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Synthesis and Fungitoxicity of Some Pyrimidine Derivatives

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A series of 12 pyrimidine derivatives were prepared and tested in vitro against growth, sporulation and nucleic acids of *Fusarium oxysporum* f. sp. *lycopersici* and *Helminthosporium oryzae*. Introduction of thiazole ring together with two aryl groups to 2-aminopyrimidine induced drastic toxicity for both fungi. Pyrimidine derivatives with aryl groups were less toxic. Nitro groups were found to enhance the toxicity of the pyrimidine derivatives especially when substituted in ortho-position of the aryl groups. Inhibition of nucleic acids synthesis of both fungi was attributed mainly to the presence of thiazole ring.

Key words: Pyrimidine derivatives, Fungitoxicity, Thiazole ring

INTRODUCTION

Many heterocyclic compounds were validated as fungicides (Nezu *et al.*, 1986; Huxley *et al.*, 1990), herbicides (Ebert and Dumford, 1976: Esser *et al.*, 1976; Cremlyn, 1978), insecticides (Kuhr and Dorough, 1976; Lowen, 1991) and bacteriocides (Hammad *et al.*, 1990; Mehta *et al.*, 1990; Awad *et al.*, 1991). The combination of nitro group(s) to the heterocycles have been elucidated to enhance the activity of the biocides (Tsuboi and Honda, 1990). Biocides having thiazolyl rings as active groups could be prepared and have been used to control some pathogens. Kunz and Schurter (1991) prepared 1,2,3-benzothiadiazole derivatives which were useful as plant fungicides, bacteriocides and virucides and were able to control *Puccinia graminis* on wheat.

Pyrimidine derivatives as a class of heterocycles were known to be fungicides. The pyrimidines ethirimol and dimethirimol were some of the earliest truly effective systemic fungicides, being translocated to all parts of the plant and giving excellent control to cereal diseases (Woodcock, 1977). O-Acylation of pyrimidines lead to active compounds which are highly effective on mildew (Cole *et al.*, 1973). Also substances that resemble the components of nucleic acids as pyrimidine analogues have had considerable success against some bacterial species (West, 1988).

The aim of the present investigation is to synthesize some of the aryl and thiazolyl pyrimidines as well as their nitro-derivatives and to evaluate their efficiency as fungicides on growth, sporulation and nucleic acids synthesis of *Fusarium oxysporum* f. sp. *lycopersici* and *Helminthosporium oryzae* which are pathogenic to tomato and rice, respectively.

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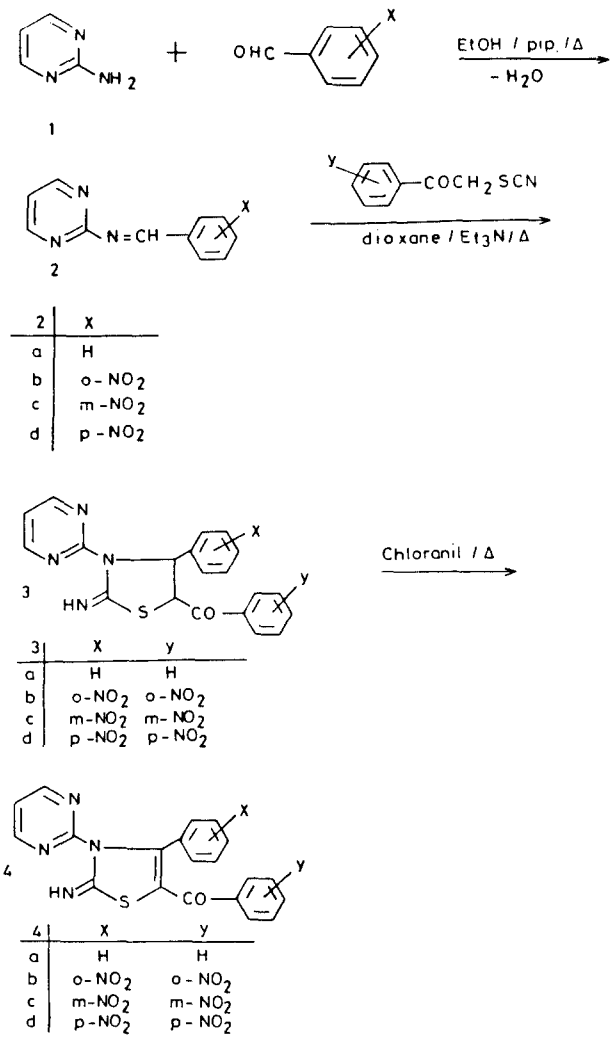
MATERIALS AND METHODS

Chemistry

2-Aminopyrimidine (**1**) condensed with appropriate aromatic aldehyde in ethanol containing few drops of piperidine as catalyst to afford the corresponding Schiff bases **2a-d** (Scheme 1). The assigned structure of **2** was interpreted from their analytical and spectral data (Tables 1 and 2). Thus, e.g., the IR spectra of 2a revealed the absorption band corresponding to the C=N group at 1640 cm^{-1}. Cyclo addition of substituted phenacyl thiocyanates on the Schiff bases **2** was carried out in refluxed dry dioxane in presence of triethylamine as catalyst to afford the corresponding thiazolidinylpyrimidines **3** in good yields. Elemental analyses as well as the spectral data of **3** (Tables 1 and 2) are in accordance with the proposed structure (Scheme 1). Heating **3** with chloranil affected dehydrogenation to yield the corresponding thiazolyl pyrimidines **4a-d**.

Bioassay

Test organisms: The fungi selected for this study were *Fusarium oxysporum* f. sp. *lycopersici* (Sacc) Snyder & Hansen and *Helminthosporium oryzae* [Cochliobolus miyabeanus] (Ito and Kuribayashi) Drechsler ex Dastur. The former organism, an important plant pathogen causing tomato wilt in Egypt, was isolated from infected



Scheme 1.

tomato plants. The latter organism was isolated from infected rice plants.

Pyrimidine derivatives: The pyrimidine derivatives used in the microbiological work were **1**, **2a-d**, **3a-d** and **4a-d.**

Concentrations of pyrimidine derivatives were prepared in aqueous ethanol to give a logarithmic series of concentrations in range 2-256 $\mu g \cdot ml^{-1}$ when further diluted tenfold with the growth media and spore suspension of the test fungi. The toxicity of the pyrimidine derivatives was determined by sporeling bioassay described by Spendley and Ride (1984) which is based on the technique of Skipp and Bailey (1976).

From the results of the assays, graphs were plotted of percentage inhibition of germ-tube growth (with respect to the controls) versus log concentration of each pyrimidine derivative. From this, the concentrations producing 50% inhibition (ED_{50}) and 100% inhibition (MLD) were directly obtained. When the ED_{50} or MLD values exceeded the maximum concentrations of pyrimidine used, extrapolation was performed when the last point was within 5% of the ED_{50} or MLD line, otherwise the result was expressed as >256 μg/ml.

Table I. Physical and analytical data of the newly prepared pyrimidine compounds*

Compd. no.	X	Y	Yield (%)	M.P. °C	Mol. Formula (Mol. wt.)
2a	H	–	88	144-5	$C_{11}H_9N_3$ (183.21)
2b	o-No_2	–	85	188	$C_{11}H_8N_4O_2$ (228.21)
2c	m-NO_2	–	83	205-6	$C_{11}H_8N_4O_2$ (228.21)
2d	p-NO_2	–	79	162	$C_{11}H_8N_4O_2$ (228.21)
3a	H	H	75	143	$C_{20}H_{16}N_4OS$ (360.43)
3b	o-NO_2	o-NO_2	71	159	$C_{20}H_{14}N_6O_5S$ (450.42)
3c	m-NO_2	m-NO_2	74	145	$C_{20}H_{14}N_6O_5S$ (450.42)
3d	p-NO_2	p-NO_2	79	161	$C_{20}H_{14}N_6O_5S$ (450.42)
4a	H	H	65	154	$C_{20}H_{14}N_4OS$ (358.41)
4b	o-NO_2	o-NO_2	61	173	$C_{20}H_{12}N_6O_5S$ (448.40)
4c	m-NO_2	m-NO_2	59	139	$C_{20}H_{12}N_6O_5S$ (448.40)
4d	p-NO_2	p-NO_2	65	153	$C_{20}H_{12}N_6O_5S$ (448.40)

*Satisfactory elemental analyes, C, ± 0.3, H, ± 0.3, N, ± 0.2 have been obtained for the newly prepared compounds.

Growth: Since some pyrimidine derivatives are lethal at relatively higher doses and others at lower doses, so comparison of the effect of pyrimidine derivatives on the growth, sporulation and nucleic acids synthesis of the test fungi was undertaken at a concentration of 64 $\mu g \cdot ml^{-1}$.

A series of conical flasks (250 ml capacity) containing 50 ml Czapek-Dox liquid medium were used for each fungus. Each of three flasks was supplemented with 64 $\mu g \cdot ml^{-1}$ of each pyrimidine derivatives. The flasks were inoculated with a 5 mm diameter agar disc cut from the margin of actively growing colonies. The flasks were incubated at 28°C for 7 days after which the produced mycelial felts were collected, washed several times with distilled water and oven-dried at 80°C till constant weight.

Sporulation: Plates of Czapek-Dox agar supplemented with 64 $\mu g \cdot ml^{-1}$ of each pyrimidine derivatives were inoculated with a 5 mm diameter agar disc of the used fungi. The plates were then incubated for 7 days at 28°C. A 1 cm^2 was cut from the margin of the colony and transferred to a vial containing 10 ml sterile distilled water. The suspension was continuously shaken for 5 min and the density of spores/ml was coun-

Table II. Spectroscopic data of the newly prepared pyrimidine compounds

Compd. no.	IR. (cm^{-1}) (selelcted bands)	^{1}H-NMR (ppm)
2a	2930 (CH arom.); 1630 (C=N)	4.21 (S, 1H, CH); 7.28-7.34 (m, 8H, arom. protons+pyrimidine-H).
2b	2925 (CH arom.); 1635 (C=N)	4.19 (S, 1H, CH); 7.31-7.36 (m, 7H; arom. protons+pyrimidine-H).
2c	2930 (CH arom.); 1635 (C=N)	4.25 (S, 1H, CH); 7.30-7.35 (m, 7H; arom. protons+pyrimidine-H).
2d	2920 (CH arom.); 1640 (C=N)	4.22 (S, 1H, CH); 7.32-7.43 (m, 7H; arom. protons+pyrimidine-H).
3a	3350-3240 (NH); 2950 (CH atom.); 1720 (CO); 1610 (C=N)	1.34 (S, 1H, CH); 1.45 (S, 1H, CH); 7.25-7.61 (m, br, 13H, arom·protons+pyrimidine-H); 10.21-10.32 (S, 1H, NH)
3b	3345-3240 (NH); 2930 (CH arom.); 1715 (CO); 1620 (C=N)	1.35 (S, 1H, CH); 1.44 (S, 1H, CH); 7.24-7.50 (m, br, 11H, arom. protons+pyrimidine-H); 10.11-10.29 (S, 1H, NH)
3c	3350-3250 (NH); 2940 (CH arom.); 1720 (CO); 1620 (C=N)	1.34 (S, 1H, CH); 1.46 (S, 1H, CH); 7.31-7.60 (m, br, 11H, arom. protons+pyrimidine-H); 10.21-10.31 (S, 1H, NH)
3d	3340-3245 (NH); 2930 (CH arom.); 1720 (CO); 1620 (C=N)	1.32 (S, 1H, CH); 1.46 (S, 1H, CH); 7.31-7.58 (m, br, 11H, arom, protons+pyrimidine-H); 10.10-10.21 (S, 1H, NH)
4a	3340-3245 (NH); 2950 (CH arom.); 1710 (CO); 1620 (C=N)	7.28-7.61 (m, br, 13H, arom. protons+pyrimidine-H); 10.13 (S, br, 1H, NH).
4b	3350-3240 (NH); 2950 (CH arom.); 1720 (CO); 1620 (C=N)	7.26-7.59 (m, br, 11H, arom. protons+pyrimidine-H); 10.10 (S, br, 1H, NH).
4c	3350-3240 (NH); 2950 (CH arom.); 1720 (CO); 1620 (C=N)	7.24-7.61 (m, br, 11H, arom. protons+pyrimidine-H); 10.11 (S, br, 1H, NH).
4d	3340-3240 (NH); 2950 (CH arom.); 1720 (CO); 1620 (C=N)	7.27-7.60 (m, br, 11H, arom. protons+pyrimidine-H); 10.12 (S, br, 1H, NH).

Table III. Measured concentrations of each pyrimidine derivative producing 50% inhibition (ED_{50}) and 100% inhibition (MLD) of *Fusarium oxysporum f* sp. *lycopersici* and *Helminthosporium oryzae*

Pyrimidine	*F. xlysporum f. sp. lycopersici*		*H. oryzae*	
derivative	ED_{50}	MLD	ED_{50}	MLD
1	>256	>256	>256	>256
2a	88	220	68	250
2b	75	188	72	201
2c	77	196	78	199
2d	80	201	73	201
3a	38	109	45	128
3b	22	81	31	99
3c	28	85	36	108
3d	31	88	38	118
4a	12	78	19	78
4b	8	21	10	63
4c	11	36	14	65
4d	11	72	14	68

ted by haemocytometer. Three plates were used for each treatment.

Nucleic acids: The nucleic acids (RNA and DNA) of each fungus were estimated in the mycelia harvested from liquid Czapek-Dox media amended with 64 µg/ml of each pyrimidine derivatives after 7 days of incubation at 28°C. The method used for quantitative determination of RNA is that of Ashwell (1957). It depends on a colorimetric analysis of the ribose sugar using orcinol reaction. The quantitative estimation of DNA was carried out according to the method of Burton (1968). It depends on measuring the color developed after treating the extracted DNA with diphenylamine reagent.

RESULTS AND DISCUSSION

Regardless to the title compound (**1**), all the pyrimidine derivatives showed a significant toxicity which is dependant on their chemical structures (Table 1). The toxicity pattern of the derivatives towards both fungi is similar although the levels of derivatives that were required to produce ED_{50} and MLD for *Helminthosporium oryzae* were almost higher than those required for *Fusarium oxysporum* f. sp. *lycopersici*. Thiazolyl pyrimidine derivatives **4a-d** were more toxic than those of **3a-d**. Derivatives of compound 2 had the least toxicity. The toxicity of the derivatives of each compound gradually increased in the order b>c>d>a. It appears that introduction of an aryl group to 2-aminopyrimidine induces a significant toxicity of the title compound towards the test fungi. Compounds containing aryloxy, arylosulfinyl and arylsulfonyl have been used as fungicides (Elbe *et al.*, 1968). Introduction of thiazole ring together with two aryl groups to 2-aminopyrimidine induces drastic increase in the toxicity for both fungi. The derivatives of unsaturated compound (compound **4**) were more potent than the corresponding saturated ones (compound **3**). The biological activity of thiazole and pyrimidine derivatives against protozoa, spirochetes was tested by Kharchuk *et al.* (1988). They found that some derivatives were very active. Singh *et al.* (1991) prepared thiazolo [2,3-C]-1,2,4-triazole derivati-

Table IV. Effect of 64 μg/ml^{-1} of each pyrimidine derivative on mycelial dry weight, sporulation and nucleic acids of *Fisaroi, oxysporum* f sp. *lycopersici*

Pyrimidine derivative	Mycelial dry weight (mg/50 ml)	Sporulation (spores×10^{-5}/ml of culture)	Nucleic acids (mg/g dry weight) DNA	RNA
Control	400.2	64.8	16.2	0.22
1	436.0	72.8	35.0	0.39
2a	354.9	39.6	32.2	0.31
2b	305.2	25.2	31.3	0.30
2c	315.1	27.4	31.6	0.30
2d	327.6	31.3	29.0	0.31
3a	265.5	26.0	11.3	0.14
3b	215.3	19.1	9.5	0.09
3c	226.4	20.4	9.0	0.11
3d	258.2	23.2	10.3	0.14
4a	136.2	8.2	8.3	0.04
4b	0.0	0.0	0.0	0.03
4c	106.3	3.4	6.5	0.04
4d	130.9	5.3	7.6	0.04
L.S.D*. at 1%	36.2	6.9	5.1	0.06
5%	25.6	4.4	4.0	0.03

*Least significant difference.

Table V. Effect of 64 μg/ml^{-1} of each pyrimidine derivative on mycelial dry weight, sporulation and nucleic acids of *Helminthosporium oryzae*

Pyrimidine derivative	Mycelial dry weight (mg/50 ml)	Sporulation (spores×10^{-5}/ml of culture)	Nucleic acids (mg/g dry weight) DNA	RNA
Control	498.1	22.2	8.1	0.13
1	558.2	36.1	19.2	0.28
2a	416.4	18.2	17.6	0.24
2b	362.6	12.6	16.5	0.20
2c	406.5	14.5	16.2	0.26
2d	411.0	15.6	14.4	0.26
3a	269.0	9.7	3.0	0.08
3b	206.0	7.0	1.6	0.07
3c	222.2	7.1	1.2	0.07
3d	231.7	9.3	1.1	0.07
4a	112.6	3.1	0.76	0.05
4b	0.0	0.0	0.0	0.00
4c	0.0	0.0	0.0	0.00
4d	3.5	2.8	0.51	0.05
L.S.D*. at 1%	37.2	5.1	3.6	0.05
5%	21.0	3.5	1.9	0.03

*Least significant difference.

ves and showed that some derivatives have fungitoxic action against *Aspergillus flavus* and *Fusarium solani.*

The lethal doses of the tested compounds were further lowered when ortho-, meta- or para- position of aryl rings was substituted with NO_2 group. The toxicity increases in the order o-NO_2>p-NO_2>m-NO_2. The nitro-group plays an important role for the antimicrobial activity. Several workers have shown that nitrophenol herbicides reduce the severity of diseases caused by plant pathogens (Porter and Rud, 1980 ; Cerkauskas *et al.*, 1985). Ismail *et al.* (1987) reported that in the phenol and phenolic acid series, toxicity increased with the molecular weight of the compounds in which the hydroxyl groups are arranged in ortho-position to one another on the benzene nucleus. The reverse being true in those which the hydroxyl groups stand in meta-position to one another. Likewise, Shortle *et al.* (1971) indicated that ortho-dihydroxyphenolic compounds were much more inhibitory to *Phialophora melinii* than were meta- or para-hydroxyphenols.

The effect of all pyrimidine derivatives on growth, sporulation and nucleic acids was tested at a concentration of 64 μg/ml^{-1}. Compound 1 allowed good mycelial growth, sporulation and nucleic acids synthesis

by the two fungi under test. This indicates that the two fungi can utilize 2-aminopyrimidine as a nitrogen source. In this connection, West (1988) found that uracil, uridine cystsine, cytidine, deoxycytidine, dihydrouracil and other pyrimidine derivatives could be utilized by *Pseudomonas fluorescens* as a sole nitrogen source.

Although all the derivatives of compound **2** caused inhibition to mycelial dry weight and sporulation of both fungi, however a clear rise in the yield of DNA and RNA was observed as compared to the control. This indicates that the nitroaryl group, introduced to 2-aminopyrimidine, is that causing the inhibition of the growth and sporulation. However, under such inhibitory stress accumulation of nucleic acids by the two fungi is enhanced indicating that the two fungi could degrade nitroarylpyrimidines, at least partially, and use the pyrimidine product as precursor for nucleic acid synthesis. Porter and Rud (1980) showed that the nitrophenol herbicide (Dinoseb and Dyanap) at 1 µg/mg significantly reduced the mycelial growth of *Sclerotinia* minor, the causal agent of *Sclerotinia* blight of peanut. Thiazolidinyl- and thiazolyl-pyrimidines (compound **3** and **4**, respectively) caused more reduction to the mycelial dry weight and sporulation of both fungi as well as RNA and DNA syntheses. This drop in the yield in spite of the high amount of nucleic acids formed by the two fungi grown on thiazole-free pyrimidines indicates that the thiazole ring is that responsible for such drop. This can be indicated by the inhibitory effect of thiazolylpyrimidine on the viral nucleic acids. The virucidal effect of 1,2,3-benzothiadiazole derivatives was proven by Kunz and Schurter (1991). Also Igwegbe and Salary (1977), working on *Sclerotium rolfsii*, showed that the primary effect of 6-methylpurine was inhibition of RNA synthesis and, later, disruption of protein synthesis.

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