

Synthesis, Characterization and Anti-Bacterial Activity of Isatin Schiff Base Derivatives

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Abstract

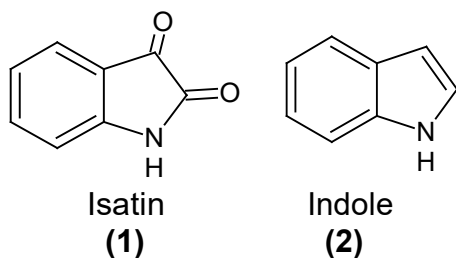
Initially, Isatin was treated with 4-amino acetanilide to form Isatin Schiff base, by the reaction of the free amino group on 4-amino acetanilide with carbonyl group of Isatin in the presence of ethanol and glacial acetic acid with the elimination of water molecule. The obtained Isatin Schiff base was then treated with substituted aromatic aldehydes in the presence of potassium hydroxide and ethanol to form various Chalcone derivatives (C1-C7). The structures of the synthesized compounds (C1-C7) were examined and confirmed using IR, Mass spectroscopy, ¹H-NMR and elemental analysis. By agar disc diffusion method, all given compounds were evaluated for their anti-bacterial activity against four bacteria at concentrations 25, 50 and 100 µg/ml. C4 and C6, were discovered to have possessed the most potent anti-bacterial activity.

Keywords: Aromatic Aldehyde, Antibacterial, Agar Disc Diffusion Method, Chalcone, Isatin, Schiff Base

1. Introduction

1.1 Isatin

Isatin (1) or 1*H*-indole-2,3-dione is an indole (2) derivative and it is an important group of heterocyclic compounds which are biologically active and of significant importance in medicinal chemistry¹. The compound isatin was first obtained as a product from the oxidation of indigo dye by nitric acid and chromic acids by Erdman and Laurent in 1846, it can be used for the synthesis of a large variety of heterocyclic compounds, examples are indoles and quinolines, and can be used as a raw material for drug synthesis².



The simple isatin nucleus possesses many biological properties like-antibacterial³, anti HIV⁴, antitubercular⁵, antitumor⁶, anti-inflammatory⁷, antioxidant⁸, antiviral⁹, anticonvulsant¹⁰ and CNS depressant activities¹¹.

Over the past years, potency of antibacterial therapy is somewhat in hesitant due to bacterial resistance to antibiotics. Quick development of drug resistant strains exerts a severe threat in present years. The lack of effective treatment is the main cause of this problem. The main challenge plunges into two parts: suitable target selection, mainly the requirement of pursuing molecular targets that are not susceptible to rapid resistance development and enhancement of chemical libraries to defeat the limitations of diversity particularly that which is essential to defeat barriers to bacterial species, especially, in gram negative organism. Even though there is advancement in expansion of antibacterial agents. The chemical reactivity and physical properties of Schiff bases are continued to be used to synthesize a large variety of various heterocyclic compounds and as a raw material for drug synthesis. Schiff bases are further known to

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possess wide spectrum of pharmacological activities such as antiviral, antimycobacterial, antibacterial, anti-inflammatory, cytotoxic, etc. Isatin shows a wide range of pharmacological activities reported in the literatures, including antiviral¹², spermicidal¹³, anti-corrosive¹⁴, analgesic¹⁵, anticonvulsant¹⁶, antioxidant¹⁷, antitubercular¹⁸, transthyretin fibrillogenesis inhibitory activity¹⁹, antidepressant²⁰ and antiepileptic²¹. The objective of the proposed study is to synthesize and evaluate the anti-bacterial activity of the compound with the aim to:

- To Synthesize Schiff base from isatin derivative.
- To incorporate the benzaldehyde derivatives to the synthesized Schiff base of isatin to form various chalcones (Figure 1).
- To characterize the new chalcone derivatives by IR, NMR, Mass spectroscopy and elemental analysis and to evaluate their anti-bacterial activity (*in vitro*)

2. Material and Methods

Melting point (mp) was determined in open capillary tubes and was uncorrected. The IR spectra were recorded in potassium bromide disks using IR Affinity-1 SHIMADZU spectrometer. The ¹H-NMR spectra were recorded on BRUKER (¹H NMR IN CDCl₃).

The Chemical shifts were recorded in parts per million (ppm) relative to TMS as an internal reference. Mass spectra were recorded on a LCMS-2010A DATA REPORT SHIMADZU instrument using Fast Atom Bombardment (FAB Positive). The progress of all the reactions were monitored by readymade silica gel plates (Merck). Iodine and UV lamp were used as a developing agent. Spectral data (IR, ¹H-NMR and Mass spectra) were confirmed the structures of the synthesized compounds and the purity of these compounds were ascertained by microanalysis.

3. General Procedure

3.1 Synthesis of Isatin Schiff's Bases

Equimolar quantity of isatin (1.47g, 0.01mol) and 4-aminoacetanilide (0.01mol) was added into absolute ethanol (20ml) and refluxed for one hour in the presence of 2-3 drops of glacial acetic acid in round bottomed flask. The solvent was then distilled off and the product obtained was recrystallized using ethanol.

3.2 Synthesis of Chalcone Derivative. C1-C7

0.01 mol of isatin Schiff base (from step 1) and 0.01 mol of substituted benzaldehyde were mixed in 30 ml of ethanol in a RBF and placed in an ice bath. To this, 10 ml of 60% KOH solution was added with continuous stirring for 3 h at room temperature. The mixture was left still at room temperature overnight. Then, it was mixed with ice distilled water (40 mL), filtered and washed. Dried in air and recrystallized from rectified methanol to get the titled compound C1-C7 (Table 1).

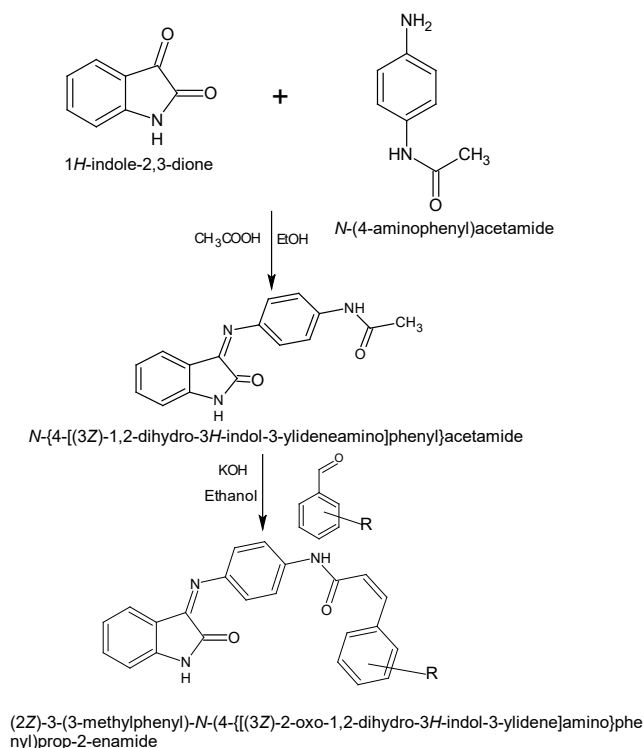


Figure 1. Synthetic scheme.

4. Scheme

Table 1. Derivatives of synthesized compounds

COMPOUND	R
4-methoxybenzaldehyde	4-OCH ₃
Benzaldehyde	H
3-bromobenzaldehyde	3-Br
4-chlorobenzaldehyde	4-Cl
4-hydroxy,3-methoxy benzaldehyde	4-OH, 3-OCH ₃
3-nitrobenzaldehyde	3-NO ₂
2-hydroxybenzaldehyde	2-OH

5. Antibacterial Activities

The standard strains of microorganisms were procured from the CSIR Instituted of Microbial Technology, Chandigarh, India. Agar disc diffusion method was used for the screening the anti-bacterial activities of all the synthesized compounds (C1-C7). And were evaluated against two Gram-positive bacteria (*Staphylococcus aureus* ATCC 6633 & *Bacillus subtilis* ATCC 6633) and two Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853 & *Escherichia coli* MTCC 724).

Bacterial strains and yeast were cultured overnight at 37°C in Muller-Hinton broth and at 30°C in nutrient agar medium respectively. Amoxicillin was used as standard drugs for anti-bacterial²¹.

In order to activate these cultures, subculture were freshly prepared, sterilized (autoclaved at 120°C for 30 min) and incubated at 37°C for 18 h to 24 h before use. A volume of 0.1 ml of each such culture was used as inoculums in all tests. The preparation of nutrient

medium (broth), subculture, agar medium and peptone water was done following the standard procedure. The discs measurements were 6.25 millimeter in diameter and obtained from Whatman filter paper. The Stock of synthesized compounds (C1-C7) were diluted in 1% dimethyl sulphoxide to give 25 µg/ml, 50 µg/ml and 100 µg/ml as final concentration. A standard reference solution for gram negative and gram-positive bacteria were made by dissolving weighed amount of amoxicillin (50µg/ml) in sterile distilled water, separately. It was then incubated at 37°C for 24h. 0.1 mL of dimethyl sulfoxide was used to maintain the control and showed no inhibition. The zone of inhibition revealed by each compound was measured in millimeter.

6. Results

6.1 Physical Data

The physical data and solubility data of all the title compounds are presented in the Table 2 and 3 respectively.

Table 2. Physical data of the title compounds C1-C7

Compound Code	Molecular Formula	Molecular Weight	M.P. (°C)	Rf Value	YIELD (%)
C1	C ₂₄ H ₁₉ N ₃ O ₃	397	228-231	0.41	81
C2	C ₂₃ H ₁₇ N ₃ O ₂	367	210-212	0.35	85
C3	C ₂₃ H ₁₆ BrN ₃ O ₂	446	233-235	0.37	79
C4	C ₂₃ H ₁₆ ClN ₃ O ₂	402	225-227	0.42	82
C5	C ₂₄ H ₁₉ N ₃ O ₄	413	241-243	0.45	81
C6	C ₂₃ H ₁₆ N ₄ O ₄	412	217-219	0.49	86
C7	C ₂₃ H ₁₇ N ₃ O ₃	383	191-193	0.44	78

Table 3. Solubility data of the title compounds C1-C7

S.NO	SOLVENT	C1	C2	C3	C4	C5	C6	C7
1	DMSO	+++	+++	+++	+++	+++	+++	+++
2	Acetic acid	+++	+++	+++	+++	+++	+++	+++
3	Ether	-	-	-	-	-	-	-
4	Toluene	++	++	++	++	++	++	++
5	Cyclohexane	++	++	++	++	++	++	++
6	Methanol	++	++	++	++	++	++	++
7	Ethanol	++	++	++	++	++	++	++
8	DMF	+++	+++	+++	+++	+++	+++	+++
9	Water	-	-	-	-	-	-	-
10	Dichloromethane	+	+	+	+	+	+	+
11	Ethyl acetate	++	++	++	++	++	++	++

+++ = freely soluble, ++ = sparingly soluble, + = slightly soluble, - = Insoluble

6.2 Spectral Data

(2Z)-3-(4-methoxyphenyl)-N-{4-[(Z)-(2-oxo-1,2-dihydro-3H-indol-3-ylidene) amino]phenyl}prop-2-enamide (C1)

IR (KBr) cm^{-1} : 1570 bending (N-H), 3270 stretching (N-H), 3060 (Ar-CH), 1320 (C-N), 1710 (C=O), 2150 (N-C=O) & 950 (OCH_3). $^1\text{HNMR}$ (CDCl_3), δ (ppm): 6.8-8.3: 12H, Ar-R. 4.8-5.0: 1H, CH=CH. 1.6-2.0: 1H, CH-C=O. 2.0-2.4: 2H, N-H. 3.2-3.5: 3H, RO-CH. Mass spectrum: 397($\text{M}^+.1$) $m/z = 379.426$.

(2Z)-N-{4-[(Z)-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]phenyl}-3-phenylprop-2-enamide (C2)

IR (KBr) cm^{-1} : 1560 bending (N-H), 3290 stretching (N-H), 3050 (Ar-CH), 1310 (C-N), 1720 (C=O) & 2160 (N-C=O). $^1\text{HNMR}$ (CDCl_3), δ (ppm): 6.7-8.3: 13H, Ar-R. 4.9-5.1: 1H, CH=CH. 1.8-2.0: 1H, CH-C=O. 2.1-2.4: 2H, N-H. 3.2-3.5: 3H, RO-CH. Mass spectrum: 368($\text{M}^+.1$) $m/z = 367.399$.

(2Z)-3-(3-bromophenyl)-N-{4-[(Z)-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]phenyl}prop-2-enamide (C3)

IR (KBr) cm^{-1} : 1600 bending (N-H), 3250 stretching (N-H), 3060 (Ar-CH), 1300 (C-N), 1720 (C=O), 630 (C-Br), & 2220 (N-C=O). $^1\text{HNMR}$ (CDCl_3), δ (ppm): 6.6-8.1: 12H, Ar-R. 4.8-5.0: 1H, CH=CH. 1.6-2.0: 1H, CH-C=O. 2.0-2.4: 2H, N-H. Mass spectrum: 447($\text{M}^+.1$) $m/z = 446.296$.

(2Z)-3-(4-chlorophenyl)-N-{4-[(Z)-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]phenyl}prop-2-enamide (C4)

IR (KBr) cm^{-1} : 1610 bending (N-H), 3240 stretching (N-H), 3020 (Ar-CH), 1340 (C-N), 1730 (C=O), 740 (C-Cl), & 2170 (N-C=O). $^1\text{HNMR}$ (CDCl_3), δ (ppm): 6.6-8.2: 12H, Ar-R. 4.8-5.2: 1H, CH=CH. 1.9-2.2: 1H, CH-C=O. 2.0-2.4: 2H, N-H. Mass spectrum: 402($\text{M}^+.1$) $m/z = 401.845$.

(2Z)-3-(4-hydroxy-3-methoxyphenyl)-N-{4-[(Z)-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]phenyl}prop-2-enamide (C5)

IR (KBr) cm^{-1} : 1680 bending (N-H), 3290 stretching (N-H), 3100 (Ar-CH), 1190 (C-N), 1750 (C=O), 3300 (C-OH), 1100 (OCH_3) & 2280 (N-C=O). $^1\text{HNMR}$ (CDCl_3), δ (ppm): 6.5-8.1: 12H, Ar-R. 5.0-5.3: 1H, CH=CH. 2.0-2.2: 1H, CH-C=O. 2.0-2.5: 2H, N-H, 3.2-

3.4: 3H, RO-CH, 3.5-3.7: 1H, R-OH. Mass spectrum: 414 ($\text{M}^+.1$) $m/z = 413.425$.

(2Z)-3-(3-nitrophenyl)-N-{4-[(Z)-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]phenyl}prop-2-enamide (C6)

IR (KBr) cm^{-1} : 1680 bending (N-H), 3250 stretching (N-H), 3100 (Ar-CH), 1220 (C-N), 1720 (C=O), 1550 (C-NO) & 2300 (N-C=O). $^1\text{HNMR}$ (CDCl_3), δ (ppm): 6.7-8.3: 12H, Ar-R. 5.0-5.2: 1H, CH=CH. 2.0-2.3: 1H, CH-C=O. 2.3-2.8: 2H, N-H. Mass spectrum: 413 ($\text{M}^+.1$) $m/z = 412.397$.

(2Z)-3-(2-hydroxyphenyl)-N-{4-[(Z)-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]phenyl}prop-2-enamide (C7)

IR (KBr) cm^{-1} : 1620 bending (N-H), 3210 stretching (N-H), 3090 (Ar-CH), 1210 (C-N), 1770 (C=O), 3550 (C-OH) & 2290 (N-C=O). $^1\text{HNMR}$ (CDCl_3), δ (ppm): 6.6-8.2: 12H, Ar-R. 4.8-5.2: 1H, CH=CH. 1.9-2.2: 1H, CH-C=O. 2.4-2.6: 2H, N-H. 3.2-3.5: 1H, R-OH. Mass spectrum: 384 ($\text{M}^+.1$) $m/z = 383.399$.

6.3 Antibacterial Activity

Table 4 represents the comparison of anti-bacterial activity of the standard drugs and synthesized compounds.

All synthesized compounds showed a significant activity against all tested bacteria

From the anti-bacterial study, it was discovered that in general the compounds with electron withdrawing moiety showed better activity compared to compounds having electron releasing moieties.

Within the electron withdrawing moiety, compounds (C3 and C4) showed good activity among other tested compounds. The compounds (C3, C4 and C6) possessed activity towards entire tested microorganism. The zone of inhibition was observed and measured, and it is in the order of (C4>C3>C6).

7. Conclusion

The chemical structure and anti-bacterial activity of the synthesized compounds reveals that in general the compounds having electron withdrawing moiety exhibit better activity than compounds having releasing moieties.

All compounds were found to show significant activity against tested bacteria. The compound C4(2Z)-3-(4-chlorophenyl)-N-{4-[(Z)-(2-oxo-1,2-dihydro-3H-

Table 4. Anti-bacterial activity of title compounds C1-C7 (zone of inhibition in mm)

Micro-organism	Conc. (µg/ml)	C1	C2	C3	C4	C5	C6	C7	Con	Std
<i>Bacillus Subtilis</i>	25	4	8	7	7	-	-	-	-	
	50	8	10	12	13	3	4	3	-	25
	100	12	16	17	18	5	7	7	-	
<i>Escherichia Coli</i>	25	5	10	9	8	1	8	3	-	
	50	8	10	14	14	2	11	6	-	28
	100	14	18	21	19	7	21	10	-	
<i>Pseudomonas Aeruginosa</i>	25	5	8	9	10	1	-	1	-	
	50	10	12	13	17	3	10	7	-	27
	100	14	18	20	22	6	20	12	-	
<i>Staphylococcus Aureus</i>	25	4	3	7	8	-	5	3	-	
	50	11	8	10	12	3	10	7	-	27
	100	14	11	16	19	6	20	12	-	

indol-3-ylidene)amino]phenyl}prop-2-enamide) showed better antibacterial activity while comparing with other synthesized compounds, which may be due to presence of electron withdrawing group at para position. Hence, this molecule may be considered as a lead molecule for antibacterial activity.

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