Synthesis and Antimicrobial Screening of Thiadiazole Derivatives

S.G. Shingade*, S.S. Shirodkar

Department of Pharmaceutical Chemistry, P.E.S’s Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi, Ponda, Goa - 403 401, India.

ARTICLE DETAILS

Article history:
Received 18 May 2016
Accepted 12 June 2016
Available online 26 June 2016

Keywords:
Schiff Base
Isatin
Antimicrobial Activity

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 isatin (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], anticoagulant [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.

1H NMR and 13C NMR spectra were recorded on Bruker Advance II 400 NMR spectrometer; chemical shifts are given in units (ppm) related to internal standard Tetramethyloxane (TMS) using solvent DMSO-d6, 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) Isonitrosoacanitainide (2a-j)

In a round-bottomed flask chloral hydrate (0.1 mol) was dissolved in 1.2 mL of water and sodium sulphate (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.

2.2.2.1 Substituted Indol-2, 3-dione (3a-j)

A round-bottomed flask containing concentrated sulfuric acid (0.1 mol) was warmed to 5°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.

2.2.2.2 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 green-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 Antimicrobial 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
5-Flouro-3-(5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-ylimino)-indoline-2-one (5d)

Orange brown, 79 % mp 238 °C, IR (KBr, cm⁻¹): 3239, 3112 (NH), 3002 (CH aromatic), 1712 (C=O), 1620 (C≡O), 1471 (C≡N), 1147 (C-F), 1028 (C≡S), 634 (C=S); H NMR (5, DMSO-d₆): 13.39 (s, 1H, isatin NH), 10.60 (s, 1H thiazole NH), 8.15 (s, 1H, Ar-H), 7.67-7.69 (d, 1H, Ar-H), 7.64-7.56 (d, 1H, Ar-H); ¹³C NMR (5, DMSO-d₆): 164.9 (1C, C=O), 158.7, 156.3 (2C, C=O), 138.7 (1C, C≡O), 118.0, 117.6, 116.3, 113.9, 113.7 (6C, Ar-C); MS, (m/z): 280 (M⁺). Analysis Calcd for C₁₅H₁₀F₂N₂O₂S: C 47.30, H 2.17, N 15.04 %; Found: C 47.26, H 2.20, N 14.86 %.

5-(3-Thioxo-4,5-dihydro-1,3,4-thiadiazol-2-ylimino)-indoline-2-one (5e)

Brown, 73 % mp 298 °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); H NMR (5, DMSO-d₆): 13.33 (s, 1H, isatin NH), 10.57 (s, 1H thiazole NH), 7.69-7.75 (5H, 2H, Ar-H); ¹³C NMR (5, DMSO-d₆): 162.5 (1C, C=O), 143.5, 142.2 (2C, C≡C), 138.2 (1C, C≡O), 124.5, 122.6, 122.4, 122.0, 110.8, 119.9 (6C, Ar-C); MS, (m/z): 262 (M⁺). Analysis Calcd for C₁₅H₁₀N₂O₂S: C 50.56, H 2.60, N 16.02 %.

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

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≡S), 815 (C=C), 665 (C=C); H NMR (5, DMSO-d₆): 13.44 (s, 1H, isatin NH), 10.71 (s, 1H thiazole NH), 7.93 (s, 1H, Ar-H), 7.30-7.29 (5H, 2H, Ar-H), 6.85-6.87 (d, 1H, Ar-H); ¹³C NMR (5, DMSO-d₆): 164.2 (1C, C=O), 143.4, 141.1 (2C, C≡C), 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 C₁₇H₁₂ClN₂O₂S: 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=C, Br), 653 (C=S); H NMR (5, DMSO-d₆): 13.48 (s, 1H, isatin NH), 10.75 (s, 1H thiazole NH), 8.19 (s, 1H, Ar-H), 8.03-8.03 (d, 1H, Ar-H), 6.85-6.87 (d, 1H, Ar-H); ¹³C NMR (5, DMSO-d₆): 183.1 (1C, C=O), 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 C₁₇BrH₁₀N₂O₂S: 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): 3232, 3178 (NH), 2999 (CH aromatic), 1735 (C=O), 1610 (C=N), 1485 (C≡C), 1058 (C≡S), 794 (C=C), 659 (C=S); H NMR (5, DMSO-d₆): 13.35 (s, 1H, isatin NH), 10.68 (s, 1H thiazole NH), 7.86 (s, 1H, Ar-H), 7.28-7.23 (d, 1H, Ar-H), 6.87-6.85 (d, 1H, Ar-H); ¹³C NMR (5, DMSO-d₆): 163.9 (1C, C=O), 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 C₁₇H₁₁ClN₂O₂S: C 44.67, H 2.04, N 14.21 %; Found: C 44.63, H 2.09, N 14.19 %.

5-Nitro-3-(5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-ylimino)-indolin-2-one (5d)

Yellowish brown, 69 %, mp 182 °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=C), 644 (C=S); H NMR (5, DMSO-d₆): 12.4 (s, 1H, isatin NH), 10.9 (s, 1H thiazole NH), 8.03 (s, 1H, Ar-H), 8.011 (s, 1H, Ar-H); ¹³C NMR (5, DMSO-d₆): 144.9 (1C, C=O), 144.2, 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 C₁₇H₁₀N₂O₃S: C 49.01, H 1.58, N 12.75 %; Found: C 49.96, H 1.56, N 12.66 %.

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 1764-1411.4 for two carbons of C. The appearance of a band between 1735-1658 cm⁻¹ for C=O of isatin in the IR spectra; a peak in 1763 C NMR spectra with a δ value between 11.8 and 11.6 for nitrogen in the N-H group. The presence of the NH group of isatin was indicated at δ 129.2-305.7 cm⁻¹ in the IR spectra. The spectrum of a singlet in the 1H NMR spectra at δ value 13.5-12.4. The NH of isatin base 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 1H NMR spectra. The presence of tautomeric form was also confirmed by a sharp band of compound C=O around 1056 cm⁻¹ and a peak at 1642-184 in 13C 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>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (µg)</th>
<th>Name of microorganism (Zone of inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>S. aureus</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>E. coli</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>S. typhi</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>V. cholerae</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>C. albicans</td>
<td>25</td>
<td>6</td>
</tr>
</tbody>
</table>

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 Sa, Sb, Sc, Sd, Se and Sf exhibited good antibacterial as well as antifungal activity. Compound Sg exhibited equipotent antibacterial activity as that of standard ciprofloxacin may be due to the presence of electron withdrawing substituent at 5th position of isatin. Compound Sh exhibited better activity as the presence of two electron withdrawing groups at 5th and 7th position. Compounds Sh showed good antibacterial activity and compound Se and Sj showed good activity as presence of nitro and bromo groups respectively.

Acknowledgment

We would like to thank Principal, PES’s Rajarang and Taraba Bandekar College of Pharmacy, Farmagudi, Ponda, Goa for providing necessary facilities to carry out the research work.

References


