Condensed Heterocycles: Synthesis and Antifungal Activity of π-Deficient Pyrimidines Linked with π-Rich Heterocycles

Rahat H. Khan* and Romesh C. Rastogi

Synthetic Organic Chemistry Division, Regional Research Laboratory, Jorhat 785 006, Assam, India

Some new substituted fused 1,3,4-oxadiazolo-, -thiadiazolo-, and thiazolo[3,2-*a*]pyrimidin-5-ones (IIa–c–Va–c) and their 7-one analogues (VIa–c) have been synthesized by the reaction of corresponding 2-aminoheterocycle synthons (Ia–c) with different Michael acceptors. All of the compounds have been compared with Dithane M-45, a commercial fungicide, for their antifungal action against *Helminthosporium oryzae*, *Aspergillus niger*, and *Fusarium oxysporium*.

INTRODUCTION

The importance of uracil and its annelated substrates is well recognized by synthetic and biological chemists (Brown, 1984; Bradshaw et al., 1987; Griengl et al., 1987; Jones et al., 1988). With the development of clinically useful anticancer (5-fluorouracil) and antiviral drugs (Heidelberger et al., 1963; Brown, 1984; Clercq, 1986; Griengl et al., 1987), there has recently been remarkable interest in the synthetic manipulations of pyrimidines (Hirota et al., 1981; Su et al., 1986). In addition to these, the diverse pharmacological properties of pyrimidines and bridgehead nitrogen heterocycles as fungicides (Matolcsy, 1971), viricides (Commner, 1951; Mercer, 1953), bactericides (Pershin et al., 1972), herbicides (Magialli et al., 1983; Brown, 1984), and leishmanicides (Wang, 1984; Ram, 1988) aroused considerable interest to synthesize π-deficient pyrimidines linked with π-rich heterocycles as a variant of the purine system, in anticipation that new compounds would exhibit pronounced antifungal properties. Sometimes the fusion of the heterocyclic nucleus enhances the pharmacological activities many fold over that of its parent nucleus. Antimicrobial properties of azoles such as oxadiazoles, thiadiazoles and thiazoles in isolated as well as fused states are well-known (Hill, 1984; Kornis, 1984; Metzger, 1984). In view of these observations and in continuation of our efforts (Khan and Rastogi, 1990) on the synthesis of nitrogen heterocycles of chemical and biological interest, we report herein the synthesis of π-deficient pyrimidines linked with π-rich heterocycles to prove how this combination could enhance antifungal action.

This paper describes the strategy to synthesize fused pyrimidine ring systems by the condensation–cyclization reaction of 2-aminoheterocycles with different Michael acceptors; the reaction sequence is outlined in Scheme I. The reaction of 2-amino-5-(2′,4′-dichlorophenyl)-1,3,4-oxa/thiadiazoles (Ia,b) and 2-amino-4-(2′,4′-phenyl)thiazole (Ic) with ethyl acetoacetate in the presence of polyphosphoric acid gave 7-methyl-2-(2′,4′-dichlorophenyl)-1,3,4-oxa/thiadiazolo[3,2-*a*]pyrimidin-5-ones (IIa,b) and 7-methyl-3-(2′,4-dichlorophenyl)thiazolo[3,2-*a*]pyrimidin-5-one (IIc), respectively. The treatment of Ia, Ib, and Ic with diethyl malonate in the presence of phosphorus oxychloride and polyphosphoric acid at elevated temperature afforded 7-chloro-2-(2′,4′-dichlorophenyl)-1,3,4-oxa/thiadiazolo[3,2-*a*]pyrimidin-5-ones (IIIa,b) and 7-chloro-3-(2′,4′-dichlorophenyl)thiazolo[3,2-*a*]pyrimidin-5-one (IIIc), respectively, whereas on refluxing Ia, Ib, and Ic in 1,2,4-trichlorobenzene with diethyl malonate and *p*-toluenesulfonic acid furnished 7-hydroxy-2-(2′,4′-dichlorophenyl)-1,3,4-oxa/thiadiazolo[3,2-*a*]pyrimidin-5-ones (IVa,b) and 7-hydroxy-3-(2′,4′-dichlorophenyl)thiazolo[3,2-*a*]pyrimidin-5-one (IVc), respectively.

Diethyl ethoxymethylenemalonate (EMME) as a synthon (Paudler and Kress, 1970) has attracted considerable interest because of its versatility as a reagent in the development of various heterocyclic systems. 6-Carboethoxy-2-(2′,4′-dichlorophenyl)-1,3,4-oxa/thiadiazolo[3,2-*a*]-pyrimidin-5-ones (Va,b) and 6-carboethoxy-3-(2′,4′-dichlorophenyl)thiazolo[3,2-*a*]pyrimidin-5-one (Vc) were obtained by condensation cyclization of Ia, Ib, and Ic with EMME, respectively. Similarly Ia, Ib, and Ic on treatment with dimethyl acetylenedicarboxylate furnished 5-carbomethoxy-2-(2′,4′-dichlorophenyl)-1,3,4-oxa/thiadiazolo-[3,2-*a*]pyrimidin-7-ones (VIa,b) and 5-carbomethoxy-3-(2′,4′-dichlorophenyl)thiazolo[3,2-*a*]pyrimidin-7-one (VIc) analogues, respectively. The structural assignments of the synthesized compounds were based on their elemental analyses and IR, PMR, and mass spectral data (Table I).

Antifungal Screening. The antifungal activity of the compounds (IIa–c–VIa–c) has been evaluated on *Helminthosporium oryzae*, *Aspergillus niger*, and *Fusarium oxysporium* at three concentrations, viz., 10, 100, and 1000 ppm, by agar growth technique (Horsfall, 1945) using Czapek's agar medium. The number of replications in each case was three, and the percentage of inhibition of mycelial growth was calculated according to the formula

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

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

The antifungal activity displayed by the compounds is summarized in Table II. The results were compared with a known commercial fungicide, Dithane M-45 (commonly known as Maneb).

RESULTS AND DISCUSSION

It is evident from the screening data (Table II) that all of the compounds (IIa–c–VIa–c) inhibited more than 65% of growth of all the test fungi at 1000 ppm concentration and hence are antifungal. The most active compounds, IIIc, IVc, Vc, and VIc, exhibited fungicidal action almost equivalent to that of Dithane M-45 at 1000 ppm concentration and inhibited 34–48% of growth of all fungal species even at 10 ppm concentration. It is noteworthy that the thiazole moiety containing compounds (IIIc–VIc) exhibited higher fungicidal activity than the oxadiazole and thiadiazole moiety containing compounds (IIIa,b–VIa,b). The

0021-8561/91/1439-2300$02.50/0

Table I. Characterization Data of Various Compounds (IIa–c–VIa–c) Prepared

compd	mp, °C	yield, %	molecular formula	found (calcd) % of C	H	N	spectral data[a–c]
IIa	205	53	$C_{12}H_7N_3O_2Cl_2$	48.5 (48.8)	2.7 (2.4)	14.4 (14.2)	IR: 1690 ^{1}H NMR: 3.25 (s, 3 H, CH_3), 6.85 (s, 1 H, H_6), 7.45–7.72 (m, 3 H, Ar H) MS: 295 (M^+)
IIb	214–215	55	$C_{12}H_7N_3OSCl_2$	46.7 (46.3)	2.4 (2.2)	13.2 (13.5)	IR: 1680 ^{1}H NMR: 3.30 (s, 3 H, CH_3), 6.95 (s, 1 H, H_6), 7.35–7.76 (m, 3 H, Ar H) MS: 311 (M^+)
IIc	210	58	$C_{13}H_8N_2OSCl_2$	50.6 (50.3)	2.7 (2.6)	9.2 (9.0)	IR: 1705 ^{1}H NMR: 3.28 (s, 3 H, CH_3), 6.90 (s, 1 H, H_6), 7.30–7.81 (M, 4 H, H_2 and Ar H) MS: 310 (M^+)
IIIa	180	45	$C_{11}H_4N_3O_2Cl_3$	41.5 (41.9)	1.5 (1.3)	13.2 (13.3)	IR: 1660 ^{1}H NMR: 6.30 (s, 1 H, H_6), 7.55–7.85 (m, 3 H, Ar H) MS: 315 (M^+)
IIIb	196	48	$C_{11}H_4N_3OSCl_3$	40.2 (39.9)	1.6 (1.2)	12.9 (12.7)	IR: 1675 ^{1}H NMR: 6.35 (s, 1 H, H_6), 7.58–7.72 (m, 3 H, Ar H) MS: 331 (M^+)
IIIc	188	42	$C_{12}H_5N_2OSCl_3$	43.9 (43.6)	1.2 (1.5)	8.2 (8.5)	IR: 1650 ^{1}H NMR: 6.29 (s, 1 H, H_6), 7.56–7.82 (m, 4 H, H_2 and Ar H) MS: 330 (M^+)
IVa	>250	62	$C_{11}H_5N_3Cl_2$	44.8 (44.4)	1.3 (1.7)	14.0 (14.1)	IR: 1700, 3300 ^{1}H NMR: 6.40 (s, 1 H, H_6), 7.12–7.45 (m, 3 H, Ar H) MS: 297 (M^+)
IVb	>250	65	$C_{11}H_5N_3O_2SCl_2$	42.0 (42.2)	1.4 (1.6)	13.1 (13.4)	IR: 1695, 3310 ^{1}H NMR: 6.75 (s, 1 H, H_6), 7.35–7.55 (m, 3 H, Ar H) MS: 313 (M^+)
IVc	>250	58	$C_{12}H_6N_2O_2SCl_2$	46.5 (46.1)	2.2 (1.9)	8.7 (9.0)	IR: 1705, 3320 ^{1}H NMR: 6.65 (s, 1 H, H_6), 6.95–7.45 (m, 4 H, H_2 and Ar H) MS: 312 (M^+)
Va	200	52	$C_{14}H_9N_3O_4Cl_2$	47.3 (47.6)	2.7 (2.5)	11.7 (11.9)	IR: 1715, 1755 ^{1}H NMR: 1.38 (t, 3 H, CH_3), 4.50 (q, 2 H, CH_2), 7.26 (s, 1 H, H_6), 7.45–7.68 (m, 3 H, Ar H) MS: 353 (M^+)
Vb	212	55	$C_{14}H_9N_3O_3SCl_2$	45.2 (45.5)	2.2 (2.4)	11.3 (11.4)	IR: 1695, 1740 ^{1}H NMR: 1.35 (t, 3 H, CH_3), 4.55 (q, 2 H, CH_2), 7.20 (s, 1 H, H_6), 7.55–7.85 (m, 3 H, Ar H) MS: 369 (M^+)
Vc	219–220	45	$C_{15}H_{10}N_2O_3SCl_2$	48.7 (48.9)	2.5 (2.7)	7.9 (7.6)	IR: 1700, 1765 ^{1}H NMR: 1.29 (t, 3 H, CH_3), 4.45 (q, 2 H, CH_2), 6.88 (s, 1 H, H_6), 7.30–7.48 (m, 4 H, H_2 and Ar H) MS: 368 (M^+)
VIa	198	48	$C_{13}H_7N_3O_4Cl_2$	46.3 (46.0)	2.4 (2.1)	12.0 (12.4)	IR: 1680, 1735 ^{1}H NMR: 3.75 (s, 3 H, $COOCH_3$), 6.95 (s, 1 H, H_6), 7.35–7.65 (m, 2 H, Ar H) MS: 339 (M^+)
VIb	208	50	$C_{13}H_7N_3O_3SCl_2$	43.5 (43.9)	1.8 (2.0)	11.5 (11.8)	IR: 1660, 1745 ^{1}H NMR: 3.80 (s, 3 H, $COOCH_3$), 6.85 (s, 1 H, H_6), 7.45–7.70 (m, 3 H, Ar H) MS: 355 (M^+)
VIc	195	55	$C_{14}H_8N_2O_3SCl_2$	47.1 (47.4)	2.0 (2.2)	7.7 (7.9)	IR: 1665, 1755 ^{1}H NMR: 3.76 (s, 3 H, $COOCH_3$), 6.84 (s, 1 H, H_6), 7.25–7.55 (m, 4 H, H_2 and Ar H) MS: 354 (M^+)

[a] IR (KBr), cm^{-1}. [b] ^{1}H NMR ($CDCl_3$–DMSO-d_6), δ. [c] MS/M^+, m/z.

overall results are not as encouraging as one would expect from the combined performance of the two biolabile nuclei, i.e., azole (oxidazole, thiadiazole, and thiazole) and pyrimidine. This may be attributed to the partial saturation in the azole nuclei resulting in the loss of planarity of the oxa/thiadiazolo[3,2-*a*]pyrimidine and thiazolo[3,2-*a*]pyrimidine ring systems. This presumption is supported by the earlier observations that compact size and planarity of a molecule often enhance its pesticidal activity (Chatt, 1956; Fischer and Summers, 1976).

Although an attempt was made to combine various toxophoric groups on these molecules with the hope of achieving compounds for better potency, the results are not very encouraging in all cases. This indicates that the activity of any compound may not be necessarily related to the numerical sum of all toxophores present in the molecule.

EXPERIMENTAL PROCEDURES

All of the melting points (mp) were taken on an electrically heated Buchi melting point apparatus and are uncorrected. IR spectra in potassium bromide disk were recorded on a Perkin-Elmer Model 337 spectrophotometer (ν_{max} in cm^{-1}), ^{1}H NMR spectra in $CDCl_3$ or DMSO-d_6 on a Varian T-60 instrument with TMS as internal standard (chemical shifts in δ), and mass spectra on an AEI-MS 30 mass spectrometer. Purity of all compounds was checked by TLC on silica gel (0.25 mm) plates using benzene–ethyl acetate as irrigant, and spots were located by iodine vapors or $KMnO_4$ spray.

7-Methyl-2-(2′,4′-dichlorophenyl)-1,3,4-oxa/thiadiazolo[3,2-*a*]pyrimidin-5-ones and 7-Methyl-3-(2′,4′-dichlorophenyl)thiazolo[3,2-*a*]pyrimidin-5-one (IIa–c). General Procedure. A mixture of freshly distilled ethyl acetoacetate (3 mL), polyphosphoric acid (11 g), and Ia–c (0.01 mol) was refluxed for 4–5 h, cooled, and neutralized with aqueous sodium

Scheme I

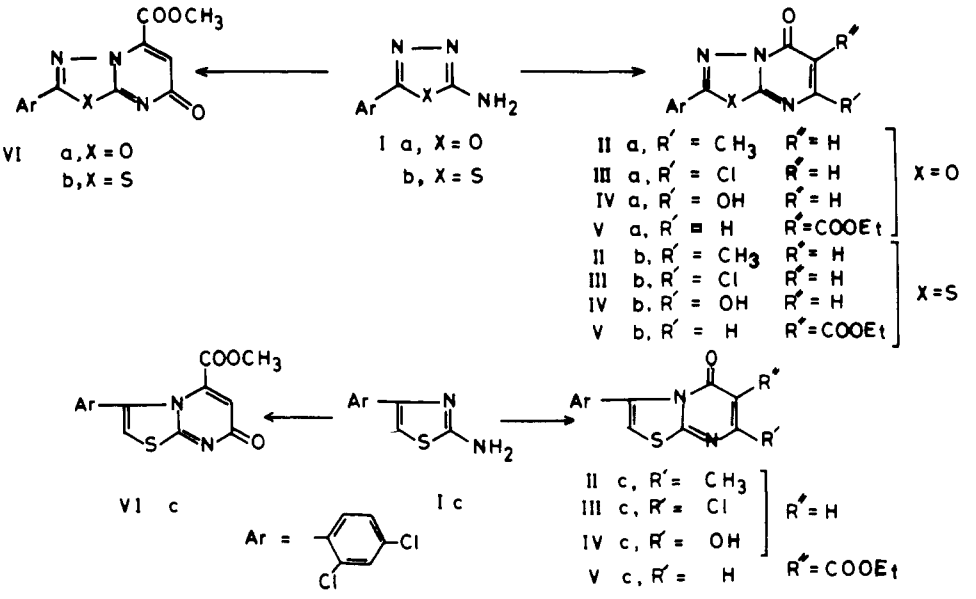

Table II. Fungicidal Screening Results of Compounds IIa–c–VIa–c

	percentage inhibition after 96 h against								
	H. oryzae at			*A. niger* at			*F. oxysporium* at		
compd	1000 ppm	100 ppm	10 ppm	1000 ppm	100 ppm	10 ppm	1000 ppm	100 ppm	10 ppm
IIa	70.5	25.8	17.8	70.4	30.4	12.8	71.3	32.1	13.1
IIb	69.8	30.8	15.8	72.2	29.6	12.2	72.2	30.6	11.2
IIc	78.5	58.4	40.1	78.8	48.8	35.4	70.7	49.3	33.8
IIIa	68.3	40.6	30.5	73.5	32.6	18.3	72.5	30.4	15.0
IIIb	65.2	50.0	40.2	75.8	53.8	39.8	75.4	49.6	47.4
IIIc	90.5	60.5	39.2	96.4	58.6	38.6	77.6	49.6	43.6
IVa	69.7	41.0	40.0	72.4	49.6	39.4	72.6	47.4	42.4
IVb	66.4	37.8	33.2	80.6	54.4	44.4	80.4	54.4	43.4
IVc	92.8	45.6	40.6	98.0	44.6	34.8	99.4	58.4	47.8
Va	72.3	50.6	21.5	73.4	46.4	42.4	72.4	49.4	42.8
Vb	70.5	40.8	18.8	77.4	48.4	36.6	76.6	50.8	48.8
Vc	97.5	58.0	38.8	98.5	53.6	47.8	99.8	53.5	46.6
VIa	74.5	45.4	25.8	75.5	42.5	39.8	74.1	41.4	27.6
VIb	66.8	57.8	26.8	80.8	59.5	48.4	88.8	58.6	47.8
VIc	95.0	60.0	45.0	99.4	44.6	33.8	99.8	53.4	47.4
Dithane M-45	100.0	80.0	64.8	100.0	80.8	66.5	100.0	85.3	68.2

carbonate. The solid thus separated was filtered, washed with water, and crystallized from ethanol to give IIa–c (Table I).

For compounds IIIa–c diethyl malonate was used in place of ethyl acetoacetate along with polyphosphoric acid and phosphorus oxychloride at 120 °C for 3–4 h (Table I).

7-Hydroxy-2-(2′,4′-dichlorophenyl)-1,3,4-oxa/thiadiazolo-[3,2-*a*]pyrimidin-5-ones and 7-Hydroxy-3-(2′,4′-dichlorophenyl)thiazolo[3,2-*a*]pyrimidin-5-one (IVa–c). General Procedure. A mixture of Ia–c (0.01 mol), diethyl malonate (3 mL), and *p*-toluenesulfonic acid (100 mg) in 1,2,4-trichlorobenzene (25 mL) was heated on an oil bath at 240 °C for 2–3 h. The reaction mixture was cooled, and the oily mass was dissolved in ethyl acetate and passed through silica gel to remove gummy material. The solvent was evaporated, and the solid mass thus obtained was refluxed in dry toluene (12 mL) with *p*-toluenesulfonic acid (50 mg) for 48 h to afford IVa–c (Table I), which were crystallized from methanol.

6-Carboethoxy-2-(2′,4′-dichlorophenyl)-1,3,4-oxa/thiadiazolo[3,2-*a*]pyrimidin-5-ones and 6-Carboethoxy-3-(2′,4′-dichlorophenyl)thiazolo[3,2-*a*]pyrimidin-5-one (Va–c). General Procedure. A solution of Ia–c (0.91 mol) and EMME (0.01 mol) in Dowtherm A (20 mL) was heated to 200 °C for 8–9 h. Dowtherm A was removed by washing with *n*-hexane. The residue obtained was purified by crystallization from a mixture of chloroform–hexane (1:1) (Table I).

Compounds VIa–c were similarly prepared but in place of EMME dimethyl acetylenedicarboxylate in 1,2-diethoxyethane was used at reflux temperature for 6–8 h (Table I).

ACKNOWLEDGMENT

We are thankful to Dr. J. N. Baruah, Director, Regional Research Laboratory, Jorhat, for providing necessary facilities and to the Analytical Division for recording spectra. We thank Dr. R. A. K. Srivastava, Biochemistry Division, who helped us to carry out the antifungal activity.

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Received for review April 5, 1991. Revised manuscript received August 9, 1991. Accepted August 23, 1991.