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Synthesis,characterization and antimicrobial activity of Schiff bases of isatin and isatin derivatives

Sourabh Bhardwaj[1,*], Lalit Kumar[2], Ruchi Verma[2] and Umesh Kumar Singh[3]

[1]Department of Prescription Audit, Indraprastha Apollo Hospital, Sarita Vihar, New Delhi, India-110076

[2]Department of Pharmacology, Sikkim Manipal Institute of Medical Sciences, Sikkim Manipal University, 5th Mile, Tadong, Gangtok, E. Sikkim, India-737102

[3]Department of Pharmaceutical Chemistry, Dr. K.N Modi Institute of Pharmaceutical Education and Research, Modinagar, Ghaziabad, Uttar Pradesh India-201204

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ABSTRACT

Several new Schiff's base derivatives were prepared by the combination of Isatin and its prepared derivatives and Isoniazid in the presence of glacial acetic acid and ethanol. All the compounds were characterized by physical and spectral studies. The newly synthesized compounds were evaluated for their antimicrobial activities.

Key words: Isatin, Schiff bases, FTIR, NMR, Antimicrobial,

INTRODUCTION

Isatin is a synthetically versatile substrate that can be used to prepare a large variety of heterocyclic compounds, such as indoles and quinolines, and as a raw material for drug synthesis.[1]

Isatin (1H-indole-2,3 Dione) is an endogenous compound that is widely distributed in mammalian tissues and body fluids. It has been identified in urine, blood and tissue using gas chromatography-mass spectrometry and, more recently, high performance liquid chromatography with an ultraviolet detector.[2]

Isoniazid (INH) is the organic compound that is the first- line antituberculosis medication in prevention and treatment. The compound was first synthesized in the early 20th century, but its activity against tuberculosis was first reported in the early 1950s and three pharmaceutical companies attempted unsuccessfully to simultaneously patent the drug.[3] Isoniazid is available worldwide, is an expensive and is generally well tolerated. It is manufactured from isonicotinic acid, which is produced from 4-methylpyridine.[4]

In recent years, Schiff bases of Isatin are reported to exhibit broad –spectrum chemotherapeutic properties such as antiviral[5], anti-tubercular[6,7], antifungal and antibacterial activities[8]. Investigation of the structure activity relationships in 1-H –indole -2,3-dione derivatives revealed that 5- halogenations[6,9], N-alkylations[7,10] were effective in causing a marked rise in activity against various bacteria, fungi, and virus. Moreover, cyclization of Isatin to 4–Thiazoline[6], 4- Thiazolidinone, and Pyridazindole was efficient in increasing antimicrobial activity.[6]

The present work is oriented towards synthesis of some Schiff bases of Isatin and its prepared derivatives by condensing Isoniazid with glacial acetic acid and ethanol. All the synthesized compounds have been characterized on the basis of their m.p., TLC, FTIR, and 1H NMR spectral data followed by its antimicrobial activity. The main aim of the present work is to find molecules such as these by synthesizing several Schiff bases from Isatin.

MATERIALS AND METHODS

MATERIALS

Isatin was purchased from Fischer Scientific, New Delhi and Isoniazid was purchased from Central Drug House, Pvt. Ltd., New Delhi and all other chemicals was purchased from the SD Fine-chemical Ltd., Mumbai, India. Strains of the bacteria were obtained from the IPC, Ghaziabad, U.P., India.

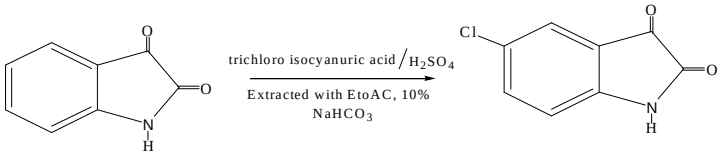
***Corresponding author.**
Sourabh Bhardwaj
Clinical Pharmacist,
Department of Prescription Audit,
Indraprastha Apollo Hospital,
Sarita Vihar, New Delhi, India-110076.

METHODS

1. Synthesis of 5-Isatin Derivatives

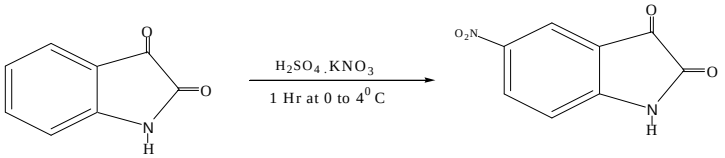
a. Synthesis of 5-Chloro Isatin derivative

The 10 m mol isatin and 3.4 m mol trichloro isocyanuric acid was dissolved in 4 ml of sulphuric acid with continuous stirring at room temperature for 5 min. The resulting solution was firstly neutralized with the addition of cold water followed by its treatment with 15 ml of ethyl acetate as an acid and 20 ml of 10% sodium bicarbonate solution as an alkaline. Then the resulting solution was filtered, washed with saturated NaCl solution and then dried using anhydrous Na_2SO_4. Then the organic solvent was re-evaporated at under reduced pressure and from this 5-chloro isatin was isolated.



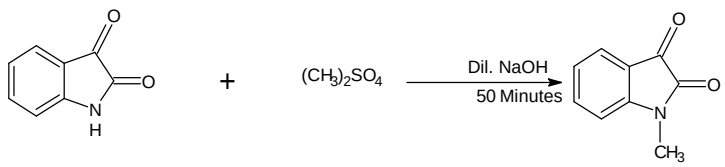
b. Synthesis of 5-Nitro Isatin derivative

3.4 m mol solution of isatin was prepared in 3.2 ml of conc. Sulphuric acid and then this solution was added drop wise into 3.4 m mol solution of nitric acid prepared in 3.8 ml of conc. Sulphuric acid over a period of 1 hour whilst the temperature was maintained between 0 to 4°C. The reaction mixture was poured into 25 ml of ice water and precipitates were collected followed by its washing with water. The crude 5-nitro isatin was purified by re-crystallization with alcohol.



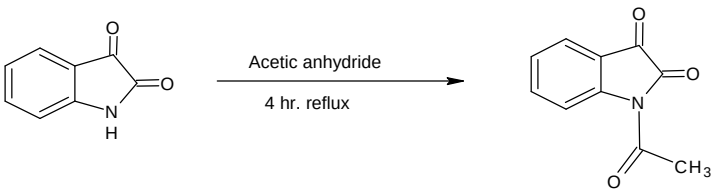
c. Synthesis of N-methyl isatin derivative

5 gm of isatin was dissolved in dilute sodium hydroxide solution then 0.62 ml of Di-methyl sulphate was added. Then whole contents were refluxed in water bath for approximately 50 minutes. After refluxing, the mixture was poured into beaker and cooled in ice. Then the content was evaporated on water bath and dried.



d. Synthesis of N-Acetyl Isatin derivative

0.735 gm of isatin was taken into the round bottom flask and to it 5.1 ml of acetic anhydride was added. Then whole of the content was refluxed for about 4 hours and then the solution was poured into beaker containing crushed ice followed by the filtration and drying of the product.[11]



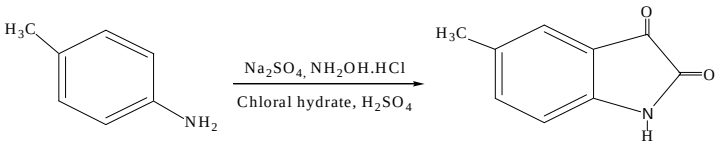
e. Synthesis of 5- Methyl Isatin derivative

Steps 1: Synthesis of isonitrosoaceto-p-toluidine from p-toluidine

9 gm of chloral hydrate was taken into the round bottom flask and dissolved in 120 ml water. To that 13 gm of sodium sulphate, a solution of 5.4 gm of p-toluidine in 30 ml of water containing 5.12 gm of concentrated hydrochloric acid (4.34 ml) to dissolve the amine and solution of 11 gm of hydroxylamine hydrochloride in 50 ml of water were added. Flask was then heated vigorously until the reaction was completed. After it, the solution containing beaker was cooled in running water followed by the filtration of reminder crystallized product with suction pump and air dried.[12]

Step 2: Synthesis of 5-methyl isatin from isonitrosoaceto-p-toluidine

18.4 gm of concentrated sulphuric acid (10.0 ml) was warmed to 50°C and 2.5 gm of dry isonitrosoaceto-p-toluidine was added in such a rate so as to keep the temperature between 60-70°C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly after the addition of isonitroso compound was finished. The solution was heated to 80°C and kept at this temperature for about 10 min to complete the reaction. Then the reaction mixture was cooled to room temperature and poured it into ten times its volume of cracked ice. After standing for 90 min, the final product was filtered with suction pump followed by washing with cold water to remove sulphuric acid and dried in air.[12]



2.Characterization of Synthesized 5-Substituted Isatin Derivatives

The Synthesized 5-substituted isatin derivatives were characterized as percent yield, color and melting point. The percent yield was determined by using equation-1 and melting point was determined by using open capillary tubes.[13]

Percent yield = $(T_p / T_m) \times 100$ ..... (1)

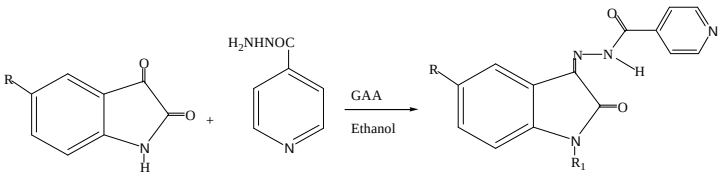
Where,

T_p = Total product found.

T_m = Total raw-material taken.

3.Scheme of Schiff Bases Prepared From Isatin and Synthesized Isatin Derivatives

In this scheme various isatin derivatives reacted with INH in presence of glacial acetic acid and ethanol to yield Isatin Schiff bases.



Isatin

R= H, NO_2, CH_3, Cl

R_1=CH_3, $COCH_3$

Schiff Bases

4.Synthesis of Schiff Bases From Isatin and its Synthesized Isatin Derivatives

A mixture of (0.01 mol) isatin derivative and (0.01 mol) INH were dissolved in 40 ml of ethanol and 2 ml of glacial acetic acid (GAA) (as a catalyst) in a round bottom flask and then whole of the substance was refluxed for about 12-14 hours and then checked for completion by Thin Layer Chromatography (TLC). After refluxing, the whole of the content was poured into cold water. At last, the precipitated product was re-crystallized.

5.Physical Characterizations of Synthesized Schiff Bases

All the prepared Schiff bases compounds were purified by successive re-crystallization using ethanol. Melting points of the synthesized compounds were determined by open capillary method and are uncorrected. The purity of the synthesized compounds was checked by performing thin layer chromatography (TLC) on pre-coated silica gel G (Merck) TLC plates using benzene: methanol (8:2) and spots were located by iodine vapors. The percent yield, colour and solubility studies of the obtained compounds were also determined.[13,14,15,16]

6.Spectral Characterizations of Synthesized Schiff Bases

The IR spectra were obtained on a Shimadzu FTIR – 8400S spectrophotometer using KBr pellets. The ^{1}HNMR spectra in DMSO-d_6 were recorded on Bruker DRX-300 MHz instrument and chemical shift were reported as parts per million (d ppm) down field using tetra-methyl silane (TMS) as internal standard. [13,14,15,16,17]

7.Antimicrobial activity of Schiff bases

All the newly synthesized compounds were evaluated for their *in-vitro* antimicrobial activity against gram negative bacteria (*Klebsiella pneumoniae*, MTCC 10031) and gram positive bacteria (*Bacillus Subtilis*, MTCC 6633). [13,14,15,18,19] Disk cup plate method was used for determination of the antimicrobial activity. Nutrient agar medium was prepared and sterilized by an autoclave. In an aseptic room, they were poured into Petri dishes to a uniform depth of 4 mm and then allowed to solidify at room temperature. After solidification, the test organisms were spread over media with the help of a sterile swab socked in bacterium and used for antibacterial study. Newly synthesized compounds (Schiff bases) were dissolved in DMF (as a control) to produce concentration 100 µg /ml, which were used for study. Gentamycin (100 µg/ml) was used as the standard. Then cup was made with the help of sterile glass borer of size 6 mm and capacity 20 µl in solidified agar in such a way that there is no overlapping of zone of inhibition and different concentrations of each compound and standard drug were placed in each separate Petri dish. Plates were kept at room temperature for half an hour for the diffusion of the sample into the agar media. The organism's inoculated Petri dishes were incubated at 37 °C for 24 hours. After incubation period, zone of inhibition produced by the samples and standard were measured. All tests were performed in triplicate.[18]

RESULTS AND DISCUSSION

Tablet 1. Characterization of Synthesized 5-Substituted Isatin Derivatives

S.No.	Prepared 5-Substituted Isatin derivatives	Color Observation	Percent yield (%)	Melting Point (m.p.) (°C)
1	5-Chloro Isatin	Dark Orange	72	247
2	5-nitro isatin	Dark Orange	47	250
3	N-methyl isatin	Dark Orange	84.5	246
4	N-Acetyl Isatin	Dark Orange	93.50	278
5	5-methyl isatin	Dark Orange	57.85	288

Table 2. Scheme for the Synthesis of Schiff Bases from Isatin and its Derivatives

S. No.	Compound	R	R_1	Isatin or Isatin derivatives (0.01 mole)	INH (mole)	Ethanol (ml)	GAA (ml)
1	4.1A	H	H	Isatin	0.01	40	2
2	4.1B	NO_2	H	5-nitro isatin	0.01	40	2
3	4.1C	CH_3	H	5-methyl isatin	0.01	40	2
4	4.1D	Cl	H	5-Chloro Isatin	0.01	40	2
5	4.1E	H	CH_3	N-methyl isatin	0.01	40	2
6	4.1F	H	$COCH_3$	N-Acetyl Isatin	0.01	40	2

1.Characterization of Synthesized 5-Substituted Isatin Derivatives

Data for the characterization study of prepared 5-substituted Isatin derivatives has ben reported in the Table-1. All the synthesized 5-substituted isatin derivatives were found in dark orange color. The percent yield of the prepared isatin derivatives were found in the range of 47.0 to 93.50%. Almost all the isatin derivatives have same melting point (m.p.) except N-Acetyl Isatin and 5-Methyl Isatin.

Table 3. Physical Characterizations of Synthesized Schiff Bases

Compound	Percent Yield(%)	Color	Solubility	Molecular Formula	Molecular Wt. (gm)	Melting Point (°C)	Refractive Index (R_f)
4.1A	95.45	Orange	DMF,DMSO	$C_{14}H_{10}N_4O_2$	266.259	270	0.83
4.1B	77.66	Yellow	DMF,DMSO	$C_{14}H_9N_5O_4$	311.257	280	0.44
4.1C	57.85	Brown	DMF,DMSO	$C_{15}H_{12}N_4O_2$	280.137	288	0.56
4.1D	53.00	Orange	DMF,DMSO	$C_{14}H_9N_4O_2Cl$	300.705	280	0.67
4.1E	69.28	Yellow	DMF,DMSO	$C_{15}H_{12}N_4O_2$	280.287	240	0.62
4.1F	93.50	Green	DMF,DMSO	$C_{15}H_{12}N_4O_3$	308.297	278	0.66

Table 4. Spectral Characterizations of Synthesized Schiff Bases

S.No	Compound	R	R_1	IR Spectral Data (KBr), (cm^{-1})	^{1}H-NMR Spectral Data (d, ppm)
1	4.1A	H	H	1640 (C=N), 1708 (CONH), 1722 (C=O), 3196 (C-H), 3232 (N-H).	6.9-7.7 (8H, Ar-H),11.38 (1H, NH), 13.63 (1H, =NHCO).
2	4.1B	NO_2	H	1521 (C-NO_2), 1678 (C=N), 1708 (CONH), 1740 (C=O), 3174 (C-H), 3262 (N-H).	6.8-7.8 (7H,Ar-H), 12.04 (1H, NH),13.63(1H,=NH-N-CO).
3	4.1C	CH_3	H	1460 (C-CH_3), 1640 (C=N), 1685 (CONH), 1703 (C=O), 2918 (C-H_{alk}), 3051 (C-H_{ar}), 3259 (N-H).	1.5 (3H , CH_3),6.8-7.8 (7H, Ar-H), 11.04 (1H, NH),13.71 (1H, =N-NH-CO).
4	4.1D	Cl	H	3564 (N-H), 1703 (C=O), 1640 (C=N), 1679 (CONH), 3236 (C-H_{ar}), 3247 (N-H).	7.8-7.9 (7H, m, Ar-H) 11.38 (1H, NH),13.97 (1H, s, C-H).
5	4.1E	H	CH_3	1734 (C=O), 1640 (C=N), 1680 (CONH), 2885 (C-H_{alk}), 3140 (C-H_{ar}), 3269 (N-H).	1.5 (3H,N-CH_3),7.5-8.5 (7H,Ar-H),11.40. (1H, =N-NH-CO) 13.96 (1H,N-H).
6	4.1F	H	$COCH_3$	1640 (C=N), 1685 (CONH), 1722 (C=O), 2956 (C-H_{alk}), 3147 (C-H_{ar}), 3259 (N-H).	6.9-8.8 (7H, Ar-H),11.50 (1H, NH) 13.90 (1H, =N-NH-CO).

Table 5. Antimicrobial Studies of Newly Synthesized Compounds

Compound → / Organism ↓	4.1A	4.1B	4.1C	4.1D	4.1E	4.1F	DMF (Control)	Gentamycin (Standard)
Klebsiella pneumoniae	13	15	13	14	14	18	-	16
Bacillus Subtilis	11	12	10	15	-	11	-	17

2.Scheme for the Synthesis of Schiff Bases from Isatin and its Derivatives

Table-2 shows the scheme for the synthesis of Schiff bases from Isatin and its derivatives. All the Schiff bases were prepared with isatin and its different derivatives, but same concentration of all the isatin and Isoniazid. All the prepared Schiff bases were coded as 4.1A, 4.1B, 4.1C, 4.1D, 4.1E and 4.1F.

3.Physical Characterizations of Synthesized Schiff Bases

Table-3 demonstrates the physical characterization of Synthesized Schiff bases. Percent yield of all the synthesized Schiff bases was found in between the range of 53.00 to 95.45%. All the newly synthesized Schiff bases show the solubility in two solvents i.e. Di-methyl formamide (DMF) and Di-methyl sulphoxide (DMSO). From the TLC studies, it was observed that the R_f value of all the synthesized compound was less than 1.0.

4.Spectral Characterizations of Synthesized Schiff Bases

The important diagnostic bands in the IR spectra were assigned and the bands positions are complied in Table-4. The IR spectra showed absorption band at 1700-1650 cm^{-1} indicated the stretching vibration of C=N (Schiff bases) which confirms to the condensation of reactants. C-H stretching vibration of $-CH_2$ appeared at 3200-3050 cm^{-1} and C=O stretching appeared at 1750-1740 cm^{-1} which indicates the condensation of reactants. The other peaks of IR spectra prove the structure of Schiff's base derivatives.

In the ^{1}H-NMR spectra, the signals due to NHCO proton were observed at 11.0-12.0 ppm as broad, present in all compounds, observed while the signals due to aromatic protons were observed at 6.0-8.8 ppm.

5.Antimicrobial Studies of Newly Synthesized Compounds

Antimicrobial activity of the synthesized compounds was done in comparison with gentamycin as standard to reveal the potency of synthesized derivatives. The two strains of bacteria *viz* negative bacteria (*Klebsiella pneumoniae*, MTCC 10031) and gram positive bacteria (*Bacillus Subtilis*, MTCC 6633) showed sensitivity at higher concentration (100 µg/ml) except the compound 4.1E which is ineffective against gram positive bacteria. Synthesized compound 4.1F shows greater activity against gram negative bacteria in comparison of standard, but less active against gram positive. Similarly, synthesized compound 4.1D shows greater activity against gram positive bacteria, less in comparison of standard. This synthesized compound (4.1D) shows almost similar activity against gram negative bacteria. The data for the antimicrobial activity of synthesized Schiff bases demonstrated in the Table-5.

CONCLUSION

The preparation procedure followed in this work for the synthesis of title compounds offers reduction in the reaction time, operation simplicity and easy work-up. All spectroscopic analysis confirmed the proposed structures for these compounds. Antimicrobial data have shown that the synthesized compounds have a significant antimicrobial activity against the tested micro-organisms.

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