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ANTIMICROBIAL ACTIVITY OF ISATIN LINKED CHALCONES

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Abstract: A series of novel isatin linked chalcones 4(a-n) was synthesized by knoevenagel reaction of N-phenyl propyl–5-substituted isatin with various acetophenone analogues and were screened for antimicrobial activity. The synthesized compounds were characterized by elemental data, IR, ^{1}HNMR, Mass spectral analysis. These compounds were also screened for their in vitro antimicrobial activity. The antimicrobial activity was experimented by cup plate method using Ciprofloxacin and Amphotericin B as standards against gram positive and gram negative bacteria and fungi respectively. The results of antimicrobial activity showed that amongst the fourteen synthesised derivatives, halogen substitution at 5th position of isatin were more potent.

Key Words: Thiophene, Pyridine, Isoquinoline, Ciprofloxacin and Amphotericin B.

INTRODUCTION

The versatile nature of heterocycles and their direct involvement in natural products has been known from century (Katritzky *et al*.,1984; Pozharskii *et al*.,2011; Vitaku *et al* 2014). Amongst the various heterocycles, the nitrogen-containing heterocycles have proven pervasive structural features and play a chief role in medicinal chemistry (Eicher *et al*., 2003; Nicolaou *et al*.,2008; Mitchell *et al*.,2012). In a large group of nitrogen containing heterocycles, indole motifs have received considerable attention due to their presence in proteins, amino acids, bioactive alkaloids, and drugs (Dolle *et al*.,1999; Sravanthi *et al*.,2016; Kaushik *et al*.,2013; Rahman *et al*.,2018). In this context, a large number of indole moieties have been investigated in the development of new effectual bioactive molecules with diverse pharmacological properties, such as antimicrobial (Sayed etal.,2014), antiviral (Abdel-Gawad *et al*.,2010), anticancer (Gali *et al*.,2015), anti-inflammatory (Srivastava *et al*.,2004), antitubercular (Khan *et al*.,2017) and antioxidant (Andreadou *et al*.,2002).Generally, the substitution at 3rd position of Isatin are known to exhibit innumerable biological activity (El-Sawy *et al*.,2013; Cao *et al*.,2014; Chen *et al*.,2014). On the other hand, the significance of N-substituted isatin derivatives are in marketed drug molecules, natural products, and marine organisms are of great extent (Biswal *et al*.,2012; Humphrey *et al*.,2006). Further Chalcones a class of compounds have ascertained to be a useful structural motif in medicinal chemistry, having applications in the development of drugs for the treatment of malaria, cancer, protozoal infections, inflammation, etc.(Nowakowska,2007). The significance of the chalcone scaffold in the biological system can be realized being a common substructure in numerous natural products belonging to the flavonoid family (Solomon *et al*.,2012). Keeping in view the diverse spectrum of biological activities of indole and chalcones, here in we report fourteen analogues of novel isatin linked chalcone derivatives synthesized, characterized in the present investigation and screened for their antibacterial and antifungal activities. Below is the structure of synthesised 3-(2-oxoethylidene)-1-(3- phenyl propyl) indole-2-one derivatives and table 1 depicts the substitution at R and R_1

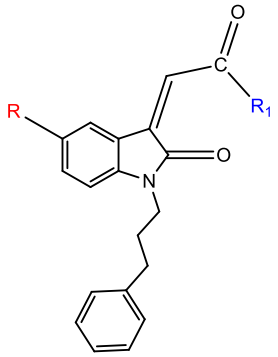
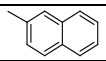
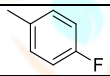
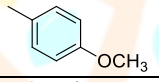
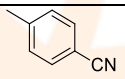
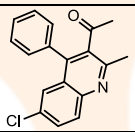


Table 1: Substitution at R and R_1 of 3-(2-oxoethylidene)-1-(3-phenylpropyl) indolin-2-one derivatives (4a-n)

Compound No	R	R_1	R	Compound No
4a	H		Cl	4h
4b	H		Cl	4i
4c	H		Cl	4j
4d	H		Cl	4k
4e	H		Cl	4l
4f	H		Cl	4m
4g	H		Cl	4n

The synthesised indole derivatives were screened for their antibacterial activity against *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29737), *Escherichia coli* (ATCC 9637), *Salmonella typhi* (ATCC 6539). The results were compared with the standard reference drug Ciprofloxacin. The synthesised compounds were evaluated for their antifungal activity against *Aspergillus niger* (MTCC-281), *Aspergillus flavus* (MTCC-1973) and compared with that of Amphotericin B.

MATERIALS AND METHODS

The antimicrobial activities of the synthesised compounds were evaluated against standard gram positive, gram negative and fungal test strains by using cup plate method (Seely *et.al.,1975*). By dissolving bacteriological peptone (0.1%), beef extract (0.5%), Sodium chloride (0.5%) in distilled water the nutrient agar was prepared and the P^H of the solution was adjusted to 7 to 7.4 by using NaOH solution (40%). It was then sterilized for half an hour at 15 lbs pressure. By dissolving peptone (1 g) and glucose (4 g) in distilled water (100 ml) and filtering Sabourauds agar was prepared for antifungal activity Agar powder (2 g) was added and sterilized for 30 minutes at 15 lbs pressure. The preparation of subculture was carried out one day earlier to test. the microorganisms were inoculated into the sterilized nutrient broth and incubated at 37°C for 18 to 20 hr. The standard Ciprofloxacin was prepared by dissolving 5 mg of ciprofloxacin in 5 ml of DMF to get a concentration of 1000 µg/1 ml. similarly 5 mg of Amphotericin B was dissolved in 5 ml of DMF to get a concentration of 1000 µg/1 ml.5 mg of each test compound was dissolved in 5 ml of DMF in serially and suitably labelled sterile test tubes thus giving a final concentration of 1000µg/ 1ml.

This method depends on the diffusion of an antibiotic from a cavity through the solidified agar layer in a petridish to an extent such that growth of the added microorganisms is prevented entirely in a circular area or zone around the cavity containing a solution of test drug. About 15-20 ml of molten nutrient agar was poured into each of the sterile Petri dishes. The cups were made by scooping out nutrient agar with a sterile cork borer of 6 mm diameter. The agar plates so prepared were divided into different sets and each set of the plates were inoculated with the suspension of particular organism by spread plate technique.

The cups of inoculated plates were then filled with 0.1 ml of the test solution; the plates were then incubated at 37ºC for 24 hr. The zone of inhibition developed, if any, was then measured for the particular compound for each organism. The solvent DMF was used as control to know the activity of the solvent. The results of antibacterial and antifungal activity are summarized in Table 2.

Table-2: Antimicrobial activity results of 4(a-n)

Zone of inhibition in mm.						
Compound	Antibacterial activity				Anti-fungal activity	
	Gram positive		Gram negative			
	B.Subtilis	S.aureus	E. coli	S. typhi	A.fumigatus	A.Niger
4a	**8±.46**	**10±.235**	**18±.136**	**14±.23**	**10.4±.38**	**8.6±.24**
4b	**17±.28**	**18±.12**	**24±.27**	**20±.272**	**18±.471**	**17.2±.34**
4c	**15±.51**	**14±.27**	**20±.17**	**18±.32**	**15.53±.24**	**14.76±.32**
4d	**12±.54**	**14**	**22±.13**	**19±.272**	**14.4±.169**	**11.33±.27**
4e	**8±.27**	**9±.24**	**16±.27**	**12±.23**	**9.9±.42**	**6.66±.45**
4f	**12±.48**	**11±.163**	**14±.15**	**16±.30**	**12.33±.27**	**11.66±.272**
4g	**23±.09**	**21±.51**	**27±.12**	**23±.26**	**20±.24**	**19±.10**
4h	**10±.26**	**12±.136**	**24±.27**	**15±.21**	**9±.23**	**10.83±.360**
4i	**24±.27**	**22±.32**	**23±.27**	**21±.42**	**18.5±.235**	**17.6±.25**
4j	**14±.47**	**15±.27**	**21±.24**	**20±.31**	**16.5±.235**	**15.33±.272**
4k	**12±.27**	**13±.23**	**25±.25**	**22±.25**	**12.86±.381**	**11.56±.24**
4l	**11±.46**	**9±.25**	**16±.23**	**17±.16**	**11.63±.25**	**11±.47**
4m	**13±.28**	**12±.24**	**17±.36**	**18±.09**	**13.76±.32**	**12.76±.32**
4n	**24±.12**	**23±.31**	**27±.22**	**25±.11**	**22±.26**	**23±.14**
Ciproflaxcin	**26**	**28**	**30**	**26**		
AmphotericinB					**28**	**30**

Values are expressed in mean±SD

RESULTS AND DISCUSSION

As many as new fourteen compounds were synthesised, characterized by their physical, analytical and spectral data. The isatin linked chalcones were then screened for antimicrobial activity by the cup plate technique. The compounds **4b, 4g, 4i, 4n,** have shown maximum zone of inhibition against *Bacillus subtilis* (ATCC 6633),, *Staphylococcus aureus* (ATCC 29737), *Escherichia coli* (ATCC 9637), and *Salmonella typhi* (ATCC 6539) . **4b** and **4i** incorporate thiophene and 4g and 4n have isoquinoline derivative in them. The report of antibacterial activity displays that, the compounds **4a, 4b, 4c, 4d, 4g, 4h, 4i, 4j, 4k, 4n,** have shown maximum zone of inhibition against gram negative bacterial strains i.e , *Escherichia coli* (ATCC 9637), and *Salmonella typhi* (ATCC 6539). The presence of methoxy group in compounds **4e** and **4h** having $–OCH_3$ as substituent displayed least activity. The synthesised compounds having halogen at 5th position of isatin nucleus and also N-substituted derivatives were found to be more potent against gram negative bacteria. The results of antifungal activity revealed that compounds **4b, 4g, 4i, 4n, have** exhibited very good antifungal activity against *Aspergillus flavus (MTCC-1973) and Aspergillus niger (MTCC-281),* remaining compounds showed moderate zone of inhibition. to evaluate the efficacy of the tested compounds under the same conditions, Ciprofloxacin is used as bacterial standards and amphotericin B is used as fungal standards for references.

CONCLUSION

By Knovenegal reaction method fourteen analogues of isatin derivatives were synthesized. The products were obtained in good yields and were also in pure form. The synthesised compounds were tested for their antimicrobial activity against Gram-positive and Gram-negative bacteria and fungal pathogenic organisms. The results were made in triplicate and the means were calculated. It was found that the activity was associated with the presence of the chlorine at the 5th position of the isatin moiety of the prepared compounds. The outcome of the activity is thiophene(4b,4i) and isoquinoline derivative(4g,4n) showed good to high activity towards the all Gram-negative bacterial strains and the two fungal tested microorganisms. All the synthesised compounds showed good to moderate activity except in compounds having methoxy as substituent but none of the compounds showed activity equal to the standard drug.

FUTURE SCOPE: Monochloro substituted 3-(2-oxoethylidene)-1-(3-phenylpropyl) indolin-2-one derivatives have shown good antimicrobial activity, if compounds of dihalogen substitution at 5th and 7th position of isatin are synthesised or if more electronegative atom is substituted at 5th position then the antimicrobial activity may be enhanced.

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CONFLICT OF INTEREST. None

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