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 generate a variety of Schiff's bases. These compounds' Mannich bases were created by reacting them with formaldehyde and secondary amine (piperidine). The compounds were all described using IR, 1H NMR spectroscopic data, and elemental analysis. The antibacterial activity of the produced compounds was determined using the tube dilution and Well plate methods. When compound UNS-3 was incubated at concentrations of 100, 50, and 25g/ml, it showed the greatest Zone of Inhibition against *Enterococcus faecalis*. When compared to the reference medication, all of the synthesised compounds demonstrated superior antimicrobial efficacy.

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

INTRODUCTION

Indoline 2, 3-dione has been reported to have extremely high activity in animals. [1] most recently 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] have been described for Schiff's and Mannich bases of Indoline 2, 3-dione. We have synthesised novel Schiff's bases of Indoline 2, 3-dione using N-(4-(4-aminophenylsulphonyl)phenyl) acetamide as part of our ongoing study on Indoline 2, 3-dione. The imino group of Indoline 2, 3-dione was reacted with formaldehyde and N-ethyl-N-methylethanamine & perhydro azine to produce the N-Mannich bases of aboveSchiff's bases.

MATERIALS AND METHODS

The melting points were calculated using a capillary melting point instrument and are uncorrected. 1H NMR data was collected on a 300 MHz Brucker DRX-300 using DMSO as an internal standard and TMS as an external standard. The FTIR8400S Shimadzu IR Spectrophotometer was used to capture the IR spectra. The elemental analysis was done on Carlo Erba 1108, and the results were within 4% of the theoretical values. The turbidity was visually compared, and the zone of inhibition was quantified. The homogeneity of the compounds was determined using Silica-G coated TLC plates (Merck) and 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 was prepared using 0.005 mol of Schiff's base containing the imino group of Indoline 2, 3-dione, 5 ml of THF, and 2 ml of 37 percent HCl. With cooling and shaking, N-ethyl-N-methylethanamine / perhydro azine (0.005mol) was added dropwise. The reaction mixture was allowed to remain at room temperature for 1 hour with intermittent shaking before being heated for 15 minutes in a boiling water bath. Finally, the reaction mixture was cooled, and the resulting product 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).

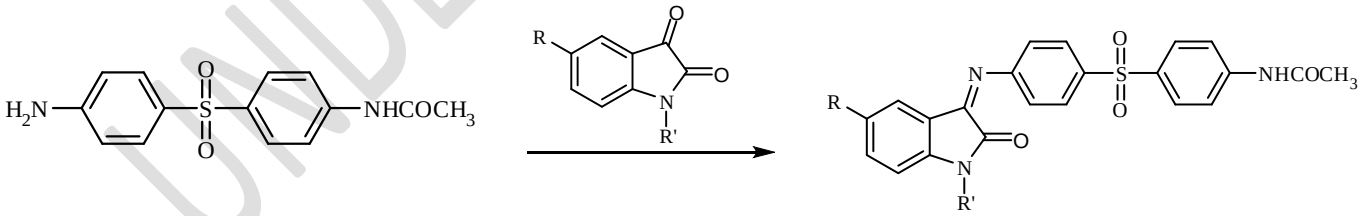
(Z)-N-(4-(4-(5-nitro-2-oxo-1-(piperidin-1ylmethyl)indolin-3-ylideneamino)phenylSulfonyl)phenyl) acetamide

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).

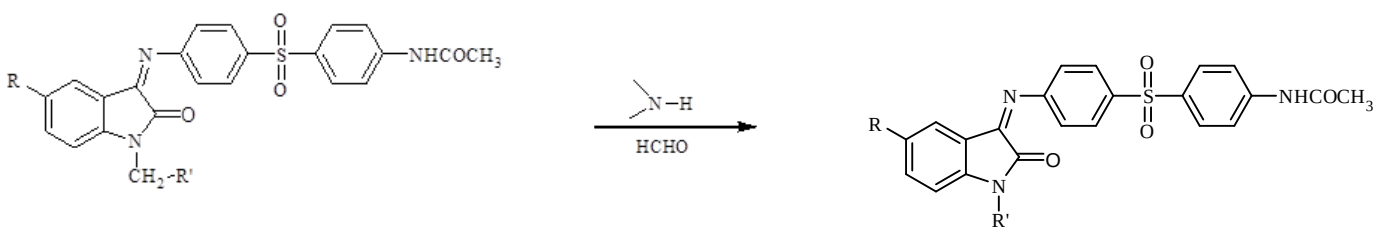
Scheme-I: Synthesis of Schiff's bases



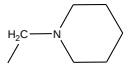
R = H, Cl, Br, NO_2

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

To begin, all laboratory instruments were sterilised. All glassware used in the assay, such as Erlenmeyer flasks, graduated cylinders, stirring rods, beakers, test tubes, petri dishes, and inoculating loops, were placed in an autoclave at 121°C under 15 psi pressure for 25 minutes using an autoclave and following aseptic technique method.

Inside the laboratory, nutrient agar medium (NAM) was created for bacterial growth and potato dextrose agar (PDA) for fungal growth. Petri dishes of standard size (100mm 15mm) are required for the entire experiment. 13 gramme powder for NAM and 24 gramme powder for PDA were combined with 1000 ml of distilled water and agitated to achieve a homogenised slurry. Following that, the NAM and PDA combination was sterilised in an autoclave at 121°C for 25 minutes at 15 psi pressure. The culture medium was then placed into petri dishes at a ratio of 20 ml/dish and left partially covered on the table to cool and harden at room temperature.

The pure isolates culture broth cultures were created by putting a loop of culture into sterile nutrient and potato dextrose broth and incubating it at 37oC for 24 hours. To generate diffused heavy lawn culture, a loop full of these broths was obtained and seeded onto sterile nutrient and potato dextrose agar plates using a sterile cotton swab. The antibacterial activity of was determined using the well diffusion technique.

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

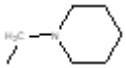
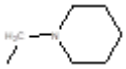
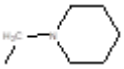
UNS-04	NO_2	$-CH_3$	146	$C_{23}H_{18}N_4O_6S$	60
UNS-05	Cl	$-CH_2-N(C_2H_5)_2$	136	$C_{27}H_{27}ClN_4O_4S$	82
UNS-06	Br	$-CH_2-N(C_2H_5)_2$	150	$C_{27}H_{27}BrN_4O_4S$	72
UNS-07	Cl		142	$C_{28}H_{27}ClN_4O_4S$	89
UNS-08	NO_2		130	$C_{28}H_{27}N_5O_6S$	88
UNS-09	Br		100	$C_{28}H_{27}BrN_4O_4S$	78

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 generate a variety of Schiff's bases. These compounds' Mannich bases were created by reacting them with formaldehyde and secondary amine (piperidine). The compounds were all described using IR, 1H NMR spectroscopic data, and elemental analysis. For antimicrobial experiments, different concentrations of 3.12, 6.25, 12.5, 25, 50, and 100 g/ml were utilised based on the MIC chosen for each microorganism. Its key characteristic is the quick placement of wells containing antibiotics on the surfaces of agar following inoculation with the organism being investigated. Overnight broth cultures that have not been diluted should never be utilised as inoculums. After 24 hours of incubation at 37oC, the plates were inspected for obvious zones of inhibition (mm) surrounding the wells impregnated with a specific dose of medication. When compound UNS-3 was incubated at concentrations of 100, 50, and 25g/ml, it showed the greatest Zone of Inhibition against *Enterococcus faecalis*. When compared to the reference medication, all of the synthesised compounds demonstrated superior antimicrobial efficacy.

CONCLUSION

The synthesis and antibacterial activity of Schiff's and N-Mannich bases of Indoline 2, 3-dione and its derivatives with N-(4-(4-aminophenylsulphonyl) phenyl) acetamide are being

investigated in this study. All of the substances tested positive for antibacterial activity against *Klebsiella pneumonia, Enterococcus faecalis, Staphylococcus aureus, Candida albicans,* and *Aspergillus flavus*. When compound UNS-3 was incubated at concentrations of 100, 50, and 25g/ml, it showed the greatest Zone of Inhibition against *Enterococcus faecalis*. The study revealed that the produced chemical demonstrated significant antibacterial efficiency and may be commercially manufactured and tested for additional pharmacological properties.

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.