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Antimicrobial, Cytotoxicity and Phytotic Effects of 7-(Sub-Phenyl)-6-(Sub-Benzoyl)-5-Methyl-2,4, 7-Trihydro-3,4,8-triazainden-1-ones

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Novel series of ring junction N-substituted pyrimidine derivatives viz. 7-(Sub-phenyl)-6-(sub-benzoyl)-5-methyl-2,4,7-tetrahydro-3,4,8-triazainden-1-ones III(1-16)have been synthesized by Cyclocondensation of 6-Methyl-5-(sub-benzoyl)-4-(sub-phenyl)-3,4-dihydro-1-H-pyrimid-2-one/thiones with amino acid such as glycine by using microwave assisted method in presence of $POCl_3$ + PPA mixture acts both as a catalyst and as a solvent which makes the process eco-friendly, economic and easy. The structures of the synthesized compounds have been conformed on the basis of IR,H^1-NMR,UV and Elemental analysis. Some of them have been screened for their antimicrobial activitity against fungal pathogens like *Aspergillus oryzae* and *Fusarium oxysporum* and bacterial pathogens like *E-coli* (Gram –ve) and *Staphylococci* (Gram +ve) . Also cytotoxicity and phytotic(antifungal) effects of some newly synthesized compounds on crop plants *Vigna radiata* (Green gram) and *Gossypium herbaceum L.* (Cotton) have been checked.

Key words: Pyrimidines, Cyclocondensation, Microwaves, Cytotoxicity, Antimicrobial activity.

The literature survey reveals that the heterocyclic compounds containing nitrogen and sulphur have gained immense importance in human life due to their variety of applications in agricultural,medicinal, pharmacological and industrial values. It has been found that ring junction N-substituted pyrimidine derivatives have been successfully tested against several pathogens and found that they possess fungicidal[1], antibacterial[2], antiviral[3], antiparasitic[4]activity etc. and also remained fore front due to their therapeutic, pharmacological[5], agro-chemicals and veterinary utility[6]. In the last few years microwave-induced organic reaction enhancement (MORE) chemistry has gained popularity as a non-conventional technique for rapid organic synthesis[7]. Many researchers have published the synthetic utility of MORE chemistry in routine organic synthesis[8] published the synthetic utility of MORE chemistry in routine organic synthesis, termed as 'e-chemistry' because the use of microwave energy offers several advantages over conventional heating techniques like many fold reduction in reaction time, easy workup and so cleaner products[9] which believed to be a step towards green chemistry.

Keeping in view the advantages of microwave heating and pyrimidines as a integral part of genetic materials viz. DNA and RNA, in the present investigation novel series of ring junction N-substituted pyrimidine derivatives viz. 7-(Sub-

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phenyl)-6-(sub-benzoyl)-5-methyl -2,4,7-tetrahydro-3,4,8-triaza- inden-1-ones III (1-16) have been synthesized by Cyclocondensation of 6-Methyl- 5-(sub-benzoyl)-4-(sub-phenyl)-3,4-dihydro-1-H-pyrimid-2-one/thiones with amino acid such as glycine by using microwave assisted method in presence of $POCl_3$ + PPA mixture[10]. Some of the newly synthesized compounds were screened for their antimicrobial activitity[11] against fungal pathogens like *Aspergillus oryzae* and *Fusarium oxysporum* and bacterial pathogens like *E-coli* (Gram –ve) and *Staphylococci* (Gram +ve) by using disc diffusion method[12],and also cytotoxicity (seed germination effect)[13] and phytotic effects[14]of some newly synthesized compounds on crop plants *vigna radiata* (Green gram) and *Gossypium herbaceum L.* (Cotton) have been checked.

MATERIAL AND METHODS

All chemicals used were of analytical grade. Melting points were measured in a open glass capillary and are uncorrected. IR spectra in KBr were recorded on instrument model spectrum one, serial number 68515; H1NMR spectra were recorded on Varian mercuryYH-300 MHz spectrometer using TMS as an internal standard and UV-Spectra on a systronic 119 spectrometer. All reactions were monitored by TLC using silica gel 60-F 254 plates. The reactions were carried out in scientific microwave oven (Scientific Microwave system model- RG31L1, 700W, 2450 MHz).Satisfactory CHN analyses were obtained for most of the compounds. Purity of the samples has been checked on HPLC and new protocol has been set up for newly synthesized compounds (Table 1).

Antimicrobial activity

The few newly synthesized compounds were screened in vitro for their antifungal and antibacterial activities against the crop plant pathogens namely *Aspergillus oryzae*, *Fusarium oxysporum*, *E-Coli* (Gram-ve), *Staphylococci* (Gram +ve) by disc diffusion method.The medium was prepared by dissolving 28 gm of ingradients in one liter of distilled water and was sterilized at 121^0C temperature and 15 Ibs/inch pressure in an autoclave for 15 minutes. After sterilization it was cooled down to50^0C and poured into sterile petri discs and allowed to solidify . The media plates were then seeded with 24 hrs. old active nutrient growth culture of the test organism in order to obtain lawn culture.Solutions having the concentrations 100 ppm and 1000 ppm of the compound to be tested were prepared in DMF .Each 0.1 ml of these solutions were added to the wells made on the culture medium using a micropipette. Then plates were incubated at 37 ^{0}C for 18-24 hrs. After incubation the zones of inhibition around the wells were checked and measured (Table -2).

Cytotoxicity(seeed germination effect) Seed infusion technique

From the literature survey it is evident that N-substituted pyrimidine derivatives have a good spectrum of fungicidal, insecticidal and cytotic effects etc. therefore , we decided to study the effect of newly synthesized compounds in the context of a agricultural crop plants.So before cytotic (antifungal) effect, the newly synthesized compound were assayed for their seed germination effect on *Gossypium herbaceum L.* (cotton) and *Vigna radiata* (Green gram) seeds by seed infusion technique.

For seed infusion, dry seeds were immersed for four hours in solvents (DMF&DMSO) containing the synthesized compounds. Seeds were then transferred to a petridish and incubated at 25-30^{o}C temperature and extent of germination was observed after 24 hrs, 48 hrs, 72 hrs (3 days), 96 hrs (4 days), 120 hrs (5 days), 144 hrs (6 days) & 168 hrs (7 days). Observations are given in (table-3). The concentration of solutions used for these experiments was 15 mg/ml.

Phytotic(antifungal)effect
Seed Soaking Method

The pots of black cotton soil were prepared on an open field by using 30x20cm HDPE bags. Required soil was sterilized by using autoclave. Before sowing, seeds were treated with synthesized compounds by immersing dry seeds in solutions of two concentrations (2mg/ml & 3 mg/ml) of DMSO solvent for four hrs. Since cytotoxicity observations shows better results of germination in DMSO solvent than DMF. The seeds of *Gossypium herbaceum L.* (cotton) and *Vigna radiata* (Green gram) under examination

were sowed in these pots(groups-B,D) separately by conventional method. These pots were divided into four group A, B, C & D,

Groups

A) Sterlize soil with untreated seeds
B) Sterlize soil with treated seeds
C) Innoculated soil* with untreated seeds
D) Innoculated soil*with treated seeds

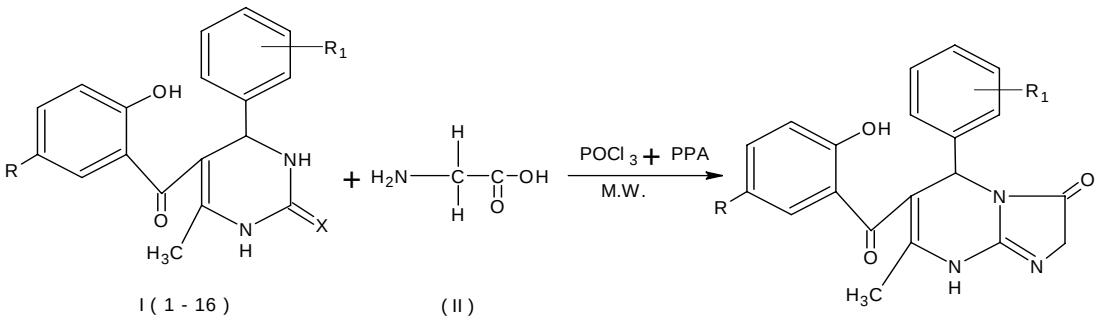
(*Sterlized soil + *Fusarium* or *Rhizoctonia* culture developed on sorghum PDA)These four groups were again grouped into two sets A_1, B_1, C_1 & D_1 and A_2, B_2, C_2 & D_2, for two fungus i.e *Fusarium oxysporum* and *Rhizoctonia*, one crop and for one concentration. The plant pots were irrigated as and when required with tap water. The plants were carefully examined and number of leaves and heights of their shoots were recorded. The data obtained was subjected to analysis of growth parameters at fortnightly intervals of 15, 30, 45 and 60 days .The observations are given in (Table-4).

RESULTS AND DISCUSSION

Antimicrobial studies of some compounds on fungal pathogens like *Aspergillus oryzae* and *Fusarium oxysporum* and bacterial pathogens like *E-coli* (Gram –ve) and *Staphylococci* (Gram +ve) have been carried out for 18-24 hours at 37 ^{0}C for 100ppm and 1000ppm. It was found that most of these compounds had shown remarkable inhibitory activity against noted pathogens in higher concentration. However, the more vigerous observation reveals that, the compounds containing chloro group were more active than those devoid of chlorogroup. The compound (10) was found to exhibit considerable activity against fungus *Fusarium oxysporum*. The compound (14) was found to exhibit considerable activity against bacteria *E. coli* (Gram –ve) and *Staphylococci* (Gram +ve) for both concentration. The compounds (12),(16) were found to exhibit promising activity against *Aspergillus oryzae* and *Fusarium oxysporum* for both concentrations.

Cytotoxicity results for compound (10) showed that the germination percentage in DMF solvent is less for cotton seed than DMSO.For compound (10) in DMSO shows the proper growth of radicle and plumule for both seeds than DMF.The main striking observation is that, control get wilted for the both seeds after 7 days. In control average radicle and plumule length is less than the treated one.

Phytotic (antifungal) study of newly synthesized compound (10) showed more antifungal activity against *Fusarium oxysporum* than *Rhizoctonia*. But antifungal activity of compound (10) was more in high concentration (3mg/ml) than low concentration (2 mg/ml) for both plants.



I (1 - 16) (II) III (1 - 16)

Where,
R = CH_3, Cl
R_1 = H, 2 - OH, 4 - OH, 4 - NO_2
X = O, S

Scheme 1.

Table 1. Synthesized 7-(Sub-phenyl)-6-(sub-benzoyl) -5-methyl-2,4,7-tetrahydro-3,4,8-triazainden-1-ones III(1-16)

Entry	R	R_1	X	M.W. time (min.)	Yield(%)	M.P. (°C)
1	$-CH_3$	H	O	1.5	85	220^0C
2	$-CH_3$	2-OH	O	1.0	87	217^0C
3	$-CH_3$	4-OH	O	1.5	86	237^0C
4	$-CH_3$	$4-NO_2$	O	1.5	82	245^0C
5	$-CH_3$	H	S	1.0	83	207^0C
6	$-CH_3$	2-OH	S	1.5	86	213^0C
7	$-CH_3$	4-OH	S	1.5	85	231^0C
8	$-CH_3$	$4- NO_2$	S	2.0	86	222^0C
9	Cl	H	O	1.5	86	213^0C
10	Cl	2-OH	O	1.0	87	232^0C
11	Cl	4-OH	O	1.0	81	217^0C
12	Cl	$4-NO_2$	O	2.0	82	238^0C
13	Cl	H	S	1.5	81	225^0C
14	Cl	2-OH	S	1.5	84	237^0C
15	Cl	4-OH	S	2.0	86	239^0C
16	Cl	$4-NO_2$	S	2.5	88	258^0C

Table 2. Antimicrobial activity synthesized 7-(Sub-phenyl)-6-(sub-benzoyl)-5methyl-2,4,7-tetrahydro-3,4,8-triazainden-1-ones III(1-16)

S. No.	Tested Compounds	Fungus (Zone of inhibition in mm)				Bacteria (Zone of inhibition in mm)			
		Aspergillus oryzae		*Fusarium oxysporum*		*E. coli* (Gram –ve)		*Staphylococci* (Gram +ve)	
		100 ppm	1000 ppm	100 ppm	1000 ppm	100 ppm	1000 ppm	100 ppm	1000 ppm
1	3	9	11	12	11	-	8	12	13
2	4	14	8	11	19	12	11	12	11
3	7	12	18	-	9	10	18	9	15
4	8	8	14	14	13	15	11	-	19
5	10	20	22	31	34	29	27	30	28
6	11	20	25	29	27	23	20	22	27
7	12	34	35	38	36	22	29	24	29
8	14	21	22	20	23	34	33	36	39
9	15	22	26	20	21	23	24	19	24
10	16	32	30	33	36	23	21	27	28

Table 3. Effect of compound (10) on seed germination

a) For Cotton Seeds Compounds	In DMSO			In DMF		
	Germination (%)	Avg. radicle length (cm)	Avg.plumule length (cm)	Germination (%)	Avg. radicle length (cm)	Avg. plumule length (cm)
Control	100	5.8	2.2	90	3.2	1.7
10	100	7.8	8.6	90	5.3	6.5

Table 3. Effect of compound (10) on seed germination

a) For Green Gram Compounds	In DMSO			In DMF		
	Germination (%)	Avg. radicle length (cm)	Avg.plumule length (cm)	Germination (%)	Avg. radicle length (cm)	Avg. plumule length (cm)
Control	100	3.2	2.7	100	2.8	2.2
10	100	8.9	18.8	100	6.9	15.2

Table 4(a). Phytotic (antifungal) effect of newly synthesized compound (10) on the growth of cultivated crops at conc. 3 mg/ml

Duration	Observations	Fungus-*Rhizoctonia*								Fungus-*Fusarim oxysporum*							
		Green Gram				Cotton				Green Gram				Cotton			
		A1	B1	C1	D1	A2	B2	C2	D2	A1	B1	C1	D1	A2	B2	C2	D2
15 Days	Germination%	80	90	80	90	100	80	80	100	100	100	70	100	100	90	70	100
	No. of leaves	7	10	8	9	9	9	5	7	8	11	11	11	9	9	7	9
	Shoot height (cm)	17	14	14	14	10	11	8	8	21	23	18	21	8	12	6	11
30 days	No. of leaves	16	17	17	18	12	19	10	14	19	26	14	24	21	21	11	15
	Shoot height	21	25	18	27	13	17	11	15	25	29	22	29	21	26	12	19
45 days	No . of leaves	22	30	21	26	18	22	12	19	28	32	21	28	27	26	19	23
	Shoot height	32	36	21	35	21	21	17	19	33	37	27	37	23	29	21	26
60 days	No of leaves	31	41	27	33	22	29	19	25	32	39	25	35	38	31	24	29
	Shoot height	34	40	29	42	24	28	21	27	36	46	29	42	29	34	27	31
90 days	No of leaves	38	45	31	48	26	33	27	29	39	49	32	54	42	38	29	34
	Shoot height	39	49	32	46	29	37	22	34	42	53	33	49	35	39	31	38

Table 4(b). Phytotic (antifungal) effect of newly synthesized compound (10) on the growth of cultivated crops at conc. 2 mg/ml

Duration	Observations	Fungus-*Rhizoctonia*								Fungus-*Fusarim oxysporum*							
		Green Gram				Cotton				Green Gram				Cotton			
		A1	B1	C1	D1	A2	B2	C2	D2	A1	B1	C1	D1	A2	B2	C2	D2
15 Days	Germination%	70	90	70	90	90	80	60	100	90	80	70	90	90	90	60	100
	No. of leaves	6	9	6	9	8	9	5	6	7	11	10	11	8	9	6	8
	Shoot height (cm)	16	12	11	13	10	11	7	8	18	21	17	20	7	11	6	10
30 days	No. of leaves	15	17	15	16	11	19	10	13	17	25	12	22	19	21	10	13
	Shoot height	19	24	18	24	12	17	10	15	24	27	20	27	20	24	11	17
45 days	No. of leaves	21	26	18	24	16	22	12	18	26	31	20	25	25	26	19	22
	Shoot height	29	35	19	31	18	21	16	19	32	36	25	36	22	25	20	25
60 days	No. of leaves	29	36	25	32	21	29	18	25	32	37	24	35	37	30	24	27
	Shoot height	33	38	27	42	22	28	19	26	35	43	27	40	26	32	26	29
90 days	No. of leaves	38	41	31	44	24	33	27	28	36	46	30	50	40	34	27	32
	Shoot height	34	42	30	45	27	36	22	31	42	48	32	46	33	37	30	37

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