

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's bases. 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 elemental analysis. 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 25 µg/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 exhibit enormous activity in mammals.[1] recently Schiff's and Mannich bases of Indoline 2, 3-dione are reported to exhibit broad spectrum 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 synthesized new Schiff's bases of Indoline 2, 3-dione with N-(4-(4-aminophenylsulphonyl)phenyl) acetamide. The N-Mannich bases of above Schiff's bases were synthesized by reacting the imino group of Indoline 2, 3-dione with formaldehyde and N-ethyl-N-methylethanamine & perhydro azine.

Materials and Methods

Melting points were determined on a capillary melting point apparatus and are uncorrected. ¹H NMR data was recorded on 300 MHz Bruker DRX-300 using DMSO with TMS as internal standard. IR spectra were recorded on FTIR 8400S Shimadzu IR Spectrophotometer. The elemental analysis was performed on Carlo Erba 1108 and was within ±4 % of the theoretical values. The turbidity was compared visually and the zone of inhibition was measured. The homogeneity of the compounds was monitored by Silica-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-dione and N-(4-(4-aminophenylsulphonyl)phenyl) acetamide were dissolved in 40 mL of ethanol. (2 ml) Glacial acetic acid was added and the reaction mixture was refluxed for about 8-10 hours. The reaction mixture was poured on to crushed ice. The crystalline product was collected by 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} . ^1H 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)phenyl Sulfonyl)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} . ^1H 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)phenyl Sulfonyl)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} . ^1H 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)phenyl Sulfonyl)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} . ^1H 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 containing the imino group of Indoline 2, 3-dione, 5 ml of THF and 2 ml of 37 % HCl was made. To this N-ethyl-N-methylethanamine / perhydro azine (0.005 mol) was added drop wise with cooling and shaking. The reaction mixture was allowed to stand at room temperature for 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)phenyl Sulfonyl)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} . ^1H 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, $-\text{CH}_2-\text{N}(\text{C}_2\text{H}_5)_2$).

(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} . ^1H 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, $-\text{CH}_2-\text{N}(\text{C}_2\text{H}_5)_2$).

(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} . ^1H 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, $-\text{CH}_2-$), 1.5-2.24 (10H, s, piperidine).

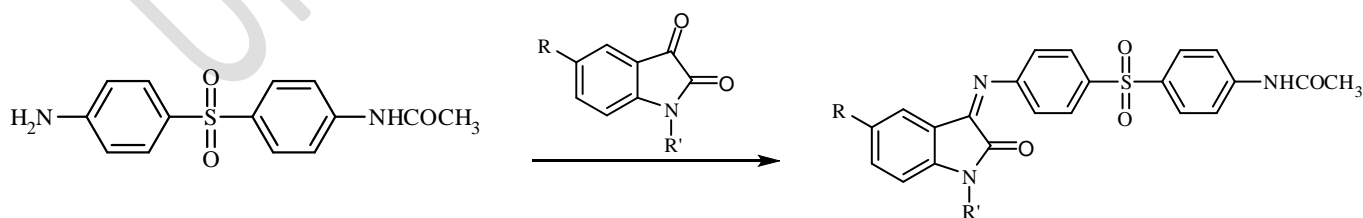
(Z)-N-(4-(4-(5-nitro-2-oxo-1-(piperidin-1ylmethyl)indolin-3-ylideneamino)phenylSulfonyl)phenyl) acetamide

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} . ^1H 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, $-\text{CH}_2-$), 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} . ^1H 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, $-\text{CH}_2-$), 1.5-2.24 (10H, s, piperidine).

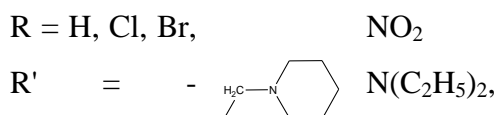
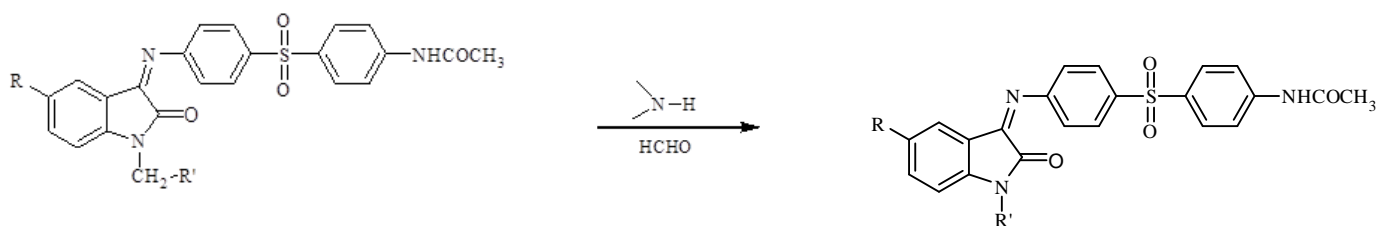
Scheme-I: Synthesis of Schiff's bases



R = H, Cl, Br, NO_2

R' = H, CH_3 , COCH_3

Scheme-II: Synthesis of N-Mannich bases



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 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°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 procedure³⁰.

Table 1: Physical Constants of Synthesized compounds

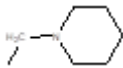
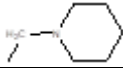
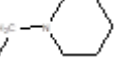
Compound Code	R	\bar{R}	M.P. (°C)	Molecular Formula	Yield (%)
UNS-01	H	H	102	C ₂₂ H ₁₇ N ₃ O ₄ S	76
UNS-02	Cl	-CH ₃	118	C ₂₃ H ₁₈ ClN ₃ O ₄ S	78
UNS-03	Br	-COCH ₃	198	C ₂₄ H ₁₈ BrN ₃ O ₅ S	68
UNS-04	NO ₂	-CH ₃	146	C ₂₃ H ₁₈ N ₄ O ₆ S	60
UNS-05	Cl	-CH ₂ -N(C ₂ H ₅) ₂	136	C ₂₇ H ₂₇ ClN ₄ O ₄ S	82
UNS-06	Br	-CH ₂ -N(C ₂ H ₅) ₂	150	C ₂₇ H ₂₇ BrN ₄ O ₄ S	72
UNS-07	Cl		142	C ₂₈ H ₂₇ ClN ₄ O ₄ S	89
UNS-08	NO ₂		130	C ₂₈ H ₂₇ N ₅ O ₆ S	88
UNS-09	Br		100	C ₂₈ H ₂₇ BrN ₄ O ₄ S	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's bases. 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 elemental analysis. There were different concentrations 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°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 antimicrobial 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.

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