under reduced pressure. The residue was washed with ethanol followed by water, and the product thus obtained was recrystallized from ethanol as shining yellowish needles. Yields, melting points, molecular formulas, and elemental analyses of compounds **IVa–f** are recorded in Table 1; spectral data are given in Table 2.

Antifungal Screening. In vitro antifungal activity of compounds **IIa–c, IIIa–f,** and **IVa–f** was evaluated against two fungal species, viz. *A. niger* and *F. 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). Six replications of controls containing the solvent (acetone/water mixture, 1:4 by volume) and Czapek's medium were provided. A standard commercial fungicide, Dithane M-45, was also tested under similar conditions for comparison. No remarkable morphological change was observed in developing fungi. The antifungal screening results are summarized in Table 3.

For the most active compounds **IVb, IVe,** and **IVf**, we ascertained whether these were fungistatic or fungicidal. Thus, following the procedure of Garber and Houston (1959), compounds **IVb, IVe,** and **IVf** were added separately to Czapek's agar medium in different Petri dishes to maintain the final concentration at their respective lethal doses (900, 800, and 825 ppm). The 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 the control dishes was recorded. Then the fungal disks were taken from the treated as well as the 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 percent inhibition compared with the dishes containing the fungal disks taken from the control dishes was recorded. The number of replicate assays in each case was three, and six replicate controls were used. It was found that the compounds **IVb, IVe,** and **IVf** caused complete inhibition of mycelial growth of the test fungi and that there was no growth in the reinoculated fungal disks taken from the treated dishes. This shows that the compounds **IVb, IVe,** and **IVf** are fungicidal.

RESULTS AND DISCUSSION

It is obvious from the antifungal screening data (Table 3) that all of the tested compounds **II–IV** significantly inhibited mycelial growth of both test fungi at higher concentration (1000 ppm) but their antifungal activity

Table 2. Spectral Data of Compounds II–IV

compd	IR ν_{max}, cm^{-1}	^1H NMR, δ (J, Hz)	MS/M$^+$, m/z
IIa	1680	7.50–8.16 (6H, m, ArH and N=CH), 8.40 (1H, s, SH)	221
IIb	1675	7.52–8.20 (5H, m, ArH and N=CH), 8.44 (1H, s, SH)	252
IIc	1670	7.51–8.22 (5H, m, ArH and N=CH), 8.42 (1H, s, SH)	266
IIIa	1610, 1635	6.72 (1H, s, NCH), 7.42–7.84 (15H, m, ArH), 9.36 (1H, br s, NH)	491
IIIb	1610, 1630	6.76 (1H, s, NCH), 7.41–7.90 (14H, m, ArH), 9.37 (1H, br s, NH)	523
IIIc	1610, 1625	6.79 (1H, s, NCH), 7.44–8.00 (14H, m, ArH), 9.39 (1H, br s, NH)	536
IIId	1605, 1635	2.33 (6H, s, 2 × Me), 6.70 (1H, s, NCH), 7.30–7.86 (14H, m, ArH), 9.33 (1H, br s, NH)	519
IIIe	1605, 1635	2.34 (6H, s, 2 × Me), 6.72 (1H, s, NCH), 7.32–7.94 (12H, m, ArH), 9.35 (1H, br s, NH)	555
IIIf	1605, 1625	2.35 (6H, s, 2 × Me), 6.76 (1H, s, NCH), 7.30–8.00 (12H, m, ArH), 9.38 (1H, br s, NH)	564
IVa	1610, 1635	1.43 (3H, t, J = 5.5, Me), 3.21 (2H, q, J = 5.5, CH_2), 6.71 (1H, s, NCH), 7.38–7.78 (10H, m, ArH)	384
IVb	1605, 1630	1.44 (3H, t, J = 5.5, Me), 3.25 (2H, q, J = 5.5, CH_2), 6.75 (1H, s, NCH), 7.36–7.89 (9H, m, ArH)	422
IVc	1605, 1625	1.45 (3H, t, J = 5.5, Me), 3.28 (2H, q, J = 5.5, CH_2), 6.77 (1H, s, NCH), 7.37–7.92 (9H, m, ArH)	439
IVd	1615, 1635	1.42 (3H, t, J = 5.5, Me), 2.32 (3H, s, o-Me), 3.19 (2H, t, J = 5.5, CH_2), 6.69 (1H, s, NCH), 7.25–7.84 (9H, m, ArH)	398
IVe	1610, 1630	1.43 (3H, t, J = 5.5, Me), 2.33 (3H, s, o-Me), 3.22 (2H, q, J = 5.5, CH_2), 6.73 (1H, s, NCH), 7.29–7.86 (8H, m, ArH)	434
IVf	1610, 1625	1.45 (3H, t, J = 5.5, Me), 2.34 (3H, s, o-Me), 3.25 (2H, q, J = 5.5, CH_2), 6.75 (1H, s, NCH), 7.28–7.87 (8H, m, ArH)	443

Table 3. Antifungal Screening Results of Compounds II–IV

	av % inhibition after 96 h against					
	A. niger at			*F. oxysporum* at		
compd	1000 ppm	100 ppm	10 ppm	1000 ppm	100 ppm	10 ppm
IIa	55	43	13	52	40	10
IIb	66	58	21	62	55	18
IIc	60	50	17	57	46	14
IIIa	70	54	28	68	53	26
IIIb	75	60	34	73	58	32
IIIc	72	55	29	69	52	26
IIId	73	57	31	71	55	29
IIIe	77	64	37	75	61	34
IIIf	75	61	33	73	58	31
IVa	82	65	43	80	63	40
IVb	100	72	51	99	70	50
IVc	84	68	40	82	65	37
IVd	88	70	45	86	68	43
IVe	100	80	55	100	78	53
IVf	100	76	53	100	73	51
Dithane M-45	100	83	67	100	85	68

decreased markedly at lower concentrations (100 and 10 ppm). The most active of these compounds, **IVb**, **IVe**, and **IVf**, displayed fungicidal action equivalent to that of Dithane M-45 at 1000 ppm concentration and inhibited the growth of both test fungi by 50-55% even at 10 ppm concentration.

In general, compounds **III** and **IV** incorporating the *s*-triazine nucleus were far more potent than their parent Schiff base **II**. The antifungal action varied marginally with the fungal species. Although some of the screened compounds, **IVb**, **IVe**, and **IVf**, were highly toxic to *A. niger* and *F. oxysporum* at higher concentration (1000 ppm), the overall results are not so encouraging as one would expect from the combined performance of the biolabile dithiocarbamate 1,3,4-thiadiazole and *s*-triazine moieties. This might be attributed to the partial saturation in the *s*-triazine nucleus resulting in the loss of planarity of the thiadiazolo-*s*-triazine ring system. This presumption is supported by the fact that compact size and planarity of a molecule often enhance its pesticidal properties (Rothwell and Wain, 1964; Singh et al., 1985; Yadav et al., 1989).

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