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Synthesis and Antimicrobial Screening of Thiadiazole Derivatives

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ABSTRACT

Isatins (**3a-j**) were prepared by using Sandmeyer method and 5-amino-1, 3, 4-thiadiazole-2-thiol (**4**) was obtained by reacting thiosemicarbazide and carbon disulphide. Schiff bases (**5a-j**) were prepared by stirring isatins (**3a-j**) with 5-amino-1, 3, 4-thiadiazole-2-thiol (**4**). The structures of synthesized compounds were confirmed by analytical (C, H, N) and spectral (FT-IR, 1H NMR, 13C NMR and Mass) data. All the synthesized compounds were screened for in vitro antimicrobial activity by agar well diffusion method and for in vitro antitubercular activity by BACTEC radiometric method using M. tuberculosis H37Rv. All the synthesized compounds **5a-j** showed better antibacterial and antifungal activity compared to reference standards ciprofloxacin and fluconazole respectively. Compound **5d** exhibited equipotent antitubercular activity compared to the reference standard streptomycin.

1. Introduction

Isatin derivatives have been found to possess potent wide spectrum of activities like antibacterial, antifungal [1-4], antitubercular [5, 6], anticonvulsant [7, 8], anticancer [9] and antioxidant [10]. A large number of thiadiazole derivatives were reported to exhibit potent antimicrobial activity [11-15]. In view of these observations and in continuation to our work on isatin [16] the work planned to synthesize novel Schiff bases by condensing substituted isatins with thiadiazole to get desired antimicrobial activity.

2. Experimental Methods

2.1 General

All chemicals and reagents used were of laboratory grade and were purchased from Sigma-Aldrich Ltd, Molychem chemicals Ltd, SD Fine chemicals Ltd., India. The melting points are uncorrected; IR spectra were recorded using KBr discs on Shimadzu IR AFFINITY-1 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Advance II 400 NMR spectrometer; chemical shifts are given in units (ppm) related to internal standard Tetramethylsilane (TMS) using solvent DMSO-d_6 at SAIF/CIL Panjab University, Chandigarh. Mass spectrum was recorded on TOF MS spectrometer at SAIF Panjab University, Chandigarh.

2.2 Synthetic Procedures

*2.2.1 General Procedure for Synthesis of Substituted Isatins [17] (**3a-J**) Isonitrosoacetanilide (**2a-J**)*

In a round-bottomed flask chloral hydrate (0.1 mol) was dissolved in 120 mL of water and sodium sulfate (0.1 mol) was added to it. A solution of substituted/unsubstituted aromatic amine (**1a-j**, 0.1 mol), 30 mL of water and 5 mL of concentrated hydrochloric acid was prepared and added to the above solution. Finally, a solution of hydroxylamine hydrochloride (0.1 mol) in 50 mL of water was added to it. The flask was then heated over a water bath for about 40-45 minutes and was further vigorously boiled for 1-2 minutes to complete the reaction. During the heating period, some crystals of isonitrosoacetanilide separated out. The solution was cooled in running water, filtered and air-dried.

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*2.2.1.1 Substituted Indol-2, 3-dione (**3a-j**)*

A round-bottomed flask containing concentrated sulfuric acid (0.1 mol) was warmed to 50 °C and to this dry isonitrosoacetanilide (**2a-j**, 0.1 mol) was added by keeping the temperature between 60–70 °C. After complete addition of the isonitrosoacetanilide the solution was heated to 80 °C for about 10 minutes. The reaction mixture was then cooled at room temperature and poured into crushed ice. The reaction mixture was allowed to stand for about one and half hour. The product was then filtered, washed several times with cold water and air dried. The crude product was recrystallized by suspending it in 10 mL of hot water and to it a solution of 0.93 g of sodium hydroxide in 2 mL of water was added. The solution was stirred and dilute hydrochloric acid was added until a slight precipitate appears. The mixture was filtered and the precipitate was rejected. The filtrate was made acidic to Congo red paper with concentrated hydrochloric acid. The solution was cooled rapidly. The product was filtered, air dried and melting point was determined.

*2.2.2 Synthesis of 5-amino-1, 3, 4-thiadiazole-2-thiol [18] (**4**)*

Thiosemicarbazide (0.022 mol) was dissolved in ethanol (100 mL) and to this anhydrous sodium bicarbonate (0.015 mol) and carbon disulphide (0.018 mol) were added. The reaction mixture was heated at 40 °C for 1 h with stirring and then was refluxed for 6-7 h at 70 °C. The mixture was cooled and excess of ethanol was distilled out. The crude product was acidified by concentrated hydrochloric acid. The greenish-yellow precipitate was filtered, washed with cold water and recrystallized from hot water.

*2.2.3 Synthesis of Schiff bases of 1, 3, 4-thiadiazole-2-thiol [19] (**5a-j**)*

Isatins (**3a-j**, 0.001 mol) were dissolved in alcohol (20 mL) and to this 5-amino-1, 3, 4-thiadiazole-2-thiol (4, 0.001 mol) was added with constant stirring. The reaction mixture was refluxed on water bath for 24-36 h in presence of few drops of glacial acetic acid. The reaction mixture was cooled and poured on crushed ice. The solid thus formed was separated by filtration and recrystallized from appropriate solvents to get respective Schiff bases of 1, 3, 4-thiadiazole-2-thiol (**5a-j**).

2.2.4 Antibacterial and Antifungal Activity (Zone of Inhibition).

The bacterial and fungal strains were procured from National Chemical Laboratory (NCL), Pune, India. The antimicrobial activity was performed by Cup-plate method [20]. All the synthesized compounds were screened against the following bacterial strains: *Bacillus subtilis* ATCC 6633, *Salmonella typhi* ATCC 19430 and *Klebsiella pneumonia* ATCC 13883 using

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ciprofloxacin as reference standard. The compounds were also screened against *Candida albicans* ATCC 10231 using fluconazole as reference standard.

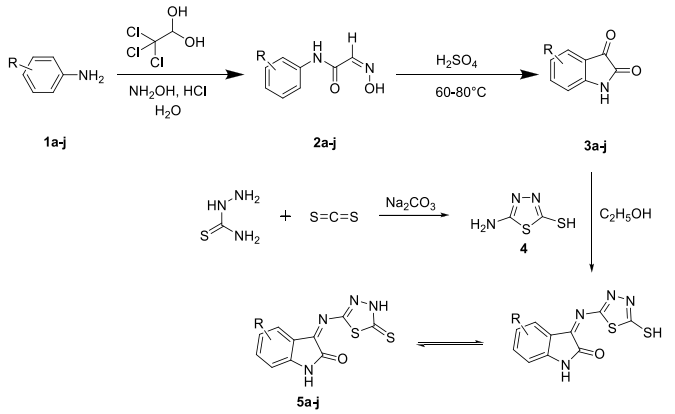
2.2.5 Antitubercular Activity

The antitubercular activity of compounds (**5a-5j**) was tested by *in vitro* BACTEC radiometric method using *M. tuberculosis* $H_{37}Rv$. The antitubercular activity of the compounds was assessed against *M. tuberculosis* using microplate alamar blue assay (MABA). Briefly, 200 µL of sterile deionized water was added to all outer perimeter wells of 96 well plates to minimized evaporation of medium in the test wells during incubation. The 96 well plates received 100 µL of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final compound concentrations tested were 100 to 0.2 µg/mL. Plates were covered and sealed with parafilm and incubated at 37 ℃ for five days. After this time, 25 µL of freshly prepared 1:1 mixture of Almar Blue reagent and 10 % tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth and pink color was scored as growth. The MIC was defied as lowest compound concentration which prevented the color change form blue to pink.

*2.2.6 Physicochemical Data of Synthesized Compounds (**5a-j**)*

*5-Chloro-3-(5-thioxo-4, 5-dihydro-1,3,4-thiadiazol-2-ylimino)-indolin-2-one (**5a**)*

Bright yellow, 81 %, mp 242 °C, IR (KBr, cm^{-1}): 3236, 3107 (NH), 2995 (CH aromatic), 1735 (C=O), 1618 (C=N), 1456 (C=C), 1026 (C=S), 815 (C-Cl), 655 (C-S); ^{1}H NMR (δ, DMSO-d_6): 13.44 (s, 1H, isatin NH), 10.71 (s, 1H thiadiazole NH), 7.93 (s, 1H, Ar-H), 7.30-7.29 (d, 1H, Ar- H), 6.85-6.87 (d, 1H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 164.2 (1C, C=S), 143.4, 141.1 (2C, C=N), 131.6 (1C, C=O), 126.4, 125.9, 116.9, 111.3, 110.9 (6C, Ar-C); MS, (m/z): 296 (M^+). Analysis Calcd for $C_{10}H_5ClN_4OS_2$: C 44.67, H 2.04, N 14.21 %; Found: C 44.69, H 1.98, N 14.17 %.



Scheme 1 Synthesis of Schiff bases (**5a-j**)

*5-Bromo-3-(5-thioxo-4, 5-dihydro-1, 3, 4-thiadiazol-2-ylimino)-indolin-2-one (**5b**)*

Orange brown, 78 %, mp 180 °C, IR (KBr, cm^{-1}): 3230, 3125 (NH), 2991 (CH aromatic), 1745 (C=O), 1612 (C=N), 1458 (C=C), 1051 (C=S), 796 (C-Br), 653 (C-S); ^{1}H NMR (δ, DMSO-d_6): 13.48 (s, 1H, isatin NH), 10.75 (s, 1H thiadiazole NH), 8.19 (s, 1H, Ar-H), 8.034-8.030 (d, 1H, Ar-H), 6.85-6.87 (d, 1H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 183.1 (1C, C=S), 158.7, 149.5 (2C, C=N), 139.6 (1C, C=O), 126.4, 119.2, 114.3, 114.2 (6C, Ar-C); MS, (*m/z*): 341 (M^+). Analysis Calcd for $C_{10}H_5BrN_4OS_2$: C 38.83, H 1.78, N 12.35 %; Found: C 38.79, H 1.81, N 12.31 %.

*6-Chloro-3-(5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-ylimino)-indolin-2-one (**5c**)*

Brown, 85 %, mp >300 °C, IR (KBr, cm^{-1}): 3292, 3178 (NH), 2999 (CH aromatic), 1735 (C=O), 1610 (C=N), 1485 (C=C), 1058 (C=S), 794 (C-Cl), 659 (C-S); ^{1}H NMR (δ, DMSO-d_6): 13.35 (s, 1H, isatin NH), 10.68 (s, 1H thiadiazole NH), 7.86 (s, 1H, Ar-H), 7.28-7.23 (d, 1H, Ar-H), 6.87-6.85 (d, 1H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 163.9 (1C, C=S), 143.7, 140.8 (2C, C=N), 132.9 (1C, C=O), 126.1, 125.8, 117.1, 111.0, 110.5, 110.1 (6C, Ar-C); MS, (*m/z*): 296 (M^+). Analysis Calcd for $C_{10}H_5ClN_4OS_2$: C 44.67, H 2.04, N 14.21 %; Found: C 44.63, H 2.09, N 14.19 %.

*5-Fluoro-3-(5-thioxo-4,5-dihydro-1,3,4-thidiazol-2-ylimino)indolin-2-one (**5d**)*

Orange brown, 79 %, mp 238 °C, IR (KBr, cm^{-1}): 3239, 3112 (NH), 3002 (CH aromatic), 1712 (C=O), 1620 (C=N), 1471 (C=C), 1147 (C-F), 1028 (C=S), 634 (C-S); ^{1}H NMR (δ, DMSO-d_6): 13.39 (s, 1H, isatin NH), 10.60 (s, 1H thiadiazole NH), 8.15 (s, 1H, Ar-H), 7.679-7.670 (d, 1H, Ar-H), 7.64-7.56 (d, 1H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 164.4 (1C, C=S), 158.7, 156.3 (2C, C=N), 138.7 (1C, C=O), 118.0, 117.8, 116.3, 116.2, 113.9, 113.7 (6C, Ar-C); MS, (*m/z*): 280 (M^+). Analysis Calcd for $C_{10}H_5FN_4OS_2$: C 47.30, H 2.17, N 15.04 %; Found: C 47.26, H 2.20, N 14.86 %.

*3-(5-Thioxo-4,5-dihydro-1,3,4-thidiazol-2-ylimino)indolin-2-one (**5e**)*

Brown, 73 %, mp 288 °C, IR (KBr, cm^{-1}): 3253, 3174 (NH), 3008 (CH aromatic), 1732 (C=O), 1618 (C=N), 1469 (C=C), 1056 (C=S), 677 (C-S); ^{1}H NMR (δ, DMSO-d_6): 13.33 (s, 1H, isatin NH), 10.57 (s, 1H thiadiazole NH), 7.69-7.55 (m, 4H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 162.5 (1C, C=S), 143.5, 142.2 (2C, C=N), 138.2 (1C, C=O), 124.5, 122.6, 122.4, 122.2, 120.8, 119.9 (6C, Ar-C); MS, (m/z): 262 (M^+). Analysis Calcd for $C_{10}H_6N_4OS_2$: C 50.56, H 2.70, N 16.08 %; Found: C 50.58, H 2.65, N 16.12 %.

*5-Methyl-3-(5-thioxo-4,5-dihydro-1,3,4-thidiazol-2-ylimino)indolin-2-one (**5f**)*

Orange brown, 73 %, mp 170 °C, IR (KBr, cm^{-1}): 3267, 3169 (NH), 2990 (CH aromatic), 1697 (C=O), 1610 (C=N), 1454 (C=C), 1056 (C=S), 684 (C-S); ^{1}H NMR (δ, DMSO-d_6): 13.13 (s, 1H, isatin NH), 10.93 (s, 1H thiadiazole NH), 7.38-7.36 (d, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 6.81-6.79 (d, 1H, Ar-H), 2.26 (s, 3H, CH_3); ^{13}C NMR (δ, DMSO-d_6): 184.4 (1C, C=S), 161.4, 159.3 (2C, C=N), 148.2 (1C, C=O), 138.6, 131.9, 131.6, 124.6, 117.6, 111.9 (6C, Ar-C), 20.1 (1C, CH_3); MS, (*m/z*): 276 (M^+). Analysis Calcd for $C_{11}H_8N_4OS_2$: C 52.34, H 3.29, N 15.26 %; Found: C 52.28, H 3.33, N 15.21 %.

*7-Methyl-3-(5-thioxo-4,5-dihydro-1,3,4-thidiazol-2-ylimino)indolin-2-one (**5g**)*

Brown, 65 %, mp 250 °C, IR (KBr, cm^{-1}): 3186, 3107 (NH), 2986 (CH aromatic), 1732 (C=O), 1602 (C=N), 1492 (C=C), 1157 (C=S), 794 (C-S); ^{1}H NMR (δ, DMSO-d_6): 12.56 (s, 1H, isatin NH), 11.09 (s, 1H thiadiazole NH), 7.38-7.36 (d, 1H, Ar-H), 7.31-7.29 (d, 1H, Ar-H), 6.97-6.93 (t, 1H, Ar-H), 2.21 (s, 3H, CH_3); ^{13}C NMR (δ, DMSO-d_6): 184.6 (1C, C=S), 159.3, 149.2 (2C, C=N), 151.5 (1C, C=O), 139.3, 122.2, 121.8, 121.5, 117.3, 115.8 (6C, Ar-C), 15.4 (1C, CH_3); MS, (*m/z*): 276 (M^+). Analysis Calcd for $C_{11}H_8N_4OS_2$: C 52.34, H 3.29, N 15.26 %; Found: C 52.32, H 3.36, N 15.27 %.

*4,5-Dichloro-3-(5-thioxo-4,5-dihydro-1,3,4-thidiazol-2-ylimino)indolin-2-one (**5h**)*

Yellow, 93 %, mp 235 °C, IR (KBr, cm^{-1}): 3295, 3169 (NH), 3004 (CH aromatic), 1702 (C=O), 1631 (C=N), 1475 (C=C), 1024 (C=S), 812 (C-Cl), 634 (C-S); ^{1}H NMR (δ, DMSO-d_6): 12.4 (s, 1H, isatin NH), 11.10 (s, 1H thiadiazole NH), 7.68-7.61 (d, 1H, Ar-H), 7.26-7.24 (d, 1H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 140.5 (1C, C=S), 140.9, 139.1 (2C, C=N), 130.2 (1C, C=O), 126.9, 124.0, 121.3, 120.5, 113.6, 111.9 (6C, Ar-C); MS, (*m/z*): 331 (M^+). Analysis Calcd for $C_{10}H_4Cl_2N_4OS_2$: C 44.67, H 2.04, N 14.21 %; Found: C 44.62, H 1.97, N 14.15 %.

*5,7-Dichloro-3-(5-thioxo-4,5-dihydro-1,3,4-thidiazol-2-ylimino)indolin-2-one (**5i**)*

Yellowish brown, 69 %, mp 162 °C, IR (KBr, cm^{-1}): 3242, 3176 (NH), 3006 (CH aromatic), 1658 (C=O), 1620 (C=N), 1454 (C=C), 1039 (C=S), 796 (C-Cl), 644 (C-S); ^{1}H NMR (δ, DMSO-d_6): 12.4 (s, 1H, isatin NH), 10.9 (s, 1H thiadiazole NH), 8.033 (s, 1H, Ar-H), 8.011 (s, 1H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 142.9 (1C, C=S), 141.2, 140.6 (2C, C=N), 138.6 (1C, C=O), 135.3, 134.9, 122.4, 121.4, 115.7, 114.1 (6C, Ar-C); MS, (*m/z*): 331 (M^+). Analysis Calcd for $C_{10}H_4Cl_2N_4OS_2$: C 40.01, H 1.53, N 12.73 %; Found: C 39.96, H 1.56, N 12.66 %.

*5-Nitro-3-(5-thioxo-4,5-dihydro-1,3,4-thidiazol-2-ylimino)indolin-2-one (**5j**)*

Brown, 81 %, mp 183 °C, IR (KBr, cm^{-1}): 3342, 3296 (NH), 3001 (CH aromatic), 1676 (C=O), 1610 (C=N), 1544 (C=C), 1530, 1358 (NO_2), 1111 (C=S), 677 (C-S), 644 (C-S); ^{1}H NMR (δ, DMSO-d_6): 12.2 (s, 1H, isatin NH), 10.6 (s, 1H thiadiazole NH), 8.2 (s, 1H, Ar-H), 7.6-7.3 (m, 2H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 142.1 (1C, C=S), 141.5, 140.3 (2C, C=N), 139.7 (1C, C=O), 134.6, 134.3, 132.8, 125.6, 118.9, 115.4 (6C, Ar-C); MS, (*m/z*): 307 (M^+). Analysis Calcd for $C_{10}H_5N_5O_3S_2$: C 43.13, H 1.97, N 18.29 %; Found: C 43.09, H 2.03, N 18.27 %.

3. Results and Discussion

3.1 Chemistry

In the present work, novel ten Schiff bases were synthesized as outlined in the Scheme 1. The substituted isatins (**3a-j**) and 5-amino-1,3,4-thiadiazole-2-thiol (**4**) were synthesized by reported procedures. Schiff bases (**5a-j**) were obtained by condensation of both the moieties.

The formation of compounds **5a-j** was evidenced by appearance of a band between 1631-1602 cm-1 for C=N in the IR spectra, presence of a peak in ^{13}C NMR spectra with a δ value between 164.4-141.1 for two carbons of C=N. The appearance of a band between 1735-1658 cm^{-1} for C=O of isatin in the IR spectra; a peak in ^{13}C NMR spectra with a δ value between 148.5-131.0 for carbonyl carbon of isatin. The presence of the NH group of isatin was indicated at 3292-3057 cm^{-1} in the IR spectra; presence of a singlet in the ^{1}H NMR spectra at δ value 13.5-12.4. The NH of thiadiazole which undergoes tautomerism was indicated by a band at 3178-2868 cm^{-1} and by a singlet peak at δ value 11.0-9.44 in ^{1}H NMR spectra. The presence of tautomeric form was also confirmed by a sharp band of C=S around 1056 cm^{-1} and a peak at 164.2-184 in ^{13}C NMR spectra.

3.2 Antimicrobial Activity

All the synthesized compounds were screened for *in vitro* antimicrobial activity by Cup-plate method and zone of inhibitions were determined. The results of the activity are presented in Table 1. The results showed that all the compounds exhibited better antibacterial and antifungal activity against the strains selected for study at 25 μg mL^{-1} than the reference standards ciprofloxacin and fluconazole respectively.

Table 1 Antimicrobial activity of Schiff bases (**5a-j**)

Compound	Concentration (μg)	Name of microorganism (Zone of inhibition in mm)				
		S. aureus	B. subtilis	K. pneumonia	S. typhi	C. albicans
5a	100	7	9	10	11	8
	50	6	7	7	10	7
	25	6	6	6	8	5
5b	100	8	8	8	11	7
	50	7	7	6	10	7
	25	6	6	5	8	6
5c	100	5	8	7	5	5
	50	5	6	7	5	5
	25	5	6	6	5	5
5d	100	8	7	6	7	8
	50	6	6	5	6	7
	25	5	5	5	5	6
5e	100	6	5	9	5	5
	50	6	5	7	5	5
	25	5	5	6	5	5
5f	100	5	8	5	8	7
	50	5	6	5	6	7
	25	5	6	5	6	7
5g	100	7	7	5	5	7
	50	6	7	5	5	6
	25	5	6	5	5	6
5h	100	8	7	8	8	5
	50	7	6	6	7	5
	25	6	5	5	5	5
5i	100	6	9	9	5	9
	50	5	5	6	5	8
	25	5	5	5	5	7
5j	100	10	10	9	5	8
	50	9	10	8	5	6
	25	8	7	7	5	5
Ciprofloxacin	100	14	14	16	15	-
	50	13	13	14	12	-
	25	12	12	12	11	-
Fluconazole	100	-	-	-	-	12
	50	-	-	-	-	11
	25	-	-	-	-	10

3.3 Antitubercular Activity

The compounds were also tested for the *in vitro* antitubercular activity by BACTEC radiometric method using *M. tuberculosis* $H_{37}Rv$. The results are presented in Table 2. All the compounds exhibited antitubercular activity at 6.25 – 50 μgmL^{-1} concentrations.

Table 2 Antitubercular activity of Schiff bases (**5a-j**)

Microorganism	Compound	MIC in μg/mL
M. tuberculosis H_{37} Rv (Vaccine strain)	**5a**	50
	5b	25
	5c	12.5
	5d	6.25
	5e	25
	5f	50
	5g	50
	5h	50
	5i	50
	5j	25
	Pyrazinamide	3.125
	Streptomycin	6.25
	Ciprofloxacin	3.125

4. Conclusion

In this study, we report synthesis and characterization of Schiff bases of 1, 3, 4-thiadiazole-2-thiol. The compounds having electron withdrawing substituent at 5th position of isatin i.e. compounds **5a**, **5b**, **5d**, **5f**, **5i** and **5j** exhibited good antibacterial as well as antifungal activity. Compound **5d** exhibited equipotent antitubercular activity as that of standard streptomycin may be due to the presence of electron withdrawing substituent at 5th position of isatin. Compound **5i** exhibited better activity as the presence of two electron withdrawing groups at 5th and 7th position. Compound **5b** exhibited good antibacterial activity and compound **5e** and **5j** showed good activity as presence of nitro and bromo groups respectively.

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