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Synthesis and Antimicrobial Evaluation of Potent 6*H*-2-Amino-4,6-diaryl-1,3-thiazines

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In a ethanolic solution of 3′,5′-dichloro-2′-fluorochalcone (**1a**) and thiourea (**2**) when refluxed with aq. KOH for 5 h 6*H*-2-amino-4,6-diaryl-1,3-thiazines is formed. Likewise the chalcones **1b**, **1c** and **1d** give the respective thiazines **5b**, **5c** and **5d**. The products are analyzed by ^{1}H and ^{13}C NMR spectra. The products are tested for antibacterial and antifungal activity.

Key Words: Antibacterial, Antifungal, 6*H*-2-Amino-4,6-diaryl-1,3-thiazines.

INTRODUCTION

Some 1,3-thiazines and their derivatives[1] are considered as important biological compounds. Generally compounds containing thiazine moiety are effective antibacterial and antifungal agents. The cephem nucleus in the cephalosporin, an antibiotic, contains 1,3-thiazine moiety. The importance of substituted 6*H*-1,3-thiazines as synthetic intermediates is related to their polydentate reactivity. They are known for their use as cepheme[2] precursors and some of their electrophilic properties[3]. There are many reports[4] about the preparation of these compounds by diene synthesis. For example, the reaction between the heterodienes and methylacrylate carried out at 12 to 15 kbar, gives the 6*H*-1,3-thiazines with good yields. A high pressure (4 + 2) cyclocondensation is a selective method for the preparation[5] of 2-heterosubstituted 6*H*-1,3-thiazines. Some 2-substituted 6*H*-1,3-thiazines can also be prepared from γ-isothiocyanatoallylchlorides[6,7]. The antibiotic effectiveness is pronounced when 1,3-thiazines are converted into ketenes. Appropriate acids such as menthoxy acetic acid, butylthio acetic acid, chloroacetic acid and benzotriazolo acetic acid are useful for generating ketenes[8-13]. In this paper we have reported the synthesis of 6*H*-2-amino-4,6-diaryl-1,3-thiazines (**5a-5d**) by the reaction between chloro,

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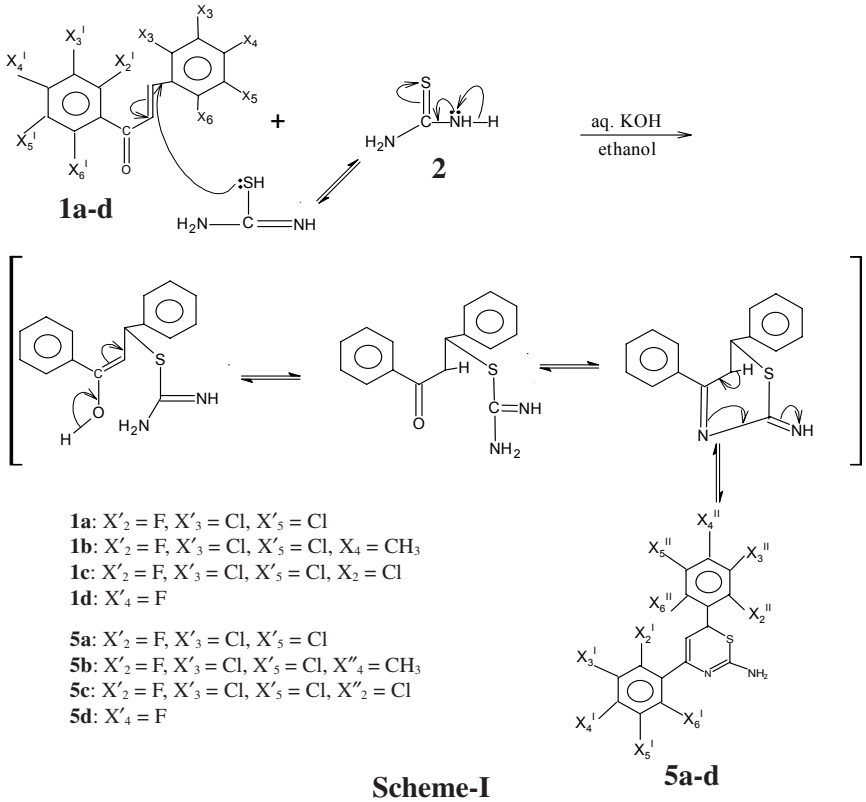
fluoro substituted chalcones (**1a-1d**) and thiourea (**Scheme-I**). The products are analyzed by ^{1}H and ^{13}C NMR spectra. Antibacterial and antifungal activity of the products are also reported.



1a: X'_2 = F, X'_3 = Cl, X'_5 = Cl
1b: X'_2 = F, X'_3 = Cl, X'_5 = Cl, X_4 = CH_3
1c: X'_2 = F, X'_3 = Cl, X'_5 = Cl, X_2 = Cl
1d: X'_4 = F

5a: X'_2 = F, X'_3 = Cl, X'_5 = Cl
5b: X'_2 = F, X'_3 = Cl, X'_5 = Cl, X''_4 = CH_3
5c: X'_2 = F, X'_3 = Cl, X'_5 = Cl, X''_2 = Cl
5d: X'_4 = F

Scheme-I

EXPERIMENTAL

Unless stated otherwise, all the melting points are uncorrected. Pet ether had a boiling range of 60-80 °C. Silica gel (100-200) was used for column chromatography and TLC plates were prepared by usual procedure. Proton NMR spectra were recorded on a Bruker AMX-400 spectrometer operating at 100 MHz. Samples were prepared by dissolving about 10 mg of sample in 0.5 mL of $CDCl_3$ containing 1 % TMS. All the chemical shifts are in reference to TMS. ^{13}C NMR spectra were recorded on a Bruker AMX-400 spectrometer operating at 400 MHz and using 10 mm sample tubes. Solution for the measurement of spectra were prepared by dissolving 0.5 g of the sample in 2.5 mL of $CDCl_3$ containing 1 % TMS. All the chemical shifts are in reference to TMS.

Preparation of 6*H*-2-amino-4-(3′,5′-dichloro-2′-fluorophenyl)-6-phenyl-1,3-thiazine (5a): A mixture of chalcone (**1a**) (1.47g, 5 mmol),

thiourea (0.38 g, 5 mmol) and KOH (0.56 g, 10 mmol in 10 mL of water) in ethanol was refluxed for 5 h. The solvent was removed under reduced pressure and the residue treated with crushed ice. The residue thus separated was subjected to column chromatography. It was first eluted with petrol to remove impurities followed by ethyl acetate-petrol (1:49) mixture. The latter eluates afforded 6*H*-2-amino-4-(3′,5′-dichloro-2′-fluorophenyl)-6-phenyl-1,3-thiazine (**5a**), which was crystallized from ethyl acetate-petrol mixture as white crystals (800 mg), m.p. 229 ºC, m.f. $C_{16}H_{11}N_2SCl_2F$. Elemental analysis (%) Calcd. (Found): C 54.39 (55.14), H 3.12 (3.10), N 7.93 (6.39).

Preparation of 6*H*-2-amino-4-(3′,5′-dichloro-2′-fluorophenyl)-6-(4″-methylphenyl)-1,3-thiazine (5b): A mixture of chalcone (**1b**) (1.54 g, 5 mmol), thiourea (0.38 g, 5 mmol) and KOH (0.56 g, 10 mmol in 10 mL of water) in ethanol was refluxed for 5 h. The solvent was removed under reduced pressure and the residue treated with crushed ice. The residue thus separated was subjected to column chromatography. It was first eluted with petrol to remove impurities followed by ethyl acetate-petrol (1:49) mixture. The latter eluates afforded 6*H*-2-amino-4-(3′,5′-dichloro-2′-fluorophenyl)-6-(4″-methylphenyl)-1,3-thiazine (**5b**), which was crystallized from ethyl acetate-petrol mixture was white crystals (900 mg), m.p. 236 ºC, m.f. $C_{17}H_{13}N_2SCl_2F$. Elemental analysis (%) Calcd. (Found): C 55.69 (55.58), H 3.69 (3.54), N 7.95 (7.63).

Preparation of 6*H*-2-amino-4-(3′,5′-dichloro-2′-fluorophenyl)-6-(2″-chlorophenyl)-1,3-thiazine (5c): A mixture of chalcone (**1c**) (1.64 g, 5 mmol), thiourea (0.38 g, 5 mmol) and KOH (0.56 g, 10 mmol in 10 mL of water) in ethanol was refluxed for 5 h. The solvent was removed under reduced pressure and the residue treated with crushed ice. The residue thus separated was subjected to column chromatography. It was first eluted with petrol to remove impurities followed by ethyl acetate-petrol (1:49) mixture. The latter eluates afforded 6*H*-2-amino-4-(3′,5′-dichloro-2′-fluorophenyl)-6-(2″-chlorophenyl)-1,3-thiazine (**5c**), which was crystallized from ethylacetate-petrol mixture was white crystals (850 mg), m.p. 234 ºC, m.f. $C_{16}H_{10}N_2SCl_3F$. Elemental analysis (%) Calcd. (Found): C 49.61 (48.86), H 2.69 (2.58), N 7.24 (7.23).

Preparation of 6*H*-2-amino-4-(4′-fluorophenyl)-6-phenyl-1,3-thiazine (5d): A mixture of chalcone (**1d**) (1.13 g, 5 mmol), thiourea (0.38 g, 5 mmol) and KOH (0.56 g, 10 mmol in 10 mL of water) in ethanol was refluxed for 5 h. The solvent was removed under reduced pressure and the residue treated with crushed ice. The residue thus separated was subjected to column chromatography. It was first eluted with petrol to remove impurities followed by ethyl acetate-petrol (1:49) mixture. The latter eluates afforded 6*H*-2-amino-4-(4′-fluorophenyl)-6-phenyl-1,3-thiazine (**5d**), which was crystallized from ethyl acetate-petrol mixture was white crys-

tals (900 mg), m.p. 238 ºC, m.f. $C_{16}H_{13}N_2SF$. Elemental analysis (%) Calcd. (Found): C 68.12 (67.61), H 4.99 (4.58), N 10.16 (9.86).

Preparation of media: Nutrient broth was used to cultivate bacteria. Agar media was prepared by adding 24 % w/v agar in the nutrient broth for making agar slants. Bacteria were sub-cultured on the nutrient agar slants. The inoculum was prepared by transferring loopfull of the corresponding organism from the stock culture into the sterile broth and incubated at 37 °C for bacterial strains. 20 mL of sterile nutrient agar media was added to each petri dish and 2 mL of 24 h broth culture of bacteria was then added to the respective plates and mixed thoroughly by rotatory motion of the plates. The respective hydrochloride (1,3-thiazines) was dissolved in water in the concentration of 10 mg/mL. The solution was maintained as a stock solution. The different concentrations (100, 200 and 500 ppm) were prepared from the stock solution. Sterile paper disc of 5 mm diameter was saturated with the three different concentrations and such discs were placed in each seeded agar plates. The petri plates were incubated at 37 °C and zones of inhibitions were measured excluding the diameter of the paper disc (5 mm). Control discs were performed with sterile water. At 500 µg/mL concentration the conventional standard antibacterial drug chloramphenicol exhibited 30 ± 0.5 mm zone of inhibition against all the test bacteria.

For the antifungal activity assay, the *in vitro* disc diffusion method is adopted. Sabouraud's Dextrose agar is used to culture the fungi. Peptone water (1 %) is used for fresh culture of all the fungi and are maintained by periodic sub culturing in fresh Sabouraud's Dextrose medium. Plates for Sabouraud's Dextrose medium are prepared with the inocula by adding 1 mL of dilute culture of the test organism. The respective hydrochlorides (1,3-thiazines) was dissolved in water in the concentration of 10 mg/mL. The solution was maintained as a stock solution. The different concentrations (100, 200 and 300 ppm) were prepared from the stock solution. Sterile paper disc of 5 mm diameter is saturated with the three different concentrations and such discs were placed in each seeded agar plates. The petri plates are incubated at 30 °C for 70 h. The inhibition zones were measured excluding the diameter of the paper disc (5 mm). At 500 µg/mL concentration the conventional standard antifungal drug ketoconazole exhibited 20 ± 0.5 mm zone of inhibition against all the test fungi.

RESULTS AND DISCUSSION

Reaction of 3′,5′-dichloro-2′-fluoro chalcone (1a) with thiourea: An ethanolic solution of chalcone (**1a**) when refluxed with 1 molecular equivalent of thiourea (**2**) in aqueous KOH solution for 5 h, gives 6*H*-2-amino-4-(3′,5′-dichloro-2′-fluorophenyl)-6-phenyl-1,3-thiazine (**5a**). The product was characterized by ^{1}H NMR and ^{13}C NMR spectra. ^{1}H NMR

spectrum showed two doublets at δ 5.08 (1H) and δ 5.28 (1H) for C_6-H and C_5-H, respectively. The multiplets in the region of δ 7.28-7.50 represents two phenyl group hydrogens at positions 4 and 6. A singlet at δ 8.13 showed the presence of -NH_2 group. ^{13}C NMR spectrum reveals the presence of two doublets at δ 57.46 (C-6) and δ 105.34 (C-5). The other carbons appear at δ 174.89 (C-N), δ 142.15 (C-4) and δ 128.06-130.34 (aromatic).

Reaction of 3′,5′-dichloro-2′-fluoro-4-methylchalcone (1b) with thiourea: An ethanolic solution of chalcone (**1b**) when refluxed with 1 mole equivalent of thiourea (**2**) in aqueous KOH solution for 5 h, gives 6*H*-2-amino-4-(3′,5′-dichloro-2′-fluorophenyl)-6-(4″-methylphenyl)-1,3-thiazine (**5b**). The product was characterized by ^{1}H NMR and ^{13}C NMR. The ^{1}H NMR spectrum showed two doublets at δ 5.07 (1H) and δ 5.25 (1H) for C_6-H and C_5-H, respectively. The multiplets in the region of δ 7.20-7.38 represents two phenyl at positions 4 and 6. A singlet at δ 7.81 showed the presence of -NH_2 group. ^{13}C NMR spectrum reveals the presence of two doublets at δ 57.22 (C-6) and δ 105.51 (C-5). The other carbons appear at δ 174.85 (C-N), δ 139.31 (C-4) and δ 128.74-130.28 (aromatic).

Reaction of 3′,5′- dichloro-2′-fluoro-2-chlorochalcone (1c) with thiourea: An ethanolic solution of chalcone (**1c**) when refluxed with 1 mole equivalent of thiourea (**2**) in aqueous KOH solution for 5 h, gives 6*H*-2-amino-4-(3′,5′-dichloro-2′-fluorophenyl)-6-(2″-chlorophenyl)-1,3-thiazine (**5c**). The product was characterized by ^{1}H NMR and ^{13}C NMR. ^{1}H NMR spectrum showed two doublets at δ 5.05 (1H) and δ 5.77 (1H) for C_6-H and C_5-H, respectively. The multiplets in the region of δ 7.20-7.39 represents two phenyl groups at positions 4 and 6. A singlet at δ 7.75 showed the presence of -NH_2 group. The ^{13}C NMR spectrum reveals the presence of two doublets at δ 58.87 (C-6) and δ 105.57 (C-5). The other carbons appear at δ 178.41 (C-N), δ 141.60 (C-4) and δ 127.72-128.17 (aromatic).

Reaction of 4′-fluorophenylchalcone (1d) with thiourea: An ethanolic solution of chalcone (**1d**) when refluxed with 1 mole equivalent of thiourea (**2**) in aqueous KOH solution for 5 h, gives 6*H*-2-amino-4-(4′-fluorophenyl)-6-phenyl-1,3-thiazine (**5d**). The product was characterized by ^{1}H NMR and ^{13}C NMR. ^{1}H NMR spectrum showed two doublets at δ 5.15 (1H) and δ 5.70 (1H) for C_6-H and C_5-H, respectively. The multiplets in the region of δ 7.22-7.38 represents two phenyl groups at positions 4 and 6. A singlet at δ 7.78 showed the presence of -NH_2 group. The ^{13}C NMR spectrum reveals the presence of two doublets at δ 51.35 (C-6) and δ 105.56 (C-5). The other carbons appear at δ 180.83 (C-N), δ 142.10 (C-4) and δ 128.15-129.13 (aromatic).

Antibacterial activity: The 6*H*-2-amino-4,6-diaryl-1,3-thiazines (**5a-5d**) are screened for their antibacterial activity. The method used for the present study is disc diffusion method suggested by Maruzella and Percival[14]. The bacterial stains used are *Vibro cholerae, Salmonella typii, Shigella flexneri, Proteus vulgaries, Escherichia coli, Staphylococous aureus, Klebsiella pneummonia, β-Heamolytic streptococcus* and *Pseudomonas*. The results are given in the Table-1. Each value is an average of three determinations. Table-1 reveals that all the four compounds (**5a-5d**) are active against all the bacteria and the compound **5b** is more active, when compared to the other three.

TABLE-1
in vitro INHIBITION PROFILE OF THE COMPOUNDS (**5a-5d**)
AGAINST TEST BACTERIA

Bacteria	Compound **5a** ppm			Compound **5b** ppm			Compound **5c** ppm			Compound **5d** ppm		
	100	200	500	100	200	500	100	200	500	100	200	500
V. cholerae	8	12	17	14	19	24	17	19	24	10	12	15
S. typii	16	19	24	20	24	29	22	25	27	19	24	29
S. flenneri	12	16	21	10	12	16	14	17	21	10	12	15
P. vulgaries	17	21	25	22	29	35	21	26	31	17	20	25
E. coli	12	18	24	16	19	24	17	19	25	15	19	24
S. aureus	17	21	26	14	17	21	Nil	Nil	Nil	17	21	27
K. pneummonia	12	16	21	10	13	19	21	26	29	17	20	23
β-H. streptococcus	24	26	30	34	37	40	Nil	Nil	Nil	18	20	25
Pseudomonas	22	29	33	22	25	29	19	24	28	20	24	26

All values are in millimeter (mm), representing the diameter of the zone of inhibition

TABLE-2
in vitro INHIBITION PROFILE OF THE COMPOUNDS (**5a-5d**)
AGAINST TEST FUNGI

Fungi	Compound **5a** ppm			Compound **5b** ppm			Compound **5c** ppm			Compound **5d** ppm		
	100	200	500	100	200	500	100	200	500	100	200	500
A. flavus	10	14	23	19	24	29	09	15	21	12	17	22
A. niger	23	25	30	27	35	37	16	24	30	23	26	31
A. fumigatus	13	15	25	15	20	26	10	15	21	12	17	26
Mucor	17	23	30	19	24	29	25	30	36	22	28	32
M. gypseum	16	20	28	20	25	30	15	18	27	25	30	33
C. albicans	15	19	27	19	23	31	15	18	23	14	17	28
Rhizopus	14	18	21	18	24	30	16	21	27	13	19	26

All values are in millimeter (mm), representing the diameter of the zone of inhibition

Antifungal activity: The inhibitory effect of the products (**5a-5d**) against selected fungi are studied in detail. The method used for this study is disc diffusion method. The fungal stains used in the study *viz.*, *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Mucor, Microsporum gypseum, Candida albicans* and *Rhizopus*. The fungal response are tabulated in Table-2. Each value is an average of three determinations. From the Table-2, it is known that compound **5b** is more active when compared to other compounds (**5a, 5c, 5d**).

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