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SYNTHESIS AND STUDY OF SOME NEW CHLOROSUBSTITUTED 1,3-THIAZINES AS GROWTH PROMOTING AGENTS ON SOME FLOWERING PLANTS

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Abstract A series of chlorinated 4-aryl-6-alkyl-1,3-thiazines (3a-f) has been synthesized by the reaction of appropriately substituted chlorochalcones (2a-b) and substituted thioureas. The newly synthesized heterocycles (3a-f) were characterized based on their chemical properties and spectroscopic data. The compounds were screened for their *in vitro* antibacterial activity using gram positive and gram-negative bacteria.

Keywords: heterocyclic synthesis, chlorochalcones, 1,3-thiazine, substituted thiourea.

Introduction

For chemistry to have its maximal effect on biology, efficient methods for the discovery of *N*-heterocyclic small molecules are in great demand in the field of chemical genetics [i-iii]. As a privileged fragment, 2,4-dihydro-1*H*benzo[*d*][1,3] thiazine is found in many compounds with remarkable biological activities or as subunit which acts as organic electroluminescent device [iv-ix]. Thiazines is an important class of heterocyclic compounds being studied by many researchers [x-xv] and reported to possess a wide spectrum of biological properties such as Ca^{2+} antagonist [xvi-xvii] blood platelet aggregation inhibitors [xviii] and antiviral [xix], antimicrobial [xx] and antihypertensive [xxi] agents. Moreover, thiazine nucleus is a pharmacophore of cephalosporin's that occupy a very important place in the field of antibiotics [xxii] and the antifungal activity of thiazine nucleus is due to the presence of thiourea linkage in its structure [xxiii].

Encouraged by the diverse biological activities of 1,3-thiazines, a series of 4-(2'-hydroxy-3',5'-dichlorophenyl)-6-alkyl-2-imino/iminophenyl-3-H/Ph-6-H-1,3-thiazines (3a-f) were prepared with an aim to obtain potential antibacterial agents.

Results and discussion

The reaction sequences for the synthesis of title compounds are shown in Scheme I. The starting chalcones 2a,b was dichlorinated at specific positions. Use of these starting materials made

available the preparation of a series of systematically substituted 4-(2'-hydroxy-3',5'-dichlorophenyl)-6-alkyl-2-imino/iminophenyl-3-H/Ph
-6-H-1,3-thiazines (3a-f). The structures of all new chalcones and 1,3-thiazines have been elucidated by elemental analyses, IR and ^{1}H NMR spectroscopic measurements.

The starting material 2-hydroxy-3,5-dichloroacetophenone (1) was obtained by a reported procedure [xxiv] and 2-hydroxy-3,5-dichloro-4-alkylchalcones (2a,b) were prepared from the reaction of 2-hydroxy-3,5-dichloroacetophenone (1) and aliphatic aldehydes (propionaldehyde, valeraldehyde). IR absorption band at 3427.7-3425.9 cm^{-1} indicated the presence of OH. A positive ferric chloride test also indicated that compounds 2a,b has a free hydroxyl group and a band at 1647.8-1635 cm^{-1} showed the presence of a conjugated carbonyl group. The ^{1}H NMR spectrum of 2a,b showed the doublet at δ 7.77-7.78 for (=CH-CO) and multiplet at δ 7.88-7.96 for (=CH-CH_2-).

These 2-hydroxy-3,5-dichloro-4-alkylchalcones 2a,b were treated with substituted thioureas (thiourea, phenylthiourea, diphenylthiourea) respectively to yield 4-(2'-hydroxy-3',5'-dichlorophenyl)-6-alkyl-2-imino/iminophenyl-3-H/Ph-6-H-1,3-thiazines (3a-f). The IR spectrum of the compounds 3a,b exhibited the band due to OH (3354-3321 cm^{-1}), imine (3436 cm^{-1}), cyclic NH (3193-3184 cm^{-1}), C=N (1562-1534 cm^{-1}), C-N (1035-1028 cm^{-1}), C-Cl (794-770 cm^{-1}). In the ^{1}H NMR spectra, it showed a singlet at δ 11.10-11.30 for one proton of imine, singlet at δ 10.80-10.98 for cyclic NH, multiplet at δ 4.9 for one proton of CH-S and δ 4.43-5.56 for ArOH.

Compounds 3c,d showed the IR absorption bands for OH (3378-3356 cm^{-1}), cyclic NH (3175 cm^{-1}), C=N (1585-1574 cm^{-1}), C-N (1035-1028 cm^{-1}), C-Cl (778-768 cm^{-1}). In the ^{1}H NMR spectra, it showed a singlet at δ 10.32-11.00 for cyclic NH, it showed a multiplet at δ 5.2-5.32 for one proton of CH-S and δ 4.58-4.70 for ArOH, which confirmed the formation of compounds 3c,d.

Further, the structures of 3e,f were also confirmed on the basis of elemental analysis, IR and ^{1}H NMR spectral data.

Experimental Section

All melting points reported were determined in open capillaries, expressed in ^{o}C and are uncorrected. Elemental analysis was performed by CDRI Lucknow and results are within ± 0.4 % of calculated values. The IR spectra of the compounds were recorded on a Perkin-Elmer 1000 spectrometer using KBr pellets and are expressed in cm^{-1}. The ^{1}H NMR spectra were recorded on a BRUKER AVANCE II 400 MHz NMR spectrometer using tetramethylsilane as internal standard and chemical shifts are expressed in δ ppm. All reagents were of highest purity commercially available. The purity of all synthesized compounds was checked by TLC using benzene-CCl_4 as developing solvent and visualized with iodine.

1 Preparation of 2-hydroxy-3,5-dichloro-4-alkylchalcone (2a-b)

To the hot solution of 2-hydroxy-3,5-dichloroacetophenone (0.01 mol) (1) and aliphatic aldehydes (propionaldehyde, valeraldehyde) respectively in ethanol (20 ml), 40 % solution of NaOH was added gradually. The mixture was stirred mechanically at room temperature for 1 hr. until it solidified. After 6-8 hrs, it was decomposed with ice-cold HCl (1:1). The precipitate thus obtained was separated by filtration, washed with sodium thiosulphate solution (10 %) and water, dried and purified by crystallization from ethanol

1a 2-Hydroxy-3,5-dichlorophenyl-4-ethylchalcone (2a)

Obtained as pale yellowish orange crystalline solid in 84 % yield, m.p. 88-90 ^{o}C; Anal. Calcd. for $C_{11}H_{10}O_2Cl_2$ (FW 244): C, 54.09; H, 4.09 %. Found: C, 53.89; H, 3.92 %; IR (KBr, cm^{-1}): 3427.7 (OH), 3065.9 (=CH str.), 2925 (CH str. in alkyl), 1647.8 (C=O), 1433.6 (CH_2 bend.), 1304.3 (CH_3 bend.), 801.7 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) (δ ppm): 0.93 (3H, t, CH_3), 2.19 (2H, m, CH_2), 7.43 (2H, dd, ArH), 7.77 (1H, d, =CH-CO), 7.88 (1H, m, =CH-CH_2), 12.61 (1H, s, OH).

1b 2-Hydroxy-3,5-dichlorophenyl-4-butylchalcone (2b)

Obtained as brown solid crystals in 86 % yield, m.p. 102-104 ^{o}C; Anal. Calcd. for $C_{13}H_{14}O_2Cl_2$ (FW 272): C, 57.35; H, 5.15 %. Found: C, 57.26; H, 5.04 %; IR (KBr, cm^{-1}): 3425.9 (OH), 3065.9 (=CH str.), 2925 (CH str. in alkyl), 1647.8 (C=O), 1433.6 (CH_2 bend.), 1304.3 (CH_3 bend.), 801.7 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) (δ ppm): 1.014 (3H, t, CH_3), 2.18-2.43 (6H, m, 3 x CH_2), 7.39 (2H, dd, ArH), 7.78 (1H, d, =CH-CO), 7.96 (1H, m, =CH-CH_2), 13.81 (1H, s, OH).

2 Preparation of 4-(2'-hydroxy-3',5'-dichlorophenyl)-6-alkyl-2-imino/iminophenyl-3-H/Ph-6-H-1,3-thiazines (3a-f)

2-Hydroxy-3,5-dichlorophenyl-4-alkylchalcones (2a,b) (0.01 mol) and subs-
tituted thioureas (thiourea, phenylthiourea, diphenylthiourea) (0.02 mol) respectively were dissolved in ethanol (30 ml). To this aq. KOH, solution (0.02 mol) was added. The reaction mixture was refluxed for three hrs, cooled, diluted with water and acidified with 1:1 HCl. The crude product obtained was filtered off, washed with water dried and purified by recrystallization from ethanol.

2a 4-(2'-Hydroxy-3',5'-dichlorophenyl)-6-ethyl-2-imino-3,6-dihydro-1,3-thiazine (3a)

Obtained as shining light brown needles in 68 % yield, m.p. 97-99 ^{o}C; Anal. Calcd. for $C_{12}H_{11}ON_2SCl_2$ (FW 301): C, 47.84; H, 3.65 %. Found: C, 47.69; H, 3.50 %. IR (KBr, cm^{-1}): 3354.1 (b, OH), 3436 (imine), 3193.4 (cyclic NH), 1562 (C=N), 1472 (C=C), 1028 (C-N), 794 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) (δ ppm): 1.01 (3H, t, CH_3), 2.15-2.30 (2H, m, CH_2), 4.43 (1H, s, Ar-OH), 4.9 (1H, m, CH-S), 7.36 (2H, dd, ArH), 10.98 (1H, s, cyclic NH), 11.10 (1H, s, imine).

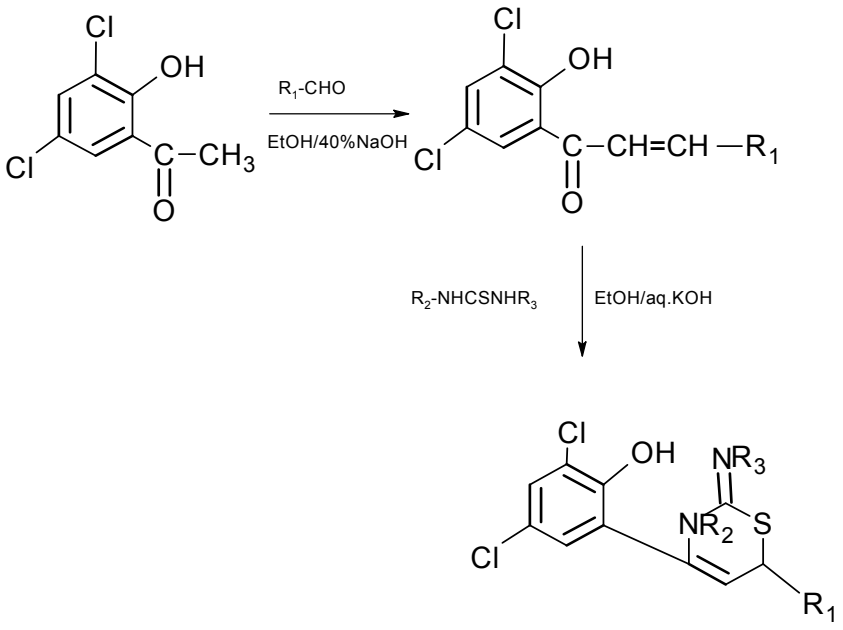
2b 4-(2'-Hydroxy-3',5'-dichlorophenyl)-6-butyl-2-imino-3,6-dihydro-1,3-thiazine (3b)

Obtained as shining brown crystals in 75 % yield, m.p. 89-91 ^{o}C; Anal. Calcd. for $C_{14}H_{15}ON_2SCl_2$ (FW 329): C, 51.06; H, 4.56 %. Found: C, 50.88; H, 4.42 %; IR (KBr, cm^{-1}): 3321.9 (b, OH), 3436 (imine), 3184.9 (cyclic NH), 1534 (C=N), 1498 (C=C), 1035 (C-N), 770 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) (δ ppm): 0.97 (3H, t, CH_3), 2.09-2.46 (2H, m, CH_2), 5.66 (1H, s, Ar-OH), 4.90 (1H, m, CH-S), 6.99 (2H, dd, ArH), 10.80 (1H, s, cyclic NH), 11.30 (1H, s, imine).

2c 4-(2'-Hydroxy-3',5'-dichlorophenyl)-6-ethyl-2-iminophenyl-3,6-dihydro-1,3- thiazine (3c)

Obtained as shining dark brown crystals in 69 % yield, m.p. 104-106 ^{o}C; Anal. Calcd. for $C_{18}H_{17}ON_2SCl_2$ (FW 379): C, 56.99; H, 4.49 %. Found: C, 56.79; H, 4.32 %; IR (KBr, cm^{-1}): 3378 (b, OH), 3175 (cyclic NH), 1574 (C=N), 1475 (C=C), 1028 (C-N), 768 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) (δ ppm): 0.91 (3H, t, CH_3), 2.18-2.33 (2H, m, CH_2), 4.58 (1H, s, Ar-OH), 5.2 (1H, m, CH-S), 7.2 (2H, dd, ArH), 10.32 (1H, s, cyclic NH).

SCHEME



R_1 = -CH_2-CH_3; -$(CH_2)_3$-CH_3
R_2 = (i) = H; (ii) = H; (iii) = Ph
R_3 = (i) = H; (ii) = Ph; (iii) = Ph

2d 4-(2'-Hydroxy-3',5'-dichlorophenyl)-6-butyl-2-iminophenyl-3,6-dihydro-1,3-thiazine (3d)

Obtained as shining dark brown solid crystals in 76 % yield, m.p. 128-130 ^oC; Anal. Calcd. for $C_{20}H_{20}ON_2SCl_2$ (FW 406): C, 59.11; H, 4.93 %. Found: C, 59.02; H, 4.82 %; IR (KBr, cm^{-1}): 3356 (b, OH), 3175 (cyclic NH), 1585 (C=N), 1464 (C=C), 1040 (C-N), 778 (C-Cl); ^1H NMR (400MHz, $CDCl_3$) (δ ppm): 0.96 (3H, t, CH_3), 2.19-2.46 (6H, m, 3 x CH_2), 4.70 (1H, s, Ar-OH), 5.32 (1H, m, CH-S), 7.41 (2H, dd, ArH), 11.0 (1H, s, cyclic NH).

2e 4-(2'-Hydroxy-3',5'-dichlorophenyl)-6-ethyl-2-iminophenyl-6H-3-phenyl-1,3-thiazine (3e)

Obtained as shining chocolate brown crystals in 76 % yield, m.p. 122-124 ^oC; Anal. Calcd. for $C_{18}H_{20}ON_2SCl_2$ (FW 382): C, 56.54; H, 5.24 %. Found: C, 56.42; H, 5.10 %; IR (KBr, cm^{-1}): 3354 (b, OH), 1562 (C=N), 1452 (C=C), 1035 (C-N), 789 (C-Cl). ^1H NMR (400 MHz, $CDCl_3$) (δ ppm): 0.96(3H, t, CH_3), 2.19-2.39 (2H, m, CH_2), 4.60 (1H, s, Ar-OH), 4.90 (1H, m, CH-S), 6.85 (2H, dd, ArH), 7.89 (10H, s, ArH).

2f 4- (2'-Hydroxy-3',5'-dichlorophenyl)- 6-butyl-2- iminophenyl-6H-3- phenyl-1,3-thiazine (3f)

Obtained as shining chocolate brown crystals in 79 % yield, m.p. 94-96 ^oC; Anal. Calcd. for $C_{24}H_{24}ON_2SCl_2$ (FW 458): C, 62.88; H, 5.24 %. Found: C, 62.73; H, 5.13 %; IR (KBr, cm^{-1}): 3370 (b, OH), 1538 (C=N), 1477 (C=C), 1039 (C-N), 794 (C-Cl); ^1H NMR (400 MHz, $CDCl_3$) (δ ppm): 0.92 (3H, t, CH_3), 2.12-2.46 (6H, m, 3 x CH_2), 4.62 (1H, s, Ar-OH), 5.0 (1H, m, CH-S), 7.72 (2H, dd, ArH), 7.64 (10H, s, ArH).

Antibacterial Activity:

The target molecules were screened for their antibacterial activity against the variety of test organisms by disc diffusion method. Initially, susceptibility testing was carried out by measuring the inhibitory zone diameters on nutrient agar (NA), with conventional paper disc method and the inhibitory zone diameters were read and rounded off to the nearest whole numbers (mm) for analysis. The inhibitory effects of compounds (2a,b and 3a-f) against these organisms are given in Table I.

The screening results indicate that compounds 3d, 3e and 3f show promising activity and compounds 2a and 2b poor activity against *P. Solanacearum*. Compounds 3f and 3b show good activity and compounds 2a and 2b low activity against *E. Amylovora*. Compounds 3b, 3d, 3e and 3f show high activity and compounds 2a, 2b, 3a and 3c low activity against *A. Tumefacience*. Compounds 3e and 3f show high activity and compounds 3a, 2b and 3b low activity against *X. Copersteris*.

The zones of inhibition of the reference compounds are also given in Table I. The result indicates the presence of methoxy, chloro, alkyl groups enhanced the antibacterial activity. However, no specific structure activity relationship could be established.

Growth promoting hormonal effects on some flowering plants:

Pre-germinated quality seeds of *Chrysanthemum coronarium, Dahlia pinnata, Verbena officinalis, Iberis amara* were procured from genuine agricultural agencies.

The sowing of seeds of all four flowering plants under examination was done in beds and in earthen pots separately by conventional method. The plants from each bed and earthen pot were divided into two groups *i.e.* A and B were designated as 'control' and 'treated' group respectively. The spraying solution of synthesized 4-(2'-hydroxy-3',5'-dichlorophenyl)-6-alkyl-2-imino/iminophenyl-3-H/Ph-6-H-1,3-thiazines (3a-e) (0.01 dilutions) were sprayed thrice at fortnightly intervals. (15, 30, 45, 60, 75, 90 days on flowering plants from group B). This was done to compare the treated plants of group B with untreated plants of controlled group A. In this context, the samples were collected after 15, 30, 45, 60, 75, 90 days after sowing with special reference to the number of leaves and height of shoots [xxv].

When the first comparison of morphological character was made between those of treated and controlled group plants, it was interesting to note that all the treated plants exhibited remarkable shoot growth and considerable increase in the number of leaves as compared to the untreated ones. When all the treated plants were compared among themselves, it was distinctly observed that the change which is dominant in *Dahlia* than *Indian Pink*. In the initial stage, growth gradually increases but after two weeks it shoots up to a considerable extend in *Dahlia* (Table II-V).

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Table I: Antibacterial activity of compounds (2a,b and 3a-f).

Antibacterial activity				
Diameter of zone of inhibition (in mm)				
Compounds	*P. Solanacearum*	*E. Amylovora*	*A. Tumefacience*	*X. Copersteris*
2a	7	4	6	5
2b	5	4	5	6
3a	8	7	5	8
3b	6	8	8	6
3c	8	7	6	9
3d	12	5	8	8
3e	10	6	12	11
3f	13	12	14	10

Table–II
4-(2'-Hydroxy-3',5'-dichlorophenyl)-6-ethyl-2-imino-3,6-dihydro-1,3-thiazine (3a).

Periodicity of the observation (in days)	*Chrysanthemum coronarium*				*Verbena officinalis*				*Iberis amara*				*Dahlia pinnata*			
	Shoot height		No. of Leaves		Shoot Height		No. of leaves		Shoot Height		No. of leaves		Shoot Height		No. of leaves	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
15	5	6	5	10	8	10	11	13	6	9	1	2	8	16	10	16
30	10	12	13	18	11	13	15	17	7	13	2	3	26	37	17	26
45	17	20	21	27	14	16	18	25	11	16	4	5	49	65	27	39
60	23	28	33	41	17	19	23	30	15	21	6	8	68	96	38	60
75	29	38	46	58	20	22	29	39	20	25	9	11	75	109	62	101
90	33	41	55	64	22	25	35	51	24	28	10	13	96	117	76	130

C = Control T = Treated

Table–III
4-(2'-Hydroxy-3',5'-dichlorophenyl)-6-butyl-2-iminophenyl-3,6-dihydro-1,3-thiazine (3d).

Periodicity of the observation (in days)	*Crysanthemum coronarium*				*Verbena officinalis*				*Iberis amara*				*Dahlia pinnata*			
	Shoot height		No. of Leaves		Shoot Height		No. of leaves		Shoot Height		No. of leaves		Shoot Height		No. of leaves	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
15	5	8	7	9	10	13	10	14	3	5	6	9	16	22	12	18
30	8	12	11	15	13	16	13	18	4	7	10	12	25	28	22	32
45	13	16	17	20	17	19	20	25	7	10	14	17	44	53	28	46
60	15	20	22	27	18	24	25	30	9	13	19	23	50	65	49	60
75	21	25	28	32	22	29	31	36	11	15	24	27	62	73	58	68
90	25	31	36	40	30	37	35	41	13	18	26	30	71	89	73	81

Table–IV
4-(2'-Hydroxy-3',5'-dichlorophenyl)-6-ethyl-2-iminophenyl-6H-3-phenyl-1,3-thiazine (3e)

Periodicity of the observation (in days)	*Crysanthemum coronarium*				*Verbena officinalis*				*Iberis amara*				*Dahlia pinnata*			
	Shoot height		No. of Leaves		Shoot Height		No. of leaves		Shoot Height		No. of Leaves		Shoot Height		No. of leaves	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
15	7	9	11	14	6	8	11	14	1	2	5	8	12	17	11	19
30	11	14	15	18	7	10	13	18	2	3	8	12	22	33	18	27
45	14	18	20	25	10	13	19	25	4	6	10	15	36	58	31	45
60	20	22	25	31	13	16	23	29	6	7	14	20	54	78	42	68
75	25	27	33	37	16	19	31	33	7	9	19	24	72	100	69	75
90	31	39	48	50	19	22	33	37	8	10	23	27	101	125	83	92

Table–V
4-(2'-Hydroxy-3',5'-dichlorophenyl)-6-butyl-2-iminophenyl-6H-3-phenyl-1,3-thiazine(3f)

Periodicity of the observation (in days)	*Crysanthemum coronarium*				*Verbena officinalis*				*Iberis amara*				*Dahlia pinnata*			
	Shoot height		No. of Leaves		Shoot Height		No. of leaves		Shoot Height		No. of Leaves		Shoot Height		No. of leaves	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
15	4	6	8	11	4	7	12	15	3	4	7	10	18	26	14	21
30	8	10	12	15	7	10	16	18	5	7	9	13	42	75	32	55
45	11	16	21	23	10	13	21	26	8	10	16	20	83	130	62	71
60	17	21	30	34	13	16	24	32	8	11	21	25	119	178	75	89
75	22	26	43	45	15	18	30	39	10	14	23	27	150	210	84	94
90	25	34	49	59	17	21	35	48	13	17	26	30	193	271	98	110