SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SCHIFF'S AND N-MANNICH BASES OF INDOLINE- 2, 3-DIONE AND ITS DERIVATIVES WITH N-(4-(4-AMINOPHENYLSULPHONYL) PHENYL) ACETAMIDE

Abstract

Indoline 2, 3-dione and substituted Indoline 2, 3-dione derivatives were reacted with N-(4-(4-aminophenylsulphonyl) phenyl) acetamide to form a series of Schiff'sbases. The Mannich bases of these compounds were synthesized by reacting them with formaldehyde and secondary amine (piperidine). All the compounds were characterized by means of their IR, 1H NMR spectroscopic data and elementalanalysis. The antimicrobial activity of the synthesized compounds was evaluated by tube dilution method and Well plate method. The maximum Zone of Inhibition was found in compound UNS-3 against *Enterococcus faecalis* when incubated at concentration of 100, 50 and 25µg/ml. All the synthesized compounds showed better anti-microbial activity compared to the reference drug.

Keywords: Indoline 2, 3-dione, Schiff's bases, Mannich bases, antimicrobial activity.

Introduction

Indoline 2, 3-dione has been found to exhibitenormous activity in mammals.[1] recentlySchiff'sand Mannich bases of Indoline 2, 3-dione are reported to exhibit broadspectrum chemotherapeutic properties such as antifungal [2-15], antibacterial [2-15], anti-HIV[6-10,16-17], anti-viral [18-19], anti-convulsant [20-23], antitubercular [24-26] and anticancer activities[27-29]. In continuation of our work on Indoline 2, 3-dione, we have synthesizednew Schiff's bases of Indoline 2, 3-dione with N-(4-(4-aminophenylsulphonyl)phenyl) acetamide. The N-Mannich bases of aboveSchiff's bases were synthesized by reacting the imino group of Indoline 2, 3-dionewith formaldehyde and N-ethyl-N-methylethanamine & perhydro azine.

Materials and Methods

Melting points were determined on a capillary melting pointapparatus and are uncorrected. 1H NMR data was recorded on300 MHz Brucker DRX-300 using DMSO with TMS asinternal standard. IR spectra were recorded on FTIR8400S Shimadzu IR Spectrophotometer. The elementalanalysis was performed on Carlo Erba 1108 and was within ±4 % of the theoretical values. The turbidity was comparedvisually and the zone of inhibition was measured. Thehomogeneousness of the compounds was monitored bySilica-G coated TLC plates (Merck), visualized by iodine vapour.

Synthesis of Schiff's base (General Method)

Equimolar quantities of 0.01 mol of Indoline 2, 3-dione/substituted Indoline 2, 3-dioneand N-(4-(4-aminophenylsulphonyl)phenyl) acetamide weredissolved in 40 mL of ethanol. (2 ml) Glacial acetic acid wasadded and the reaction mixture was refluxed for about 8-10 hours. The reaction mixture waspoured on to crushed ice. The crystalline product was collectedby filtration/ vacuum filtration, dried and recrystallised.

(Z)-N-(4-(4-(2-oxoindolin-3-ylideneamino)phenyl Sulfonyl) phenyl) acetamide

IR (KBr) 3468 (NH str), 1733 (C=O str), 1640 (C=N str),1330 anti, 1127 Syn (O=S=O str)cm^{-1}. ^{1}H NMR (DMSO)ppm. 7.03-7.9 (12H, m, Ar-H),8.0 (1H, s, -NH-CO-), 2.02 (3H, s, CH_3).

(Z)-N-(4-(4-(5-chloro-1-methyl-2-oxoindolin-3 ylideneamino)phenylSulfonyl)phenyl)acetamide

IR (KBr) 3468 (NH str), 1730 (C=O str), 1640 (C=N str),1330 anti, 1127 Syn (O=S=O str), 730 (C-Cl str)cm^{-1}.^{1}H NMR (DMSO)ppm. 7.4-7.9 (11H, m, Ar-H), 8.0 (1H, s, -NH-CO-), 2.02-2.78 (6H, m, CH_3).

(Z)-N-(4-(4-(1-acetyl-5-bromo-2-oxoindolin-3-ylideneamino)phenylSulfonyl)phenyl) acetamide

IR (KBr) 3408 (NH str), 1733 (C=O str), 1640 (C=N str),1330 anti, 1127 Syn (O=S=O str), 620 (C-Br str)cm^{-1}.^{1}H NMR (DMSO)ppm. 7.4-7.9 (11H, m, Ar-H), 8.0 (1H, s, -NH-CO-), 2.02-2.40 (6H, m, CH_3).

(Z)-N-(4-(4-(1-methyl-5-nitro-2-oxoindolin-3-ylideneamino)phenylSulfonyl)phenyl) acetamide

IR (KBr) 3460 (NH str), 1730 (C=O str), 1640 (C=N str),1330 anti, 1127 Syn (O=S=O str), 1519 (N-O str)cm^{-1}.^{1}H NMR (DMSO)ppm. 7.4-8.53 (11H, m, Ar-H), 8.0 (1H, s, -NH-CO-), 2.02-2.78 (6H, m, CH_3).

Synthesis of N-Mannich bases (General Method)

A slurry consisting of 0.005 mol of Schiff's base containingthe imino group of Indoline 2, 3-dione, 5 ml of THF and2 ml of 37 % HCl was made. To this N-ethyl-N-methylethanamine / perhydro azine (0.005mol) was added drop wise with cooling and shaking. Thereaction mixture was allowed to stand at room temperaturefor 1 hour with occasional shaking and then it was heated on a boiling water bath for 15 minutes. Finally,the reaction mixture was cooled and the product obtained was recrystallised from petroleum ether.

(Z)-N-(4-(4-(5-chloro-1-((diethylamino)methyl)-2-oxoindolin-3-ylideneamino)phenylSulfonyl)phenyl) acetamide

IR (KBr) 3440 (NH str), 1740 (C=O str), 1640 (C=N str),1330 anti, 1127 Syn (O=S=O str), 2935 (C-H str) 720 (C-Cl str)cm^{-1}.^{1}H NMR (DMSO)ppm. 7.4-7.9 (11H, m, Ar-H), 8.0 (1H, s, -NH-CO-), 2.02 (3H, s, CH_3), 1.0-4.03 (12H, m, -CH_2-N(C_2H_5)$_2$).

(Z)-N-(4-(4-(5-bromo-1-((diethylamino)methyl)-2-oxoindolin-3-ylideneamino)phenylSulfonyl)phenyl) acetamide

IR (KBr) 3436 (NH str), 1733 (C=O str), 1630 (C=N str),1330 anti, 1127 Syn (O=S=O str), 2932 (C-H str), 620 (C-Br str)cm^{-1}.^{1}H NMR (DMSO)ppm. 7.4-7.9 (11H, m, Ar-H), 8.0 (1H, s, -NH-CO-), 2.02 (3H, s, CH_3), 1.0-4.03 (12H, m, -CH_2-N(C_2H_5)$_2$).

(Z)-N-(4-(4-(5-chloro-2-oxo-1-(piperidin-1ylmethyl)indolin-3-ylideneamino)phenylSulfonyl)phenyl) acetamide

IR (KBr) 3439 (NH str), 1742 (C=O str), 1638 (C=N str),1330 anti, 1127 Syn (O=S=O str), 2935 (C-H str), 720 (C-Cl str)cm^{-1}.^{1}H NMR (DMSO)ppm. 7.4-7.9 (11H, m, Ar-H), 8.0 (1H, s, -NH-CO-), 2.02 (3H, m, CH_3), 4.03 (2H, s, -CH_2-), 1.5-2.24 (10H, s, piperidine).

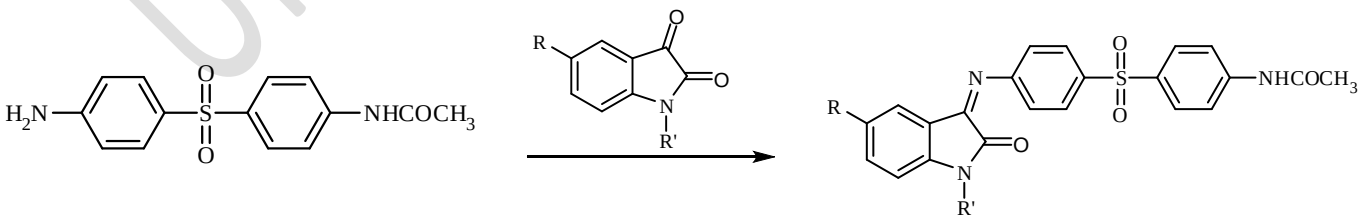
(Z)-N-(4-(4-(5-nitro-2-oxo-1-(piperidin-1ylmethyl)indolin-3-ylideneamino)phenylSulfonyl)phenyl) acetamide

IR (KBr) 3235 (NH str), 1738 (C=O str), 1620 (C=N str),1330 anti, 1127 Syn (O=S=O str),2910 (C-H str), 1530 (N-O str)cm^{-1}.^{1}H NMR (DMSO)ppm. 7.4-8.53 (11H, m, Ar-H), 8.0 (1H, s, -NH-CO-), 2.02 (3H, m, CH_3), 4.03 (2H, s, -CH_2-), 1.5-2.24 (10H, s, piperidine).

(Z)-N-(4-(4-(5-bromo-2-oxo-1-(piperidin-1ylmethyl)indolin-3-ylideneamino)phenylSulfonyl)phenyl) acetamide

IR (KBr) 3434 (NH str), 1730 (C=O str), 1630 (C=N str),1330 anti, 1127 Syn (O=S=O str), 2930 (C-H str), 620 (C-Br str)cm^{-1}.^{1}H NMR (DMSO)ppm. 7.4-7.9 (11H, m, Ar-H), 8.0 (1H, s, -NH-CO-), 2.02 (3H, s, CH_3), 4.03 (2H, s, -CH_2-), 1.5-2.24 (10H, s, piperidine).

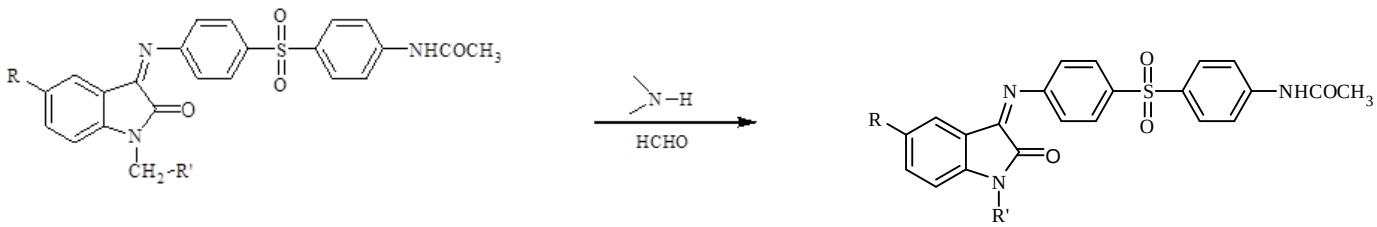
Scheme-I: Synthesis of Schiff's bases



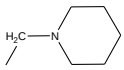
R = H, Cl, Br, NO_2

R' = H, CH_3, $COCH_3$

Scheme-II: Synthesis of N-Mannich bases



R = H, Cl, Br, NO_2

R' = -  $N(C_2H_5)_2$,

Antimicrobial Screening Antimicrobial activity of compound

Microbes used

The reference microbial species; Microbial Type Culture Collection (MTCC) of *Klebsiella pneumonia* (MTCC- 432), *Staphylococcus aureus* (MTCC- 3160), *Enterococcus faecalis* (MTCC-439), *Candida albicans* (MTCC-183), *Aspergillus flavus* (MTCC-277) were collected from bacteriology unit of the microbiology laboratory.

Media preparation

At first, all instruments which were used in laboratory were made sterile, all glassware's like Erlenmeyer flask, graduated cylinders, stirring rods, beakers, test tubes, petri dishes, inoculating loops, that were used in the assay were placed in an autoclave at 121°C under 15 psi pressure for 25 min by using Autoclave and followed aseptic technique method.

Nutrient agar media (NAM) was prepared for growing of bacteria and potato dextrose agar (PDA) for growing of fungus inside the laboratory. The standard size (100mm× 15mm) petri dishes as required for whole experiment. For preparation of NAM, 13 gram powder and for PDA, 24 gram powder was mixed with 1000 ml of distilled water and stirred to obtain homogenized mixture. After which, NAM and PDA mixture were placed in an Autoclave under 15 psi pressure, at 121°C for 25 min for sterilization of media. After that poured the culture media into petri dishes at ratio of 20 ml/dish and was left half covered on the table to let the agar cool down and solidify at room temperature.

Broth cultures of the pure isolates culture were prepared by transferring a loop of culture into sterile nutrient and potato dextrose broth and incubated at 37^{o}C for 24 hours. A loop full was taken from these broths and seeded onto sterile nutrient and potato dextrose agar plates through sterile cotton swab to develop diffused heavy lawn culture. The well diffusion method was used to determine the antimicrobial activity of given sample using standard procedure [30].

Table 1: Physical Constants of Synthesized compounds

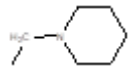
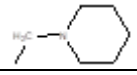
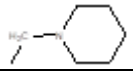
Compound Code	R	Ŕ	M.P. (^oC)	Molecular Formula	Yield (%)
UNS-01	H	H	102	$C_{22}H_{17}N_3O_4S$	76
UNS-02	Cl	$-CH_3$	118	$C_{23}H_{18}ClN_3O_4S$	78
UNS-03	Br	$-COCH_3$	198	$C_{24}H_{18}BrN_3O_5S$	68
UNS-04	NO_2	$-CH_3$	146	$C_{23}H_{18}N_4O_6S$	60
UNS-05	Cl	$-CH_2-N(C_2H_5)_2$	136	$C_{27}H_{27}ClN_4O_4S$	82
UNS-06	Br	$-CH_2-N(C_2H_5)_2$	150	$C_{27}H_{27}BrN_4O_4S$	72
UNS-07	Cl		142	$C_{28}H_{27}ClN_4O_4S$	89
UNS-08	NO_2		130	$C_{28}H_{27}N_5O_6S$	88
UNS-09	Br		100	$C_{28}H_{27}BrN_4O_4S$	78

Table 2: Antimicrobial activity of UNS-1 to UNS-9 against *Klebsiella pneumonia*

Concentration (µg/ml)	
	UNS-1
100	13±0.47
50	10±0.47
25	6±0
	UNS-2
50	9±0.47
25	8±0.47
12.5	6±0
	UNS-3
25	8±0.47
12.5	7±0.47
6.25	6±0
	UNS-4
100	14±0.47
50	12±0.47
25	6±0
	UNS-5
50	11±0.47
25	10±0.47
12.5	6±0
	UNS-6
100	16±0.47
50	11±0.47
25	6±0

	UNS-7
100	13±0.47
50	11±0.47
25	9±0.47
	UNS-8
50	11±0.47
25	10±0.47
12.5	6±0
	UNS-9
50	10±0.47
25	8±0.47
12.5	6±0

Table 3: Antimicrobial activity of UNS-1 to UNS-9 against *Enterococcus faecalis*

Concentration (µg/ml)	
	UNS-1
50	34±0.47
25	33±0.47
12.5	6±0
	UNS-2
12.5	34±0.47
6.25	25±0.94
3.12	6±0
	UNS-3
100	35±0.47
50	34±0.47
25	23±0.47
	UNS-4
100	25±0.94
50	21±0.47
25	6±0
	UNS-5
50	21±0.47
25	20±0.47
12.5	6±0
	UNS-6
100	30±0.94
50	28±0.47
25	6±0
	UNS-7
50	20±0.47
25	17±0.47
12.5	6±0
	UNS-8
100	30±0.94
50	28±0.47
25	19±0.47

	UNS-9
12.5	33±1.41
6.25	24±0.47
3.12	6±0

Table 4: Antimicrobial activity of UNS-1 to UNS-9 against *Staphylococcus aureus*

Concentration (µg/ml)	
	UNS-1
25	13±0.47
12.5	12±0.47
6.25	10±0.47
	UNS-2
100	14±0.47
50	13±0.47
25	7±0.47
	UNS-3
50	16±0.47
25	10±0.47
12.5	7±0.47
	UNS-4
12.5	24±0.94
6.25	16±0.47
3.12	12±0.47
	UNS-5
50	25±0.47
25	22±0.47
12.5	14±0.94
	UNS-6
100	24±0.47
50	13±0.47
25	6±0
	UNS-7
25	29±0.47
12.5	20±0.47
6.25	6±0
	UNS-8
12.5	27±0.47
6.25	23±0.47
3.12	12±0.47
	UNS-9
50	27±0.47
25	23±0.47
12.5	6±0

Table 5: Antimicrobial activity of UNS-1 to UNS-9 against *Candida albicans*

Concentration (µg/ml)	
	UNS-1
100	15±1.24
50	13±0.47
25	7±0.47
	UNS-2
100	14±0.81
50	11±0.47
25	7±0.47
	UNS-3
50	15±0.47
25	12±0.47
12.5	8±0.47
	UNS-4
100	16±0.47
50	14±0.47
25	9±0.47
	UNS-5
12.5	9±0.47
6.25	8±0.47
3.12	6±0
	UNS-6
100	13±0.94
50	11±0.47
25	6±0
	UNS-7
50	14±0.47
25	12±0.47
12.5	9±0.47
	UNS-8
100	17±0.47
50	14±0.47
25	10±0.94
	UNS-9
100	14±0.47
50	12±0.47
25	6±0

Table 6: Antimicrobial activity of UNS-1 to UNS-9 against *Aspergillus flavus*

Concentration (µg/ml)	
	UNS-1
100	9±0.47
50	8±0.47
25	6±0
	UNS-2
50	8±0.81
25	7±0.47
12.5	6±0
	UNS-3
12.5	9±0.47
6.25	7±0.47
3.12	6±0
	UNS-4
100	8±0.47
50	7±0.47
25	6±0
	UNS-5
50	9±0.47
25	7±0.47
12.5	6±0
	UNS-6
100	8±0.94
50	7±0.47
25	6±0
	UNS-7
50	9±0.47
25	8±0.47
12.5	6±0
	UNS-8
100	9±0.47
50	8±0.47
25	6±0
	UNS-9
50	9±0.47
25	8±0.47
12.5	6±0

Results and Discussion

Indoline 2, 3-dione and substituted Indoline 2, 3-dione derivatives were reacted with N-(4-(4-aminophenylsulphonyl) phenyl) acetamide to form a series of Schiff'sbases. The Mannich bases of these compounds were synthesized by reacting them with formaldehyde and secondary amine (piperidine). All the compounds were characterized by means of their IR, 1H NMR spectroscopic data and elementalanalysis. There were different concentration used

which are 3.12, 6.25, 12.5, 25, 50 and 100 µg/ml according to MIC selected for each microbes, for antimicrobial studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37^{o}C for 24 hr. and then examined for clear zones of inhibition (mm) around the wells impregnated with particular concentration of drug. The maximum Zone of Inhibition was found in compound UNS-3 against *Enterococcus faecalis* when incubated at concentration of 100, 50 and 25µg/ml. All the synthesized compounds showed better anti-microbial activity compared to the reference drug.

CONCLUSION

In present investigation synthesis and antimicrobial activity of Schiff's and N-Mannich Bases of Indoline 2, 3-dione and Its derivatives with N-(4-(4-aminophenylsulphonyl) phenyl) acetamide. The all compounds UNS 1 to UNS 9 showed comparable antimicrobial potential against *Klebsiella pneumonia*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus flavus.* The maximum Zone of Inhibition was found in compound UNS-3 against *Enterococcus faecalis* when incubated at concentration of 100, 50 and 25µg/ml. The Results of study concluded that the prepared compound showed potent antimicrobial effectiveness and can be synthesized commercially, and evaluated for other pharmacological activity.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Somogyi L. Transformation of is a tin 3-acylhydrazones under acetylating conditions: Synthesis and structure elucidation of 1, 5|-disubstitutedacetylspiro [oxindole-3, 2-[1, 3, 4] oxadiazolines].Bull. Chem. Soc. Jpn. 2001; 74: 873□881.
2. Pandeya SN, Sriram D. Synthesis and screening for antibacterialactivity of Schiff's and Mannich bases of Isatin and its derivatives.Acta Pharm. Turc. 1998; 40: 33-38.

3. Sarangapani M, Reddy VM. Synthesis and antimicrobial activity of1□[(N, N-disubstituted amino) methyl]-3-[(2-phenyl-3, 4-dihydro-4-oxoquinazoline-3-yl] indole-2-one. Indian J. Heterocycl. Chem.1994; 3: 257-260.
4. Varma RS, Nobels WL. Antiviral, antibacterial and antifungal activities of isatin N-Mannich bases. J. Pharm. Sci., 1975; 64: 881-882.
5. Pandeya SN, Sriram D, Nath G, DeClercq E. Synthesis andantimicrobial activity of Schiff and Mannich bases of isatin and its derivatives with pyrimidine. Farmaco. 1999; 54: 624-628.
6. Pandeya SN, Sriram D, Nath G, De Clercq E. Synthesisantibacterial, antifungal and anti HIV activity of Schiff's and Mannich bases of isatin with N-[6-Chlorobenz thiazole-2-yl]thiosemicarbazide. Indian J. Pharm. Sci. 1999; 61: 358-361.
7. Pandeya SN, Sriram D, Nath G, De Clercq E. Synthesisantibacterial, antifungal and anti HIV evaluation of Schiff and Mannich bases of isatin derivatives with 3-amino-methylmercaptoquinazolin-4(3H) one. Pharm. Acta Helv. 1999; 74: 11-17.
8. Pandeya SN, Sriram D, Nath G, De Clercq E. Synthesis, antibacterial, antifungal and anti HIV activities of Schiff andMannich bases derived from isatin derivatives and N-[4-(4-chlorophenyl) thiazol-2-yl] thiosemicarbazide. Eur. J. Pharm. Sci.1999; 9: 25-31.
9. Pandeya SN, Yogeeswari P, Sriram D, De Clercq E, Pannecouque C, Witvrouw M. Synthesis and Screening for Anti□HIV Activity of some N□Mannich Bases of Isatin Derivatives. Chemotherapy.1999; 45: 192-96.
10. Pandeya SN, Sriram D, Nath G, De Clercq E. Synthesis, antibacterial, antifungal and anti-HIV activities of NorfloxacinMannich bases. Eur. J. Med. Chem. 2000; 35: 249-255.
11. Pandeya SN, Sriram D, Nath G, De Clercq E. Synthesis, antibacterial, antifungal and anti□HIV evaluation of Schiff and Mannich bases of isatin and its derivatives with triazole. Arzneim Forsch. Drug. Res. 2000; 50: 55-59.
12. Pandeya SN, Yogeeswari P, Sriram D, Nath G. Synthesis andantimicrobial activity of N-Mannich bases of 3-[Nsulphadooximino] isatin and its methyl derivatives. Bull. Chim.Farm. 1998; 137: 321-324.
13. Ravichandran V, Mohan S, Kumar SK. Synthesis and antimicrobialactivity of Mannich bases of Isatin and its derivative with 2-[(2, 6-dichlorophenyl)amino]phenylacetic acid. ARKIVOC. 2007; (xiv):51-57.

14. Patel A, Bari S, Telele G, Patel J, Sarangapani M. Synthesis andantimicrobial activity of some new Isatin derivatives. Iran. J.Pharm. Sci. 2006; 4: 249-254.
15. Jarrahpour A, Khalili D, Clercq De E, Salmi C, Brunel M J.Synthesis, antibacterial, antifungal and antiviral activity evaluation of some new bis-Schiff bases of Isatin and their derivatives. Molecules. 2007; 12: 1720-1730.
16. Bal TR, Anand B, Yogeeswari P, Sriram D. Synthesis andevaluation of anti-HIV activity of Isatin β-thiosemicarbazone derivatives. Bioorg. Med. Chem. Let. 2005; 15: 4451-4455.
17. Sriram D, Bal TR, Yogeeswari P. Aminopyrimidino isatin anlogues: Desigen of novel non-nucleoside HIV-1 reverse transcriptaseinhibitors with broad spectrum chemotherapeutic properties. J.Pharm. Pharmaceut. Sci. 2005; 8(3): 565-577.
18. Singh SP, Shukla SK, Awasthi LP. Synthesis of some 3-(4|-nitrobenzoyl-hydrzone)-2-indolinones as potential antiviral agents.Curr. Sci. 1983; 52: 766-769.
19. Selvam P, Murgesh N, Chandramohan M, Clercq De E, Keyaerts E,Vijgen L, Maes P, Neyts J, Ranst M V. In vitro antiviral activity ofsome novel Isatin derivative against HCV and SARS-CoV viruses. Ind. J. Pharm. Sci. 2008; 70(1): 91-94.
20. Pandeya SN, Smith S, Stable JP. Anticonvulsant andSedative□hypnotic activities of N-substituted isatin-3-semicarbazones. Arch. Pharm. 2002; 4: 129-134.
21. Verma M, Pandeya SN, Singh KN, Stables JP. Anticonvulsant activity of schiff bases of Isatin derivatives. Acta Pharm. 2004; 54:49-56.
22. Smitha S, Pandeya SN, Stables JP, Ganpathy S. Anticonvulsant andsedative-hypnotic activities of N-acetyl/methyl Isatin derivatives.Sci. Pharm. 2008; 76: 621-636.
23. Sridhar SK, Pandeya SN, Stables JP, Ramesh A. Anticonvulsantactivity of hydrazones, Schiff and Mannich bases of Isatinderivatives. Euro. J. Pharm. Sci. 2002; 16: 129-132.
24. Fadl AT, Bin-Jubair FAS. Anti-tubercular activity of Isatinderivatives. Int. J. Res. Pharm. Sci. 2010; 1(2): 113-126.
25. Hussein MA, Fadl TA, Hussein A. Synthesis and antitubercular activity of mannich bases derived from Isatin, isonicotinic acid hydrazone. Pharm Sci; Assiut University 2005; 28: 131-136.
26. Karali N, Gursoy A, Kandemirli F, Shvets N, Kaynak F B, Ozbey Suhelyla, Koalishyn V, Dimoglo A. Synthesis and structure antituberculosis activity relationship of 1H-indole-2,3-dionederivatives. Bioorg. Med. Chem. 2007; 15: 5888-5904.

27. Vine KL, Locke JM, Ranson M, Pyne SG, Bremner J B. In vitrocytotoxicity evaluation of some substituted Isatin derivatives. Bioorg. Med. Chem. 2007; 15: 931-938.
28. Yogeeswari P, Sriram D, Kavya R, Tiwari S. Synthesis and in-vitro cytotoxicity evaluation of Gatifloxacin Mannich bases. Biomed. and pharmacother. 2005; 59: 501-510.
29. Hussain MM, Islam N Khar R, Islam Md R. Cytotoxicity study ofdimethyl Isatin and its heterocyclic derivatives. Bangladesh J.Pharmacol. 2007; 2: 66-70.
30. Hawkey PM, Lewis, DA. Medical Bacteriology: A PracticalApproach. Oxford University Press, Oxford. 1994, pp. 181-194.