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Original article

SYNTHESIS, ANTIMICROBIAL SCREENING AND BETA LACTAMASE INHIBITORY ACTIVITY OF 3-(3-CHLORO-4-FLUOROPHENYLIMINO) INDOLIN-2-ON AND 5-CHLORO INDOLIN-2-ON DERIVATIVES.

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Abstract

*A series of various substituted 3-(3-chloro-4-fluorophenylimino)indolin-2-on and 5-chloro-3-(3-chloro-4-fluorophenylimino)indolin-2-on Mannich base derivatives (**IIIa-f**) were synthesized. All the synthesized compounds were characterized on the basis of UV, IR, and ^{1}H NMR spectral data. These synthesized compounds were tested for their antibacterial activities against Gram +ve and Gram –ve bacteria and beta lactamase inhibitory activity which found as moderate to good for all synthesized compounds. The compound **IIId** was most active against all strains of bacteria as compared to synthetic compounds.*

Key words: *Indolin-2-on, Antibacterial activity, Beta lactamase inhibitors.*

3-(3-chloro-4-fluorophenylimino) indolin-2-on ve 5-chloro indolin-2-on Türevlerinin Beta Laktamaz İnhibitor Aktivitesi, Antimikrobiyal Etkileri ve Sentezleri

Farklı substitute 3-(3-chloro-4-fluorophenylimino)indolin-2-on ve 5-chloro-3-(3-chloro-4 fluorophenylimino)indolin-2-on' ın Mannich bazlı türevleri (IIIa-f) sentezlenmiştir. Sentezlenmiş olan tüm komponentler UV, IR ve ^{1}H NMR'ın spectral verileri yardımı ile karakterize edilmiştir. Sentezlenen komponentlerin antibakteriel aktivitesi, Gram + ve Gram – bakterilere karşı test edilmiş ve beta laktamaz inhibitor aktiviteleri incelenmiştir. Sentezlenen komponentlerin bu etkileri ortalama ile iyi arasında değişmektedir. Sentetik komponentlerle karşılaştırıldığında IIId olarak belirtilmiş olan komponentin tüm bakteri suşlarına karşı en etkili komponent olduğu görülmüştür.

Anahtar kelimeler: *İndolin-2-on, Antibakteriyel aktivite, Beta laktamaz inhibitörleri.*

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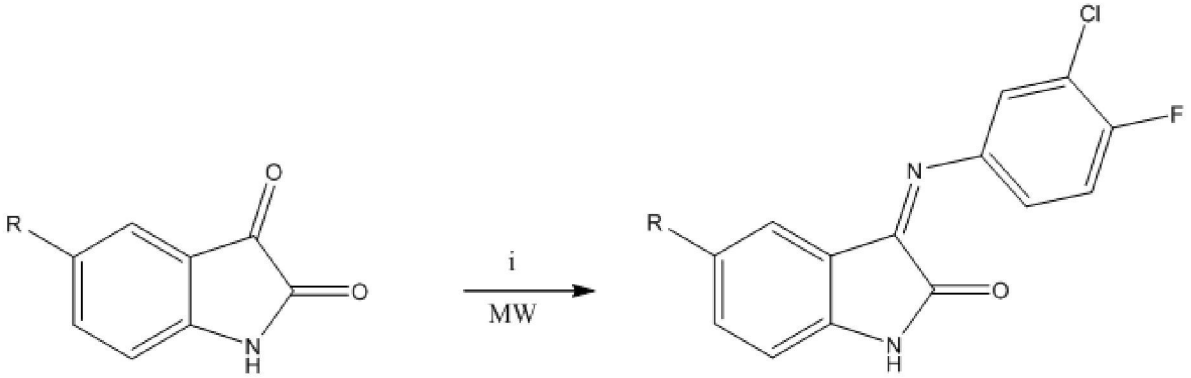
INTRODUCTION

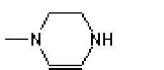
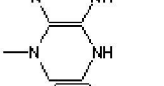
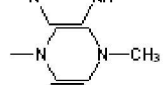
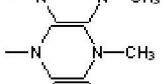
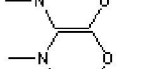
Isatin is an endogenous molecule identified in human beings that has anticonvulsant, tuberculostatic, analgesic and various other pharmacological activities. Extensive literature review has been made regarding the activities of the isatin, especially for its anticonvulsant, analgesic and anti-inflammatory activity. The synthetic versatility of the molecule has stemmed from the interest in the biological and pharmacological properties of the molecule and its derivatives. Microwave irradiation method was used for the synthesis of compounds IIa-b. Schiff base & Mannich base of isatin were reported to possess antimicrobial activity and various other pharmacological activities. Extensive literature review has been made regarding the activities of the isatin, especially for antimicrobial activity (1-10).

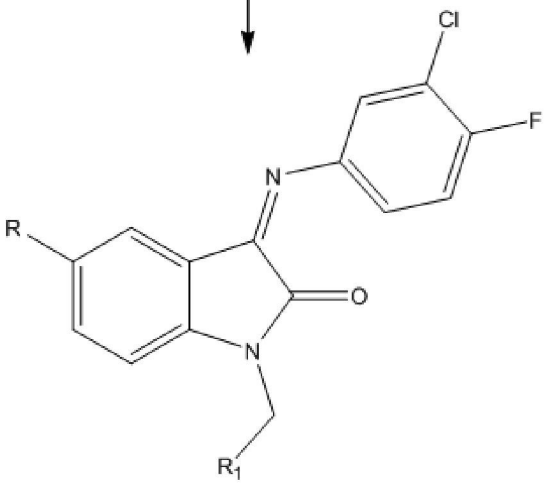
The main aim is to synthesize, perform beta lactamase inhibitory activity by iodometric assay and to carry antibacterial activity of titled compounds. In the present study Schiff base of 3-chloro-4-fluro aniline with isatin were synthesized and then their Mannich base derivatives with secondary amine such as morpholine, piperazine and N-methylpiperazine were synthesized



Ia-b **IIa-b**

ii

Comp. Code	R	R_1
IIa	H	--
IIb	Cl	--
IIIa	H	
IIIb	Cl	
IIIc	H	
IIId	Cl	
IIIe	H	
IIIf	Cl	



IIIa-f

Scheme 1. i= ethanol, GAA (Glacial acetic acid); ii= THF (tetrahydro furon), formaline solution, secondary amines (piperazine, N-methyl piperazine, morpholine,).

by using formaldehyde. All synthesized compounds were screened for antibacterial and beta lactamase inhibitory activities.

EXPERIMENTAL

All reagents were obtained from Sigma Aldrich and Loba Chem Ltd. (India). All the solvents used in these studies were dried and distilled before use. Melting points (m.p.): Veego VMP-PM digital melting point apparatus, and are uncorrected, TLC: Solvent used benzene : ethanol (9:1), UV spectra : Shimadzu Pharmspec 1700, UV-VIS spectrophotometer, IR spectra: Shimadzu 8400 S, FT-IR, ^{1}H NMR spectra: 300 MHz JEOL NMR Spectrophotometer.

*General procedure for synthesis of Schiff base of isatin and chloroisatin **(II a-b)***

Equimolar quantities of substituted isatin **Ia/Ib** (0.005mol) and chloro-fluroaniline were dissolved in warm ethanol containing 1ml of glacial acetic acid. The reaction mixture was irradiated in a microwave oven at 80% intensity with 30s per cycle. The number of cycle in turn depended on the completion of the reaction, which was checked by TLC. After completion of the reaction the mixture was poured in crushed ice. The resulting precipitate was filtered recrystallized and dried from ethanol.

*General procedure for synthesis of Mannich Base **(III a-f)***

A slurry consisting of the Schiff base of substituted isatin **IIa/IIb** (0.005 mol), THF (5 ml) & 37% formalin (2 ml) was added. To this piperazine/ N-methyl piperazine/ morpholine (0.005mol) was added drop wise with cooling and shaking. The reaction mixture was allowed to stand at room temperature for 1 hr with occasional shaking after which it was warmed on a steam bath for 15 min. At the end of the period the contents were cooled and the product was obtained, which was further recrystallized and dried from ethanol.

*3-(3-chloro-4-fluorophenylimino)indolin-2-on **IIa***

% Yield: 69; m.p. 225-227°C; UV λ_{max}: 247 nm; IR (KBr) (cm^{-1}): 756 (C-Cl), 914 (C-F), 1342 (C-N), 1731 (C=O), 3031 (Ar-CH), 3156 (NH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 4.15 (s, 2H, CH_2), 6.9-8.0 (m, 7H, Ar-H); Anal. Calcd. for $C_{14}H_8ClFN_2O$: C, 61.22; H, 2.94; N, 10.20. Found: C, 61.01; H, 2.91; N, 10.31.

*5-chloro-3-(3-chloro-4-fluorophenylimino)indolin-2-on **IIb***

% Yield: 70; m.p. 187-189°C; UV λ_{max}: 253 nm; IR (KBr) (cm^{-1}): 773 (C-Cl), 1042 (C-F), 1604 (C=N), 1730 (C=O), 3099 (Ar-CH), 3365 (NH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.9-7.6 (m, 6H, Ar-H); Anal. Calcd. for $C_{14}H_7Cl_2FN_2O$: C, 54.40; H, 2.28; N, 9.06. Found: C, 54.26; H, 2.25; N, 9.11.

*3-(3-chloro-4-fluorophenylimino)-1-(piperazin-1-ylmethyl)indolin-2-on **IIIa***

% Yield: 72; m.p. 159-162°C; UV λ_{max}: 242.5 nm; IR (KBr) (cm^{-1}): 796 (C-Cl), 867 (C-F), 1265 (C-O), 1344 (C-N), 1656 (C=N), 1730 (C=O), 2960 (C-H), 3353 (NH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.67 (t, 8H, CH_2), 4.19 (s, 2H, CH_2), 6.9-7.9 (m, 7H, Ar-H). Anal. Calcd. for $C_{19}H_{18}ClFN_4O$: C, 61.21; H, 4.87; N, 15.03. Found: C, 61.11; H, 4.91; N, 15.10.

*3-(3-chloro-4-fluorophenylimino)-5-chloro-1-((piperazin-1-yl)methyl)indolin-2-on **IIIb***

% Yield: 76; m.p. 197-199°C; UV λ_{max}: 244 nm; IR (KBr) (cm^{-1}): 775 (C-Cl), 1049 (C-F), 1604 (C=N), 1732 (C=O), 3101 (Ar-CH), 3367(NH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.31 (t, 4H, CH_2), 3.63 (t, 4H, CH_2), 4.17 (s, 2H, CH_2), 6.9-7.6 (m, 6H, Ar-H); Anal. Calcd. for $C_{19}H_{17}Cl_2FN_4O$: C, 56.03; H, 4.21; N, 13.79. Found: C, 56.00; H, 4.15; N, 13.85.

3-(3-chloro-4-fluorophenylimino)-1-((4-methylpiperazin-1-yl)methyl)indolin-2-on ***IIIc***

% Yield: 70; m.p. 182-184°C; UV λ_{max}: 243 nm; IR (KBr) (cm^{-1}): 757 (C-Cl), 831 (C-F), 3050 (Ar-CH), 2948 (C-H), 1728 (C=O), 1606 (C=N), 1344 (C-N) , 1263 (C-O); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.13 (s, 3H, CH_3), 2.45 (t, 8H, CH_2), 4.13 (s, 2H, CH_2), 6.9-7.6 (m, 7H, Ar-H); Anal. Calcd. for $C_{20}H_{20}ClFN_4O$: C, 62.10; H, 5.21; N, 14.48. Found: C, 61.99; H, 5.19; N, 14.55.

5-chloro-3-(3-chloro-4-fluorophenylimino)-1-(piperazin-1-ylmethyl)indolin-2-on ***IIId***

% Yield: 80, m.p. 193-195°C; UV λ_{max}: 245.5 nm; IR (KBr) (cm^{-1}): 776 (C-Cl), 1047 (C-F), 1607 (C=N), 1728 (C=O), 2954 (C-H), 3033 (Ar-CH), 3174 (Ar-CH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.06 (s, 3H, CH_3), 2.61 (t, 8H, CH_2), 4.08 (s, 2H, CH_2), 6.9-7.6 (m, 6H, Ar-H); Anal. Calcd. for $C_{20}H_{19}Cl_2FN_4O$: C, 57.02; H, 4.55; N, 13.30. Found: C, 56.78; H, 4.51; N, 13.41.

3-(3-chloro-4-fluorophenylimino)-1-(morpholinomethyl)indolin-2-on ***IIIe***

% Yield: 68, m.p. 176-178°C; UV λ_{max}: 244 nm; IR (KBr) (cm^{-1}): 759 (C-Cl), 867 (C-F), 3355 (Ar-CH), 3196 (Ar-CH), 1731 (C=O), 1610 (C=N), 1352 (C-O), 1261 (C-N); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.31 (t, 4H, CH_2), 3.57 (t, 4H, CH_2), 4.21 (s, 2H, CH_2), 6.9-7.6 (m, 7H, Ar-H); Anal. Calcd. for $C_{19}H_{17}ClFN_3O_2$: C, 61.09; H, 3.97; N, 10.35. Found: C, 61.01; H, 4.01; N, 10.31.

3-(3-chloro-4-fluorophenylimino)-5-chloro-1-(morpholinomethyl)indolin-2-on ***IIIf***

% Yield: 74, m.p. 208-210 °C; UV λ_{max}: 243.5 nm; IR (KBr) (cm^{-1}): 775 (C-Cl), 1049 (C-F), 3101 (Ar-CH), 3196 (Ar-CH), 1733 (C=O), 1604 (C=N); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.43 (t, 4H, CH_2), 3.55 (t, 4H, CH_2), 4.23 (s, 2H, CH_2), 6.8-8.0 (m, 6H, Ar-H); Anal. Calcd. for $C_{19}H_{16}Cl_2FN_3O_2$: C, 55.90; H, 3.95; N, 10.29. Found: C, 55.99; H, 3.90; N, 10.38.

Antimicrobial activity

The nutrient agar media (for antibacterial activity) were prepared in conical flasks and sterilized in autoclave. Suspensions of different micro organisms (inoculums) were prepared in different tubes in sterile distilled water. When the temperature of the sterile molten media reached to 40-45 ^{0}C, 0.5 ml of the inoculum was added, mixed and poured immediately into the sterile petri plates (10 cm diameter) and labeled accordingly. The agar was then allowed to solidify. The wells were prepared in plates using sterilized borer of 6 mm in diameter (3 wells/plate). About 50 µL of the control (Ethanol), sample solutions (II a-b) and (III a-f) 300 µg/ml) and the standard drugs (Amoxicillin 300µg/ml) were transferred into the wells in each plate using micropipettes. The plates were then refrigerated for 1 hr to occur prediffusion and the plates were then transferred to the incubator (temperature maintained at 37^{0}C) and were kept in incubator for 24 hr. The zones of inhibition were measured as average of 3 readings (11).

Beta lactamase inhibitory assay

All reagents were equilibrated to 30^{0}C in a water bath before adding them to the reaction tubes (20 x 150 mm. Pyrex test tubes) in the following order: first 1 ml of gelatin solution (1 per cent c. p. grade, E. Merck in 0.1*M* phosphate buffer, pH 7.0), 50 µl of enzyme, 1 drop of starch solution (1 per cent soluble starch), 1 ml of Penicillin solution (Crystalline Sodium Penicillin G procured as Benzyl penicillin IP, Alembic Ltd.) 1660 u /mg, dissolved in 0.1M phosphate buffer, pH 7.0, to contain not less than 5,000 u/ml), 3 ml of sample solution (II a-b) and (III a-f) and finally add 2 ml of iodine (0.01*N* iodine in 0.1*M* potassium iodide). Then the time of decolorization of iodine was recorded with a stop-watch, after addition of substrate blank should always be determined using solvent in place of sample solution.

Unit: Penicillinase activity is expressed in Pollock and Torriani unit. One unit is that amount of enzyme which will hydrolyze 1 μM Sodium Penicillin G in one hour at pH 7.0 at 30^0C (12,13).

RESULTS AND DISCUSSION

A series of various *5-H/chloro-3-(3-chloro-4-fluorophenylimino)indolin-2-on* Mannich base derivatives **(IIIa-f)** were synthesized. All the synthesized compounds were characterized on the basis of UV, IR, and ^{1}H NMR spectral data which confirming their respective structures. The derivatives obtained were as *Z* confirmation. All the synthesized compounds (50 μg/ml) were screened for their antibacterial activities against against *E. coli, P. aeruginosa, S. typhi, S. aureus* by agar diffusion method using Amoxicillin as a standard drug and the average diameter of zone of inhibition was recorded as shown in Table 1. All the synthesized compounds were screened for β-lactamase inhibitory activity were Penicillinase corresponding to time of decolorization of 2 ml of 0.01 *N* iodine was recorded and are shown in Table 2. All of the synthesized compounds were found as moderate to
good for antibacterial and β-lactamase inhibitory activity. The compound **IIId** was most active against amongst all the synthesized compounds and none of the compounds was found to good as compared with the standard drugs.

Table 1. Antibacterial screening result (zone of inhibition in mm) of title derivatives.

Compound	Zone Of Inhibition(mm) n=3			
	E.coli	*P.aeruginosa*	*S. typhi*	*S.aureus*
IIa	14	10	27	27
IIb	12	15	18	15
IIIa	19	23	17	16
IIIb	25	20	20	26
IIIc	19	18	11	25
IIId	10	11	12	15
IIIe	23	21	18	17
IIIf	26	23	24	22
Amoxicillin	28	30	29	30
Control	10	10	10	10

Table 2. Penicillinase corresponding to time of decolorization of 2 ml of 0.01 N iodine.

Sl. No.	Comp. Code	Time for decolorization of I_2 in Sec.	Activity u/ml	% Inhibition
1	**Control**	79.5	75.47	-
2	**Potassium Clavulanate**	240.50	24.94	66.95
3	**IIa**	131.0	45.80	39.31
4	**IIb**	170.2	35.25	53.29
5	**IIIa**	105.1	57.08	24.36
6	**IIIb**	200.6	29.91	60.39
7	**IIIc**	120.2	49.91	33.86
8	**IIId**	170.6	35.16	53.41
9	**IIIe**	198.5	30.22	59.95
10	**IIIf**	196.9	30.47	59.62

CONCLUSION

Various substituted *5-H/chloro-3-(3-chloro-4-fluorophenylimino)indolin-2-on* Mannich base derivatives **IIIa-f** were synthesized and characterized by spectral data. All the synthesized compounds were screened for their antibacterial activity and β-lactamase inhibitory activities which found as moderate to good as compared with standard drugs. These results suggest that further modification of these molecules may give really good molecules which may act as broad spectrum antibiotic.

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