Ring Transformation of Michael Adducts of 4-Benzylidene-5-oxazolones and 2-Amino-1,3,4-thiadiazoles to Antifungal 6,7-Dihydro-5*H*-thiadiazolo[3,2-*a*]pyrimidin-5-ones

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Michael addition of 2-amino-5-aryl[(aryloxy)methyl]-1,3,4-thiadiazoles Ia–c to 4-benzylidene-5-oxazolones IIa–d followed by ring transformation of the Michael adducts IIIa–l yielded new bicyclic compounds, 6,7-dihydro-2-aryl[(aryloxy)methyl]-7-aryl-6-benzamido-5*H*-1,3,4-thiadiazolo[3,2-*a*]-pyrimidin-5-ones IVa–l. Compounds IIIb and IVa–l were compared with Dithane M-45, a commercial fungicide, for their antifungal activity against *Aspergillus flavus* and *Fusarium solani*.

INTRODUCTION

Pyrimidine derivatives, including those where the pyrimidine structure constitutes a part of the condensed ring system, are well-known to be involved in various metabolic processes and consequently are biolabile. Thus, besides other useful biological activities, pyrimidine derivatives have been reported to possess bactericidal (Maekawa et al., 1975), fungicidal (Russo et al., 1981), and herbicidal (Maggiali et al., 1983) activities. Similarly, 1,3,4-thiadiazoles condensed with other heterocycles have been reported as potential antifungal and antibacterial agents (Singh et al., 1982, 1983; Yadav et al. 1989a,b; Russo et al., 1981; Maekawa et al., 1975).

In view of the above papers and our desire to develop new antifungal agents of high potency, we fused biolabile pyrimidine and 1,3,4-thiadiazole structures to probe how far this combination could be successful. The investigation appeared interesting, as the compounds IVa–l reported herein have been synthesized for the first time.

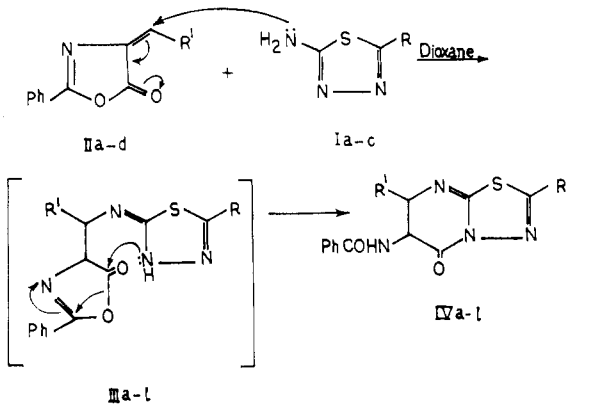
The reaction sequence leading to the formation of IVa–l is outlined in Scheme I. 2-Amino-1,3,4-thiadiazoles Ia–c were refluxed with 4-benzylidene-5-oxazolones IIa–d in dioxane for 20–22 h to furnish the new compounds IVa–l in 70–85% yields. The starting materials, thiadiazoles Ia–c, were prepared by cyclodehydration of appropriate 1-acylthiosemicarbazides with concentrated H_2SO_4 (Maffii et al., 1958), and the 4-benzylidene-5-oxazolones IIa–d were obtained from hippuric acid and the corresponding aromatic aldehydes following the standard procedure (Vogel, 1956).

Structural assignments of the synthesized compounds were based on their elemental analyses, IR, ^{1}H NMR, and mass spectra (Tables I and II). Of the tested compounds IIIb and IVa–l, the compounds IVj and IVl displayed antifungal activity of the order of Dithane M-45 [a commercial fungicide, manganous ethylenebis(dithiocarbamate) with zinc ions] at 1000 ppm concentration against *Aspergillus flavus* and *Fusarium solani*.

EXPERIMENTAL PROCEDURES

Melting points were determined in open glass capillaries and are uncorrected. IR spectra in KBr were recorded on a Perkin-Elmer 157 infrared spectrophotometer (ν_{max} cm^{-1}). ^{1}H NMR spectra were recorded on a EM-360L (90 MHz) NMR spectrometer in $CDCl_3$ using TMS as an internal reference; chemical shifts are expressed in δ (ppm). Mass spectra were recorded on a JEOL JMS-D 300 instrument.

Scheme I



R: Ia, IIIa-d, IVa-d, C_6H_5, Ib, IIIe-h, IVe-h, $C_6H_5OCH_2$, Ic, IIIi-l, IVi-l, 4-$ClC_6H_4OCH_2$.

R': IIa, IIIa, IIIe, IIIi, IVa, IVe, IVi, C_6H_5, IIb, IIIb, IIIf, IIIj, IVb, IVf, IVj, 4-ClC_6H_4, IIc, IIIc, IIIg, IIIk, IVc, IVg, IVk, 4-FC_6H_4; IId, IIId, IIIh, IIIl, IVd, IVh, IVl, 4-$CH_3OC_6H_4$.

Table I. Analytical Data for Compounds IVa–l

compd	yield, %	mp, °C	formula	found (calcd), % C	H	N
IVa	70	154	$C_{24}H_{18}N_4O_2S$	67.70 (67.61)	4.11	13.26 (13.15)
IVb	74	180–182	$C_{24}H_{17}ClN_4O_2S$	62.43 (62.54)	3.52	12.02 (12.16)
IVc	72	188	$C_{24}H_{17}FN_4O_2S$	64.65 (64.87)	4.92	12.52 (12.61)
IVd	80	146	$C_{25}H_{20}N_4O_3S$	65.58 (65.79)	4.46	12.09 (12.28)
IVe	76	152	$C_{25}H_{20}N_4O_3S$	65.66 (65.79)	4.30	12.16 (12.28)
IVf	78	184–185	$C_{25}H_{19}ClN_4O_3S$	61.24 (61.16)	3.68	11.25 (11.42)
IVg	71	196	$C_{25}H_{19}FN_4O_3S$	63.41 (63.29)	3.95	11.65 (11.81)
IVh	82	144–145	$C_{26}H_{22}N_4O_4S$	64.28 (64.20)	4.39	11.36 (11.52)
IVi	75	142–143	$C_{25}H_{19}ClN_4O_3S$	61.02 (61.16)	3.98	11.32 (11.42)
IVj	73	176–177	$C_{25}H_{18}Cl_2N_4O_3S$	57.22 (57.14)	3.56	10.45 (10.67)
IVk	70	160–162	$C_{25}H_{18}ClFN_4O_3S$	58.92 (59.00)	3.44	11.12 (11.01)
IVl	85	208–209	$C_{26}H_{21}ClN_4O_4S$	59.78 (59.94)	4.15	10.84 (10.76)

2-Amino-5-aryl[(aryloxy)methyl]-1,3,4-thiadiazoles

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Table II. Spectral Data of the Compounds IVa–l

compd	IR (KBr) $\nu_{C=O}$, cm^{-1} pyrimidinone	IR (KBr) $\nu_{C=O}$, cm^{-1} amido	^1H NMR ($CDCl_3$) δ (J, Hz)	MS/M^+ m/z
IVa	1660	1615	3.98 (1 H, d, J = 4, C_7-H), 4.86 (1 H, dd, J = 4, 8, C_6-H), 6.84–8.20 (15 H, m, aromatic H), 8.56 (1 H, br, s, NH)	426
IVb	1655	1610	3.90 (1 H, d, J = 4, C_7-H), 4.82 (1 H, dd, J = 4, 8, C_6-H), 6.86–8.22 (14 H, m, aromatic H), 8.56 (1 H, br, s, NH)	460, 462
IVc	1660	1615	3.96 (1 H, d, J = 4, C_7-H), 4.88 (1 H, dd, J = 4, 8, C_6-H), 6.88–8.20 (14 H, m, aromatic H), 8.58 (1 H, br, s, NH)	444
IVd	1655	1615	3.76 (3 H, s, OCH_3), 3.94 (1 H, d, J = 4, C_7-H), 4.80 (1 H, dd, J = 4, 8, C_6-H), 7.00–8.24 (14 H, m, aromatic H), 8.58 (1 H, br, s, NH)	456
IVe	1655	1610	3.98 (1 H, d, J = 4, C_7-H), 4.86 (1 H, dd, J = 4, 8, C_6-H), 5.22 (2 H, s, OCH_2), 6.80–8.24 (15 H, m, aromatic H), 8.58 (1 H, br, s, NH)	456
IVf	1660	1610	3.96 (1 H, d, J = 4, C_7-H), 4.88 (1 H, dd, J = 4, 8, C_6-H), 5.26 (2 H, s, OCH_2), 6.86–8.20 (14 H, m, aromatic H), 8.56 (1 H, br, s, NH)	490, 492
IVg	1655	1610	3.92 (1 H, d, J = 4, C_7-H), 4.86 (1 H, dd, J = 4, 8, C_6-H), 5.24 (2 H, s, OCH_2), 6.88–8.22 (14 H, m, aromatic H), 8.60 (1 H, br, s, NH)	474
IVh	1660	1615	3.76 (3 H, s, OCH_3), 3.96 (1 H, d, J = 4, C_7-H), 4.84 (1 H, dd, J = 4, 8, C_6-H), 5.20 (2 H, s, OCH_2), 6.80–8.24 (14 H, m, aromatic H), 8.58 (1 H, br, s, NH)	486
IVi	1660	1615	3.90 (1 H, d, J = 4, C_7-H), 4.78 (1 H, dd, J = 4, 8, C_6-H), 5.20 (2 H, s, OCH_2), 7.00–8.20 (14 H, m, aromatic H), 8.60 (1 H, br, s, NH)	490, 492
IVj	1660	1615	3.90 (1 H, d, J = 4, C_7-H), 4.84 (1 H, dd, J = 4, 8, C_6-H), 5.26 (2 H, s, OCH_2), 6.86–8.24 (13 H, m, aromatic H), 8.58 (1 H, br, s, NH)	524, 526, 528
IVk	1655	1615	3.92 (1 H, d, J = 4, C_7-H), 4.86 (1 H, dd, J = 4, 8, C_6-H), 5.22 (2 H, s, OCH_2), 6.88–8.22 (13 H, m, aromatic H), 8.60 (1 H, br, s, NH)	508, 510
IVl	1660	1610	3.76 (3 H, s, OCH_3), 3.96 (1 H, d, J = 4, C_7-H), 4.84 (1 H, dd, J = 4, 8, C_6-H), 5.24 (2 H, s, OCH_2), 6.86–8.22 (13 H, m, aromatic H), 8.58 (1 H, br, s, NH)	520, 522

(Ia–c). These were prepared according to the method of Maffii et al. (1958) which involves the cyclodehydration of 1-acylthiosemicarbazides with concentrated H_2SO_4. The compounds Ia–C agreed well with their analytical data already reported in literature (Maffii et al., 1958; Singh, 1966; Shukla and Agrawal, 1982).

4-Benzylidene-5-oxazolones (IIa–d). Following the standard procedure (Vogel, 1956) hippuric acid was treated with the appropriate aromatic aldehyde in acetic anhydride to furnish IIa–d. The compounds IIa–d agreed well with their analytical data already reported in literature (Blatzzi and Davis, 1962; Yadav et al., 1988).

6,7-Dihydro-2-aryl[(aryloxy)methyl]-7-aryl-6-benzamido-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-ones (IVa–l). An equimolar mixture of Ia–c and IIa–d was dissolved in a minimum amount of dioxane, and the solution was refluxed for 20–22 h. The reaction mixture was concentrated, cooled, and poured into water. The yellowish precipitate thus obtained was washed with water and recrystallized from ethanol to afford light yellowish needles of the desired products IVa–l. Yield, melting point, molecular formula, and elemental analysis of compounds IVa–l are recorded in Table I; the spectral data are given in Table II.

Isolation of Michael Adduct (IIIb). An equimolar mixture of Ib and IIb was refluxed in dioxane for 16 h, and then the reaction mixture was concentrated, cooled, and poured into water. The product thus obtained was recrystallized from ethanol.

IIIb: mp 172–173 °C dec; IR 1785 cm^{-1} ($\nu_{C=O}$); ^1H NMR δ 4.96 (1 H, d, J = 4, 4-H oxazolone ring), 4.32 (1 H, d, J = 4, NCH), 6.86–8.20 (14 H, m, aromatic H) 9.86 (1 H, br s, NH). Anal. Calcd for $C_{24}H_{17}ClN_4O_2S$: C, 62.54; H, 3.69; N, 12.16. Found: C, 62.46; H, 3.45; N, 12.04. Compound IIIb was converted into IVb in 90% yield by refluxing in dioxane for 3 h.

Antifungal Screening. The antifungal activity of the compounds IIIb and IVa–l was evaluated against *A. flavus* and *F. solani*. The pure cultures of the tested fungi, the pathogenicity of which was already verified, were obtained from the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi. Agar (bacteriological grade), supplied by Sharabhai M. Chemicals, was used as such. Antifungal screening was done by the usual agar plate technique (Horsfall, 1945) at 1000, 100, and 10 ppm concentrations using Czapek's agar medium. The compounds were applied as suspensions in an acetone–water mixture (20:80 v/v). One milliliter of the test suspension was thoroughly mixed with 9 mL of the medium by rotating the plates on table top, and then the mixture was allowed to set. A fungal disk of 5-mm diameter was 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 (Raper et al., 1968) and was inoculated in the center of each Petri plate in an 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 mixture (20:80 v/v) 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

$$\% \text{ inhibition} = [(C - T) \times 100]/C$$

where C is the average diameter of fungal colony (millimeters) in control plates and T is the average diameter of fungal colony (millimeters) in treated plates.

Dithane M-45, a commercial fungicide, was also tested under similar conditions for comparison. The antifungal-screening results of compounds IIIb and IVa–l are summarized in Table III.

RESULTS AND DISCUSSION

The formation of thiadiazolopyrimidinones IVa–l involves the Michael addition of 2-amino-1,3,4-thiadiazoles Ia–c to 4-benzylidene-5-oxazolones IIa–d, which undergo intramolecular nucleophilic attack of the nitrogen

Table III. Antifungal Screening Results of Compounds IIIb and IVa–l

compd	av % inhibition after 96 h against *A. flavus* 1000 ppm	100 ppm	10 ppm	*F. solani* 1000 ppm	100 ppm	10 ppm
IIIb	46	30	12	47	32	15
IVa	71	36	21	62	40	26
IVb	78	48	28	70	49	30
IVc	76	42	25	63	41	28
IVd	74	40	24	68	46	27
IVe	79	56	32	72	51	26
IVf	84	64	46	80	61	40
IVg	82	60	38	76	58	34
IVh	81	59	39	77	54	36
IVi	86	62	41	83	56	42
IVj	94	68	52	96	66	50
IVk	87	65	46	85	63	48
IVl	91	64	44	95	60	46
Dithane M-45[a]	95	82	66	100	86	73

[a] Commercial fungicide.

atom of the thiadiazole ring (N-4) at the carbonyl carbon (C-5) of the oxazolone nucleus with the simultaneous cleavage of the oxazolone ring to yield IVa–l (Scheme I). This conclusion was based on the observation that the intermediate compound IIIb could isolated in 48% yield (see Experimental Procedures) during the reaction, and it was converted into IVb in 90% yield by refluxing in dioxane for 3 h.

From the antifungal-screening data (Table III) it is obvious that compounds IVa–l inhibited more than 60% growth of both the test fungi at 1000 ppm concentration and hence are antifungal. Of these, the most active compounds IVj and IVl exhibited antifungal action almost equivalent to but not better than that of Dithane M-45 at 1000 ppm concentration and inhibited 44–52% growth of both fungal species even at 10 ppm concentration.

In spite of the fact that IIIb has a preformed open-chain skelton of pyrimidine ring, it was less potent than its successor IVb where ring closure has resulted in a more planar and compact system. This is in conformity with the earlier observations that the compact size and planarity of a molecule often inhance its pesticidal activities (Fischer and Summers, 1976; Chatt et al., 1956; Rothwell and Wain, 1964).

It is, however, noteworthy that the introduction of a chloro, fluoro, or methoxy group in the aryl moiety of these compounds tends to augment the antifungal action. Likewise, the introduction of a chloro group was far more effective than that of a fluro or methoxy group. Presumably, this is due to the greater lipophilic character of the chloro group relative to that of the fluoro or methoxy group, which favors the permeation of the compound through lipoid layers of the fungal cell wall. The antifungal activity varied marginally with the fungal species.

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