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Synthesis, Characterization And Antimicrobial Activity of Novel Isatin Carboxamide Derivative

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ABSTRACT

synthetic medicines developed are popular even among younger generations Isatin or 1H-indole-2,3-dione is an indole derivative. Isatin is an important class of heterocyclic compounds. Recently, heterocyclic compounds analogues and their derivatives have attracted strong interest in medicinal chemistry due to their biological and pharmacological properties. The structure have offered a high value of diversity that is proven useful for the development of new medicinal drugs and improved potency,less toxicity and good pharmacological activity. Isatin has a wide varity of pharmacological **activities such as an antimicrobial, anticancer, antiviral, anticonvulsant, anti-inflammatory and analgesic etc. The synthesized compounds were screened for their in-vitro growth inhibiting activity against different strains of bacteria Staphylococcus aureus and Escherichia coli at 50,100,150 and200 µg/ml concentrations by using agar well diffusion technique. The results were compared with the standard antibiotics Ciprofloxacin (100 µg/ ml). The newly synthesized compound produced significant action against the bacterial strains at 100 µg/ml concentration.**

Keywords: Melting point, TLC, H[1]NMR, IR, Mass spectroscopy, Antimicrobial Evaluation and Agar well diffusion technique.

INTRODUCTION

Heterocyclic chemistry deals with heterocyclic compounds wich are widely distributed in nature and are essential to life.the majority of known molecules are heterocyclic and heterocycles dominate the fields of biochemistry, medicinal chemistry, dyestuffs and photographic[1]. The isatin is a class of heterocyclic compounds. Isatin has also been found to be plants, animals and fungi[2-4].It is a one of the component of coal tar[5.] It prepared from cyclizing the condensation product of chloral hydrate, aniline and hydroxylamine in sulfuric acid. This reaction is called the Sandmeyer isonitrosoacetanilide isatin synthesis[6]. Isatin exists in two forms, lactam form and lactimform. Both are derivatives of 2, 3-dihydroindole. This is an example for amido-imidol tautomeric system[7]. It may be prepared from cyclizing the condensation product of chloral hydrate, aniline and hydroxylamine in sulfuric acid. This reaction is called the sandmeyer isonitrosoacetanilide isatin synthesis[8].Isatin have been identified as interesting compounds that are collaborate with various biological activities[9]. In the present study we have planned to prepare N-(3-chlorophenyl) - Isatin-5-carboxamide and elucidate the structure by physical, chemical, spectral analysis and evaluate the antimicrobial activity[14-16].

Materials:
All the chemicals used in the synthesis were of laboratory grade (Sri Venkateswara Scientifics, Parsigutta and Hyderabad). The melting points were determined in open capillary on Veego (VMP-D) electronic apparatus and the errors were rectified. The IR spectra of the synthesized compound were recorded on Perkin Elmer BX2 FT-IR spectrophotometer in potassium bromide (anhydrous IR grade) pellets.NMR spectrum was recorded in DMSO using Bruker AV 500 ultra shield NMR instrument. A UV spectrum was recorded on a Specords 100 diode array spectrometer. Progress of the reactions was monitored using TLC, which was performed on glass slides (2x1.5cm) coated with silica gel-G using ethyl acetate: hexane (1:9) as the solvent system and the spots were visualized under UV light.

Methods:

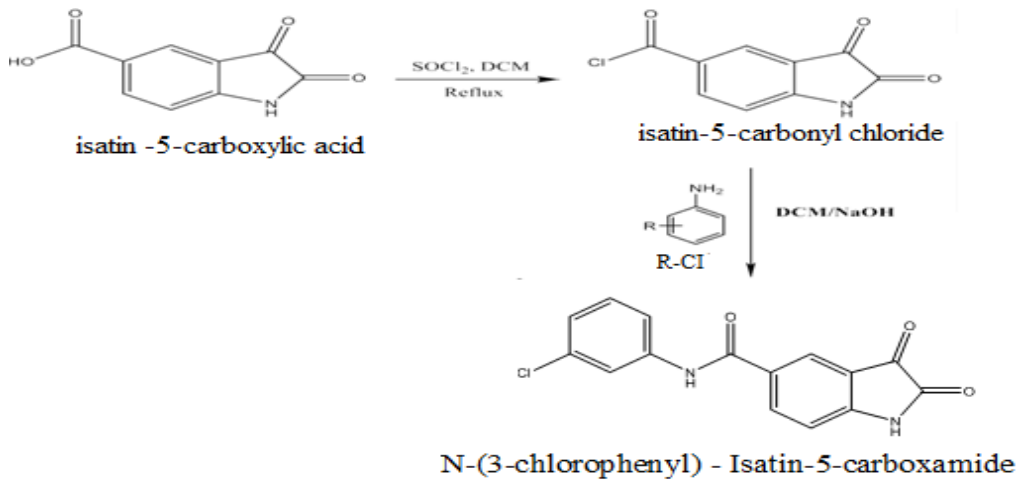
(STEP – 1): Synthesis of Isatin-5-carbonyl chloride[10]:

In a 100 mI two-necked glass round bottomed flask, 10mmol of isatin-5-carboxylic acid dissolved in 10ml dichloromethane and the RBF cooled to 0^0C. To this solution, 30mmol of thionyl chloride was added drop wise from the addition funnel fitted to the RBF. Reaction mixture was initially heated until all the evolution of gas ceases and then refluxed for 1.5hrs. Later the unreacted thionyl chloride distilled off directly from the reaction mass under vacuum with rotary evaporator and crude product of isatin -5- carbonyl chloride collected and directly utilized in the next reaction .

(STEP – 2): Synthesis of N-(3-chlorophenyl) - Isatin-5-carboxamide [11-13]:

10mmol of 3 chloro aniline were placed in a 100ml round bottomed flask and mixed with 10%sodium hydroxide solution. To this mixture 10mmol of isatin -5- carbonyl chloride pre-dissolved in 10ml of dichloromethane was added drop wise and stirred at room temperature until all the isatin-5-carbonyl chloride consumed. The reaction was monitored through TLC with 1:9 ethyl acetate and hexane. After reaction completion the reaction mass was worked up with dilute acetic acid to quench any excess sodium hydroxide, then washed with brine solution and product extracted with dichloromethane, vacuum evaporated and recrystallized from ethanol.

SCHEMATIC REPRESENTATION



Antimicrobial activity[17, 18]:

The antimicrobial activity of synthesized compound was determined by well plate or agar diffusion technique.

Preparation of media:

Wash the hands and wear gloves, sterilize all equipments then take 1gm of poptone bacteriological powder,1gm beef extract,2.5 gm agar and0.5gm sodium chloride then make the volume 100 ml with distilled water.Then heat todissolve completely and sterilize by using autoclave.

Screening for antimicrobial activity:

The in vitro antimicrobial activity was carried out against 24 hours old cultures of bacteria. The different strains of bacteria *Staphylococcus aureus* Gram positive bacteria and *Escherichia coli* Gram negative bacteria are used. Pure cultures of the test microorganisms were procured from the compounds were tested at the concentrations of 50,100,150 and 200 µg/ml and solutions were prepared by dissolving in distilled water. The petri dishes used for antibacterial screening were incubated at 37±1 ºC for 48 hours. The results were compared to Ciprofloxacin (100 µg/ml) for antibacterial activity, respectively by measuring zone of inhibition in mm.

RESULT AND DISCUSSION

Physical and Chemical Method:

The TLC of the synthesized compound was shown in figure-1. The synthesized compound, R_f value was found to be 0.84in Ethyl acetate: Hexane (1:9). It is light yellow crystals. Melting point: 200-201^{o}C. Molecular formula: $C_{15}H_9ClN_2O_3$.

Figure-1

Ultraviolet Spectrum:

About 200 mg of synthesized compound was dissolved in 10ml of anhydrous methanol. From this solution 0.1 ml of solution was taken and makes up to 10 ml with methanol. This was again made up to 10 ml with methanol by taking 1 ml of the above sample. This sample was scanned from 200nm-800nm. The synthesized compound melting point (150-170^{o}C) showed an intense λ max at 325 nm and absorbance is 0.9. Figure-2 represents the λmax of the synthesized compound.

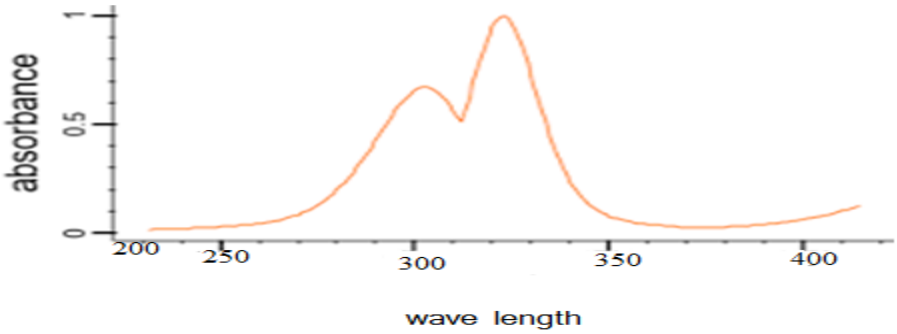


Figure-2

Infrared Spectrum:

The IR spectrum of synthesized compound is shown in fig: 3.The interpretation of various peaks obtained in the IR spectra of synthesized compound an enlisted below. Nmax in cm^{-1}: 1605.5 (C=N), 3280.1(NH), 3060.3(=C–H), 1290.5(C-N), 1540.3(C=C).

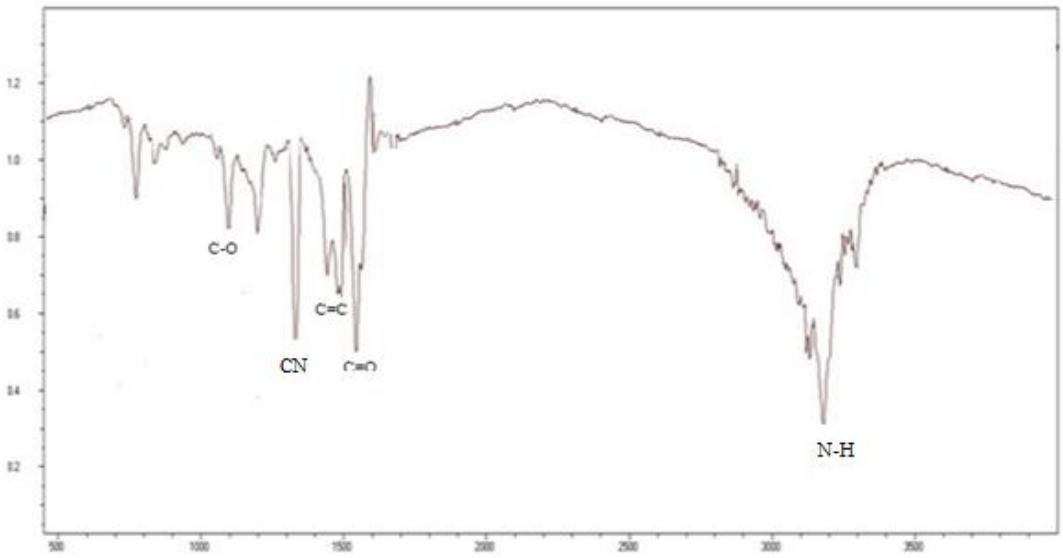


Figure-3

NMR Spectrum:

The ^{1}H NMR spectrum of synthesized compound is shown in figure- 4.The interpretation of various signals obtained in the ^{1}H NMR spectrum of synthesized compound is an enlisted below. (500 MHz, DMSO-d6) δ 10.10 (s, 1H), 8.81 (s, 1H), 8.02 (q, J = 3.9 Hz, 1H), 7.89 (dd, J = 6.6, 1.7 Hz, 1H), 7.47 – 7.39 (m, 2H), 3.04 (s, 3H), 2.59 (s, 3H).

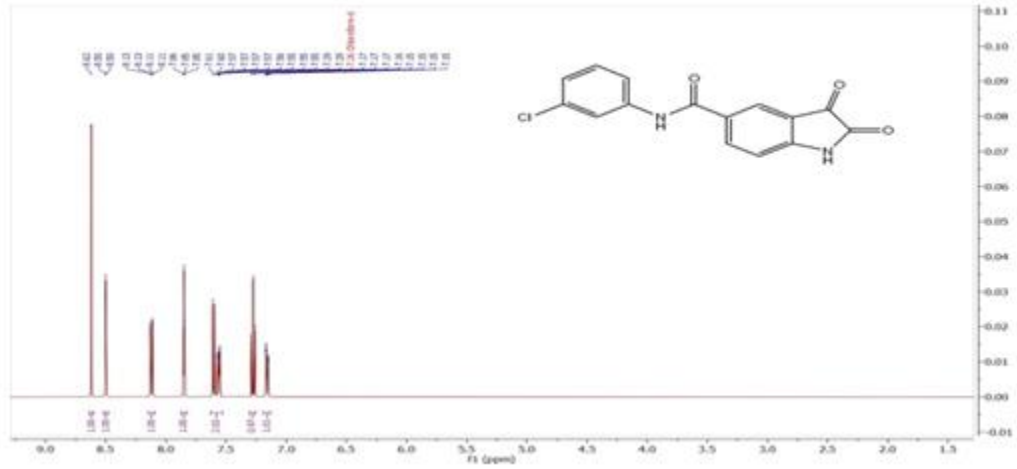


Figure-4

Mass spectrum:

The Mass spectrum of synthesized compound is shown in figure- 5. **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_9ClN_2O_3$ ([M + H]$^{+}$): 300.70, found 301.55.

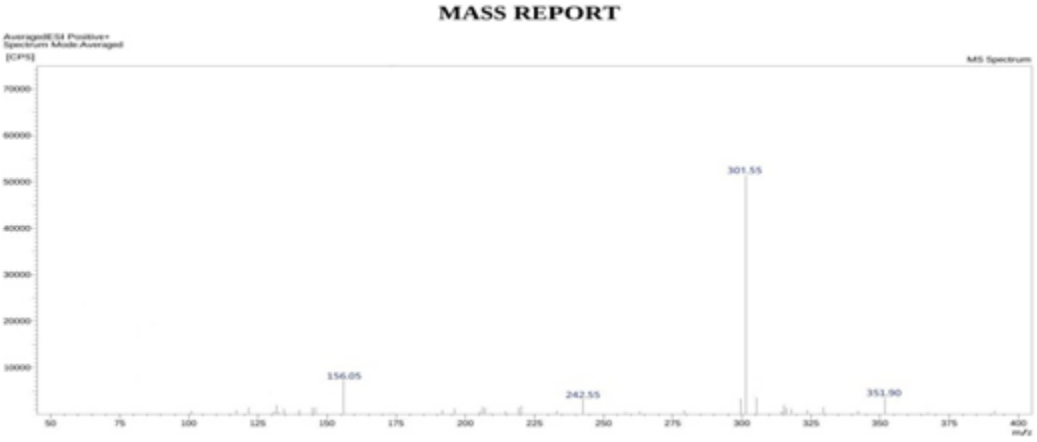


Figure-5

Antimicrobial activity:

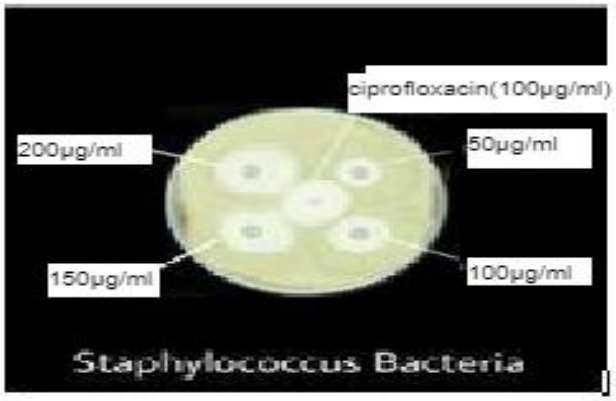
The antibacterial screening results were represented in Table 1.Table no 1 showing evaluation of in vitro antibacterial activity of N-(3-chlorophenyl) - Isatin-5-carboxamide.

Table-1

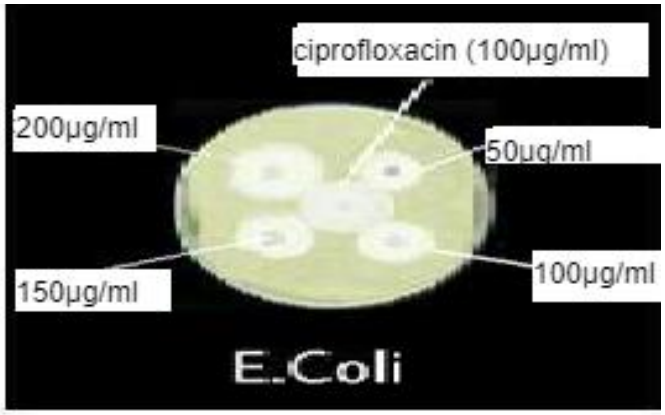
	Zone of inhibition (mm)	Zone of inhibition (mm)
Test Compound	Gram positive	Gram negative

	Staphylococcus aureus				*E.coli*			
	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml
N-(3-chlorophenyl) - Isatin-5-carboxamide	9.5	9.7	11.2	13.9	9.2	10.5	11.1	13.5
Ciprofloxacin (100µg/ml)	14.5				14.2			

Ciprofloxacin (100µg/ml) was used as positive reference standard antibiotic.
According to table, the minimum inhibitory concentration (MIC) revealed that the 200 µg/ml drug was showing good antimicrobial activity against gram negative and gram positive bacteria.



Antibacterial activity of the synthesized compound and Ciprofloxacin antibiotic against *Staphylococcus aureus*
Figure – 6



Antibacterial activity of the synthesized compound and Ciprofloxacin antibiotic against *E.coli*
Figure – 7

CONCLUSION

The synthesized compound, melting point: 200^0c -201^0c, pale yellow crystals on the U.V. studies indicated an isatin nucleus in the compound. The ^{1}H NMR spectrum of synthesized compound showed the presence of 9-hydrogen or proton present in the compound. Finally the IR spectrum confirms the functional groups present in the compound and mass

confirmed the molecular weight of the compound. Based on the Rf values, ^{1}H NMR, IR and MASS studies the structure of synthesized compound has been characterized as N-(3-chlorophenyl) - Isatin-5-carboxamide. A perusal of the literature reveals reports isatin nucleus having antimicrobial activity. Our synthesized compound was having isatin basic nucleus. So we are concluding according to result and discussion our compound also having antimicrobial activity.

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