Antifungal Activity of New 1,3,4-oxadiazolo[3,2-*a*]-*s*-triazine-5,7-diones and Their 5-thioxo-7-ones

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N^1- and N^3-(4-fluorophenyl) ureas (**III a**−**e**) were cyclocondensed with ethyl chloroformate and CS_2/KOH to yield 2-aryl-6-(4-fluorophenyl)-1,3,4-oxadiazolo[3,2-*a*]-*s*-triazine-5,7-diones (**IVa**−**e**) and their 5-thioxo-7-ones (**Va**−**e**), respectively. The compounds **III**−**V**(**a**−**e**) have been compared with Dithane M-45 for their fungitoxic action against *A. niger* and *F. oxyporum*, and the results have been correlated with the structural features of the tested compounds.

Keywords: *A. niger; F. oxysporum; czapekas agar; DMSO-d_6*

Many 1,3,4-oxadiazoles are associated with a broad spectrum of pesticidal activity (Meek, 1972; Stachler and Sachse, 1977; Rhone-Poulenc, 1962). Similarly, many *s*-triazine derivatives have attained significance in agriculture as herbicides and fungicides, including Simazine (2-chloro-4,6-bis-(ethylamino)-*s*-triazine), Atrazine (2-chloro-4 ethylamino-6-isopropylamino-*s*-triazine), and others. In 1955, Gysin and co-workers discovered the phytotoxic and plant growth regulating properties of a series of amino derivatives of triazines (Gast et al., 1955, 1956). In view of these facts, and with the hope of achieving pesticidal compounds of high potency, we have fused the biolabile 1,3,4-oxadiazole with *s*-triazine nuclei to probe how this combination could enhance the pharmacological activity. Thus, the title nitrogen-bridged heterocyclic systems bearing 5,7-dione (**IVa**−**e**) or 7-one-5-thione functions have been synthesized and evaluated for their antifungal activity. Further, all of these compounds possess fluoroaryl moiety which might be expected to enhance the fungicidal action.

The reaction sequence leading to the title compounds is depicted in Scheme I. All of the compounds have been evaluated for their general pesticidal activity, and two compounds have been found to have positive antifungal activity.

EXPERIMENTAL PROCEDURES

All melting points were determined in open glass capillaries and are reported uncorrected. Infrared spectra in KBr were recorded on a Perkin-Elmer 157 infrared spectrophotometer. ^{1}H NMR spectra were recorded on a EM-360 L (60 MHz) NMR spectrometer in DMSO-d_6 using TMS as an internal reference; chemical shifts are expressed in δ (ppm). All the compounds have given satisfactory elemental analytical results (C, H, and N) and IR spectra.

2-Amino-5-aryl-1,3,4-oxadiazoles (Ia−**e).** These compounds were prepared by oxidative cyclization of aldehyde semicarbazones with bromine in glacial acetic acid in the presence of anhydrous sodium acetate. All five compounds (**Ia**−**e**) have already been reported in the literature (Gibson, 1982; Gehlen and Moeckle, 1962; Hoggarth, 1949).

Ethyl-*N*-(5-aryl-1,3,4-oxadiazol-2-yl)carbamates (IIa−**e).** To **Ia** (6.44 g, 0.04 mol) in pyridine (160 mL) was added ethyl chloroformate (4.78 g, 0.044 mol) and triethylamine (10 mL), and the mixture was refluxed for 1 h. It was poured into dilute HCl (320 mL, 50%; V/V), and the carbamate **IIa** thus obtained was recrystallized from ethanol, yield 7.00 g (75%), mp 205−207 °C. Found: C, 56.5; H, 4.7; N, 17.9. $C_{11}H_{11}N_3O_3$ requires C, 56.7; H, 4.7; N, 18.0%. IR: 1730 cm^{-1} υ C=O. Compounds **IIb**−**c** were similarly prepared and were recrystallized from ethanol.

***N*^{1}- and *N*^{3}-(4-fluorophenyl)ureas (IIIa**−**e).** An equimolar mixture of **II** and 4-fluoroaniline in ethanol was refluxed for 16 h and the solvent was distilled off. The residue was washed with water and recrystallized from ethanol to furnish the following **III** in 59−67% yield.

IIIa. mp 230−31°. Found: N, 18.6. $C_{15}H_{11}FN_4O_2$ requires N, 18.8%. IR: 3290 (υ N−H), 1660 cm^{-1} (υ C=O).

IIIb. mp 225−27°. Found: N,16.9. $C_{15}H_{10}ClFN_4O_2$ requires N, 16.8%. IR: 3300 (υ N−H), 1665 cm^{-1} (υ C=O).

IIIc−e were similarly prepared, recrystallized from ethanol, and characterized.

2-Aryl-6-(4-fluorophenyl)-1,3,4-oxadiazolo [3,2-*a*]-*s*-triazine-5,7-diones (IVa−**e).** To a solution of **III** (0.01 mol) in pyridine (40 mL) was added ethyl chloroformate in an ice-bath. The mixture was stirred at room temperature for 2 h and then refluxed for 1 h. The contents were treated with 1N KOH (40 mL) and the product thus precipitated was recrystallized from ethanol, yield 69−78%. The following compounds were prepared.

IVa. mp 241 °C. Found: C, 59.5; H, 2.6; N, 17.4. $C_{16}H_9FN_4O_3$ requires C, 59.7; H, 2.7; N, 17.3%. IR: 1705 cm^{-1} (υ C=O). ^{1}H NMR: 7.00−8.10 (9H, m, aromatic H).

The compounds thus synthesized are given in Table 1 with their characterization data.

2-Aryl-6-(4-fluoropheny1)-5-thiox-1,3,4-oxadiazolo [3,2-*a*]-7-ones (Va−**e).** A mixture of **IIIa** (2.98 g, 0.01 mol), ethanol (40 mL), and carbon disulfide (1.52 g, 0.02 mol) was refluxed for 6 h and concentrated to a small volume. The contents were poured into ice-cold water and acidified with dilute HCl to give the desired product **Va** in 76% yield; mp 215−17 °C (EtOH). Found: N, 15.8; S, 9.4%. $C_{16}H_9FN_4O_2S$ requires N, 15.9; S 9.4%.

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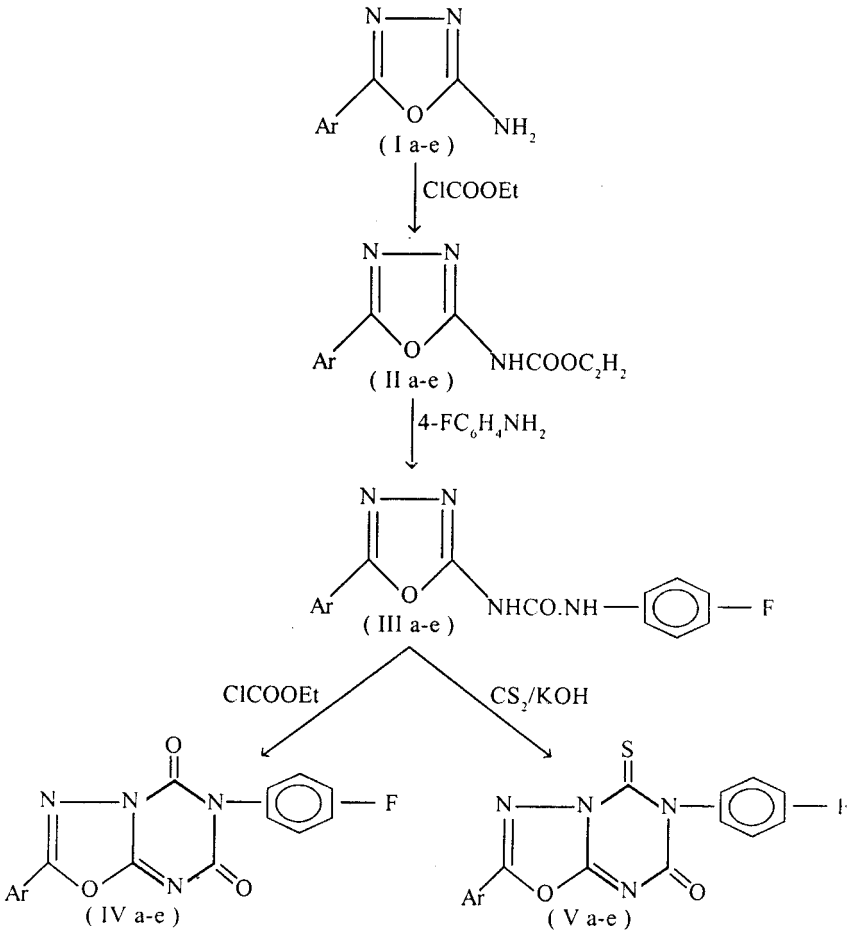
Scheme 1



a, Ar = C_6H_5 ; b, Ar = 2-ClC_6H_4 ; c, Ar = 4-ClC_6H_4 ;
d, Ar = 2-$MeOC_6H_4$; e, Ar = 4-$MeOC_6H_4$

IR: 1695 (υ C=O), 1090 cm^{-1} (υ C=S); ^1H NMR: 7.00−8.10 (9H, m, aromatic H).

Other compounds (**Vb**−**e**) were similarly synthesized and their characterization data are given in Table 1. Spectral data for compounds **IV**−**V** (**a**−**e**) are given in Table 2. **Antifungal Screening.** The compounds **III**−**V**(**a**−**e**) were screened for their antifungal activity against *A. niger* and *F. oxyporum* for poisoned food technique (Horsfall, 1945) at 1000, 100, and 10 ppm concentrations using Czapekas agar medium. Suspension of the compounds in an acetone and water mixture (20:80, V/V) was applied. A standard, commercial fungicide Dithane M-45, was also tested under similar conditions for comparison, and the results are summarized in Table 3.

RESULT AND DISCUSSION

Characterization of Test Compounds. All compounds are reported in Table 1 and had satisfactory elemental analyses. The structural assignments of the compounds were based on their IR and ^1H NMR spectra. The IR spectra of the type **III** compounds characteristics band in the region 3260−3300 and 1660−1675 cm^{-1} attributable to υN−H and υC=O, respectively. The disappearance of these bands in the IR spectra of the type **IV** and **V** compounds is comparable with their assigned structure. Similarly, the absence of peaks above δ8.00 ppm in the ^1H NMR spectra of the compounds **IV** and **V** supports their structures, as signals for N−H were present at above δ9.0 and 13.0 ppm in the ^1H NMR spectra of their precursors **III**.

Effect of Compounds of the Type III−V on Mycilial Growth of Fungi. The antifungal activity displayed by the compounds **III**−**V(a**−**e)** is summarized in Table 3 at 1000, 100, and 10 ppm concentrations. Out of these, compounds **IVb**, **Vb,** and **Vc**, at 1000 ppm, exhibited fungitoxicity similar to that of Dithane M-45 against both of the fungi. However, activity of the compounds decreased markedly at lower concentrations (100 and 10 ppm) except **IVb**, **Vb**, and **Vc**, which inhibited 42−45% growth of the fungal species even at 10 ppm.

Comparison of fungitoxicity of compounds **IV(a**−**e)** with the corresponding **V(a**−**e)** clearly indicates that the latter (containing >c=o and >c=s functions together) are more active than the former (incorporating only >c=o groups). This is in confirmity with the earlier observations that the combination of >c=o and >c=s functions sometimes works better than either alone (Horsfall and Rich, 1951). In general, the compounds **IV(a**−**e)** and **V(a**−**e)** are more potent than their parent ureas (**IIIa**−**e**). This might be attributed to the more planar and compact structure (Singh et al., 1981; Summer 1976; Chatt et al., 1956; Rothwell and Wain, 1963) of these compound condensed heterocycles than

Table 1. Yield, Melting Point, Molecular Formulas, and Elemental Analysis of Compounds III−V (a−e)

compound no.		R	yield (%)	mp (°C)	molecular formula	found (calculated), % C	H	N
III	**a**	C_6H_5	65	230	$C_{15}H_{11}FN_4O_2$	60.5 (60.4)	3.70 (3.69)	18.6 (18.8)
	b	2-ClC_6H_4	62	227	$C_{15}H_{10}ClFN_4O_2$	54.1 (54.0)	3.1 (3.0)	16.9 (16.8)
	c	4-ClC_6H_4	66	225	$C_{15}H_{10}ClFN_4O_2$	53.9 (54.0)	2.9 (3.0)	16.70 (16.8)
	d	2-$OMeC_6H_4$	59	210	$C_{16}H_{13}FN_4O_3$	58.1 (58.2)	3.95 (3.96)	17.1 (17.0)
	e	4-$OMeC_6H_4$	67	215	$C_{16}H_{13}FN_4O_3$	58.0 (58.2)	3.97 (3.96)	17.2 (17.0)
IV	**a**	C_6H_5	69	241	$C_{16}H_9FN_4O_3$	59.5 (59.7)	2.6 (2.7)	17.4 (17.3)
	b	2-ClC_6H_4	70	250	$C_{16}H_8ClFN_4O_3$	53.2 (53.0)	2.3 (2.2)	15.7 (15.6)
	c	4-ClC_6H_4	73	248	$C_{16}H_8ClFN_4O_3$	53.1 (53.0)	2.2 (2.2)	15.7 (15.6)
	d	2-$OMeC_6H_4$	75	246	$C_{17}H_{11}FN_4O_4$	57.5 (57.6)	3.2 (3.0)	15.9 (15.8)
	e	4-$OMeC_6H_4$	78	245	$C_{17}H_{11}FN_4O_4$	57.4 (57.6)	3.1 (3.0)	15.9 (15.8)
V	**a**	C_6H_5	76	216	$C_{16}H_9FN_4O_2S$	57.8 (57.9)	2.54 (2.55)	15.8 (15.9)
	b	2-ClC_6H_4	82	198	$C_{16}H_8ClFN_4O_2S$	56.1 (56.0)	2.7 (2.6)	16.1 (16.0)
	c	4-ClC_6H_4	84	228	$C_{16}H_8ClFN_4O_2S$	55.9 (56.0)	2.5 (2.6)	16.2 (16.0)
	d	2-$OMeC_6H_4$	81	235	$C_{17}H_{11}FN_4O_3S$	55.1 (55.0)	2.8 (2.9)	15.1 (15.0)
	e	4-$OMeC_6H_4$	86	243	$C_{17}H_{11}FN_4O_3S$	55.2 (55.0)	2.7 (2.9)	15.2 (15.0)

Table 2. Spectral Data of Compounds IV−V(a−e)

compound no.		IR (KBr)·Cm^{-1} C=O	C=S	^1H NMR (DMSO-d_6)
IV	**a**	1705	−	7.00−8.10 (9 H, m, aromatic H)
	b	1710	−	7.00−8.00 (8 H, m, aromatic H)
	c	1705	−	6.90−8.00 (8 H, m, aromatic H)
	d	1715	−	3.76 (3 H, s, OCH_3), 6.95−7.50 (8 H, m, aromatic H)
	e	1705	−	3.78 (3 H, s, OCH_3), 7.00−7.80 (8 H, m, aromatic H)
V	**a**	1695	1090	7.00−8.10 (9 H, m, aromatic H)
	b	1700	1095	7.20−8.20 (8 H, m, aromatic H)
	c	1705	1100	7.10−8.10 (8 H, m, aromatic H)
	d	1700	1090	3.76 (3 H, s, OCH_3), 6.90−7.00 (8 H, m, aromatic H)
	e	1695	1100	3.80 (3 H, s, OCH_3), 7.00−8.00 (8 H, m, aromatic H)

Table 3. Fungicidal Screening Results of Compounds III−V[a]

compound		A. niger 1000 ppm	100 ppm	10 ppm	F. oxysporum 1000 ppm	100 ppm	10 ppm
III	**a**	68	37	17	6	37	17
	b	70	42	22	67	40	20
	c	75	45	23	72	41	18
	d	68	41	20	66	38	20
	e	71	44	21	70	43	20
IV	**a**	82	38	29	80	38	26
	b	97	51	40	96	49	38
	c	88	48	36	87	47	35
	d	78	46	32	76	44	30
	e	80	49	33	78	48	32
V	**a**	92	53	42	91	53	41
	b	98	58	45	98	58	45
	c	98	56	44	97	56	44
	d	94	50	43	92	49	42
	e	93	49	42	91	48	41
Dithane M-45		100	85	68	100	80	67

[a] Average inhibition of fungal growth (%) at stated concentration $(mg/liter)^{-1}$.

their precursors, as well as to the presence of an additional >C═O or >C═S group (Horsfall and Rich, 1951).

All of the compounds were more active against *A. niger* than against *F. oxysporium*, but the difference was marginal.

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