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An Antibacterial, Antifungal and Anthelmintic Evaluations of Some Synthesized Chalcone Derived Benzimidazoles

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The Chalcone nucleus based natural plant products have various biological activities due to their highly reactive a, b unsaturated carbonyl group. If these medicinal plants not traceable in nature we must go through new synthetic drugs as a source of chalcone analogues. So we synthesized a series of chalcone derived benzimidazoles by condensation of 2-acetyl benzimidazole in alkali with various aromatic aldehydes. The synthesized chalcones were screened for their in-vitro antimicrobial activity using disc diffusion and broth micro-dilution assays. The synthesized chalcones shows good activity against the tested fungal species. They have better activity against gram positive bacteria but shows poor activity against gram negative bacteria. The In-vitro anthelmintic screening of all chalcones exhibited significant potency when compared to standard drug albendazole.

Key words: Chalcones, Benzimidazolyl chalcones, Antibacterial, Antifungal & anthelmintic.

The Chalcone analogues are basic nucleus of various natural plant products such as flavonoids, isoflavonoids, aurones, tetralones, aziridines and clavicin etc.This nucleus analogue is an integral part of various natural medicinal plant constitutions such as terpeneoids, dicoumarol, flavokawain, digitalis glycosides [1] and vitamin K etc. However in recent years the introduction of new synthetic chalcone derived drugs has out placed that of natural products such as warfarin for dicoumarol. The Chalcone analogues are a chemical class that has depicted antioxidant[2], antiviral, antibacterial [3], antifungal [4], insecticidal, antitrichomonal, antitubercular [5],antileishmanial, antimalarial, inhibit eosinophilia (asthma), antiseptical [6], analgesic/anti-inflammatory [7], cyclooxygenase inhibitors [8], anti ulcerogenic, prostaglandin binding, antiallergic, anaesthetic, antiplasmodial [9], hypotensive, antifibrogenic , immuno suppression,antineoplastic, cytotoxicity, anticancer [10], antiproliferative [11], antileukemic, antitumor [12] ,trypsin inhibitor, anxiolytic [13] and anthelmintic [14] activities.

Benzimidazole nucleus is structurally related to purine nucleoside bases and it is found in some natural products, such as vitamin B_{12} , marine natural product namely makaluvamins etc. The literature survey reports have been revealed that the 2- substituted 1H-benzimidazole compounds has reported to possess antibacterial [15], antifungal [16], antitubercular , antiviral [17], antiulcer and anthelminitic [18,19] activities . The benzimidazolyl chalcones also reported to have potent antimicrobial [20] properties. So we planned to merge the chalcone nucleus with benzimidazole at 2nd position to exhibit some better bioloical activity, mainly to screen for their in-vitro antimicrobial and anthelminitic activities.

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EXPERIMENTAL

Synthesis of 1-(1H-benzoimidazol-2-yl) ethanol (3)

The lactic acid (1) and ortho-phenylene diamine (2) were refluxed for one and half an hour. The mixture was cooled, 25% potassium hydroxide solution was added until the product (3) was just alkaline to litmus.

Synthesis of 1-(1H-benzoimidazol-2-yl)ethanone (4)

The 0.01 molar (3) was taken in 10ml of 50% sulphuric acid and 20% potassium dichromate mixture, refluxed for one hour and allowed to cool at room temperature and poured, in to a ice cold water with stirring to get a product(4).

Synthesis of benzimidazolyl chalcones (6a-j)

The 0.01 molar (4) in 10ml 30% potassium hydroxide with 0.012 molar of various aromatic aldehydes namely, para-anisaldehyde (5a) , benzaldehyde (5b) , cinnamaldehyde (5c), ortho-chloro benzaldehyde (5d), para-(dimethyl amino) benzaldehyde (5e), para-fluorobenzaldehyde (5f), ortho-nitro benzaldehyde (5g), para-nitro benzaldehyde (5h) , ortho-hydroxybenzaldehyde (5i) and 4-hydroxy 3-methoxybenzaldehyde (5j) separately, then refluxed for 2 hours with water bath, allow cooling to the room temperature and pouring the mixture in to a beaker containing ice cold water, with stirring to get a product namely, 1-(1H-benzoimidazol-2-yl)-3-(4-methoxyphenyl) propenone (6a),1-(1H-benzoimidazol-2-yl)-3-Phenyl Propenone (6b), 1-(1H-benzoimidazol-2-yl)-5-Phenyl Pentadienone (6c), 1-(1H-benzoimidazol-2-yl)-3-(2-Chloro Phenyl) Propenone (6d), 1-(1H-benzoimidazol-2-yl)-3-(4-(dimethyl amino) Phenyl) Propenone (6e), 1-(1H-benzoimidazol-2-yl)-3-(4-fluoro Phenyl) Propenone (6f), 1-(1H-benzoimidazol-2-yl)-3-(2-nitrophenyl) Propenone (6g), 1-(1H-benzo imidazol-2-yl)-3-(4-nitrophenyl) Propenone (6h), 1-(1H-benzoimidazol-2-yl)-3-(2-hydroxy phenyl) propenone (6i) and1-(1H-benzoimidazol-2-yl)-3-(4-hydroxy-3-methoxy phenyl) propenone (6j). All the above products were collected by filtration with ice cold water, dried and recrystallised from 90%ethanol. The structures of the synthesized compounds were verified by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral analysis.

Antimicrobial activity

Micro-organisms

Two strains of bacteria used were Klebsiella aerogenes MTCC-39, and *Escherichia coli* MTCC 1302. Three fungal species were used, these being *Aspergillus niger* MTCC 2425*, Candida albicans* MTCC *183* and the Penicillium citrinum MTCC 1256. All bacterial strains were cultivated in Mueller Hinton Agar (MHA) media and fungi species were cultivated in Sabouraud Dextrose Agar (SDA) media. The stock culture was maintained on agar slant at 4 °C. These microbial strains were subculture on a fresh appropriate agar plate 24 hrs prior to any antimicrobial test.

Agar diffusion assay

The antimicrobial activity was carried out by using 100 µl of suspension of the tested microorganisms containing 2.0×10^{6} CFU/ml for bacteria and 2.0×10^{5} CFU /ml spores for fungal strains. This microbial suspension was used to inoculate by flooding the surface of MHA and SDA plates for bacteria and fungi respectively. Then sterilized discs were prepared at 30µg/disc for synthesized chalcones and 10µg/disc for standard antibiotics. A discs have synthesized chalcones were prepared with only the corresponding volume of dimethylsulphoxide was used as negative control. The petri plates were then incubated at 37°C for bacteria and 28°C for fungi species. The antimicrobial activity was evaluated by measuring the diameter of the zones of inhibition around the disc.

Broth micro-dilution assay

The minimum inhibitory concentrations (MIC) were determined by twofold serial micro broth dilution method in Mueller Hinton or Sabouraud dextrose broth media. The synthesized chalcones and standard antibiotics were dissolved in 50% dimethylsulphoxide aqueous solution. All the compounds were diluted two fold concentration from 0.2 to 204 µg and the starting inoculums of 2.0×10^{7} CFU/ ml were used. The test tubes were incubated at 37°C for bacteria and 28°C for fungi. The lowest concentration of the drug displaying no visible growth was considered as the MIC.

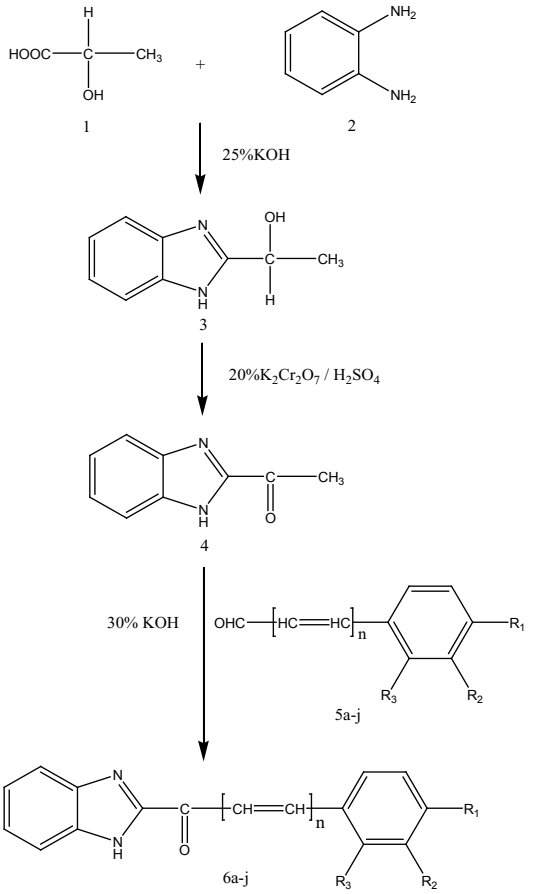
Anthelmintic Investigation

The south Indian adult earth worms *Pheretima posthuma of* 7-9cm in length and 0.2-0.3 cm in width were selected for the invitro anthelminitic activity due to its anatomical

and physiological resemblance with the gastro intestinal worm parasites of human beings. The earth worms washed with normal saline solution to remove all fecal and adhering soil materials before they were released in to petridishes. The standard drug albendazole and tested benzimidazoles were prepared at a doses level of 10, 30,50 mg by dissolving in, about 0.5ml of dimethylsulphoxide, the volume was made-up to 15 ml with normal saline, then poured into petridishes. The five earth worms were taken in each petridishes. Then, the time taken for the induction of complete paralysis and time taken for death of individual earthworms were observed. The control group was observed that the worms were still alive up to 48 hours.

RESULTS AND DISCUSSION

In this study, series of substituted benzimidazolyl chalcones were prepared and evaluated for their antimicrobial activities using disc diffusion and micro broth dilution assays, the results are displayed in Table 2. The chalcones 6d, 6f, 6g and 6h were showed effective zone of inhibition against Klebsiella. All the chalcones displayed less effective zone of inhibition against *E. coli* except 6f. The chalcones 6d, 6f, 6g and 6h exerted potent invitro antifungal activity against *Aspergillus,* Penicillium and *Candida species.* Observations showed that chalcones 6a, 6c, 6e and 6j had better activity against *Aspergillus,* Penicillium and *Candida species* respectively. All the tested chalcones showed good zone of inhibition against fungi *species* and gram positive bacteria, Klebsiella except the 6b and 6i. The most of the chalcones exhibited better activity against *Candida* than the *Aspergillus* and Penicillium strains. Only chalcone 6f have a potent activity



Scheme 1:

Table 1. Physical analysis of synthesised benzimidazolyl Chalcones

Chalcones	Molecularformula	R_1	R_2	R_3	n	Melting Point^{O}C	R_f value	Yield %
6a	$C_{17}H_{14}N_2O_2$	OCH_3	H	H	1	203–204	0.60	65
6b	$C_{16}H_{12}N_2O$	H	H	H	1	183–184	0.54	62
6c	$C_{18}H_{14}N_2O$	H	H	H	2	195–196	0.57	66
6d	$C_{16}H_{11}ClN_2O$	H	H	Cl	1	210–211	0.62	72
6e	$C_{18}H_{17}N_3O$	$N(CH_3)_2$	H	H	1	214–215	0.65	75
6f	$C_{16}H_{11}FN_2O$	F	H	H	1	187–188	0.68	81
6g	$C_{16}H_{11}N_3O_3$	H	H	NO_2	1	222–223	0.43	52
6h	$C_{16}H_{11}N_3O_3$	NO_2	H	H	1	236–237	0.41	58
6i	$C_{16}H_{12}N_2O_2$	H	H	OH	1	190–191	0.84	55
6j	$C_{17}H_{14}N_2O_3$	OH	OCH_3	H	1	229–230	0.80	60

Solvent system (TLC): chloroform: 90% ethanol (8:2)

against all microbial *species*.

When structure activity relationship studies are concerned, the antimicrobial activity might be increased by the presence of electronegative substituents such as NO_2, fluoro and chloro groups at ortho or para (R_1 or R_3) position on the chalcone benzene ring, while polar electron donating substituent OH group at ortho or para (R_1 or R_3) position decreases activity. Furthermore, compounds bearing nonpolar electron donating $-N(CH_3)_2$ or OCH_3 groups as substituents at para or meta (R_3 or R_2) position on the benzene ring, both enhanced the activity. It was observed that para substituted analogues are more potent than ortho or meta substituted chalcones. Moreover extended conjugated analogue (6c) also slightly stimulates to enhance antimicrobial activity.

In vitro anthelminitic screening results in the Table 3, data displays chalcone 6a, 6c, 6d, 6g, 6h and 6i showed appreciable activity. It indicates that

Table 2. Antimicrobial activity & Minimum inhibition concentration of the synthesized benzimidazolyl chalcones 6 a-j and reference antibiotics

Groups	Inhibition zone diameters in (mm)					Minimum inhibition concentration (µg)				
	K A	E C	A N	*C A*	P C	K A	E C	A N	*C A*	P C
6a	7.7	5.8	9.5	10.7	10.1	25.6	102.4	6.4	6.4	6.4
6b	5.3	4.0	7.8	9.0	8.2	102.4	204.8	25.6	6.4	12.8
6c	6.6	4.5	8.2	9.5	8.7	51.2	204.8	12.8	6.4	12.8
6d	8.0	6.2	10.0	11.2	10.5	12.8	51.2	6.4	3.2	6.4
6e	7.5	5.6	9.5	10.3	9.7	25.6	102.4	6.4	6.4	6.4
6f	11.5	8.3	13.6	15.3	14.7	3.2	12.8	3.2	1.6	3.2
6g	8.8	6.5	10.2	11.5	10.4	12.8	51.2	6.4	3.2	6.4
6h	10.2	7.4	12.5	13.8	13.1	6.4	25.6	3.2	3.2	3.2
6i	5.0	3.7	7.2	8.7	8.0	102.4	204.8	25.6	12.8	25.6
6j	7.3	5.2	9.0	9.8	9.3	25.6	102.4	6.4	6.4	6.4
CIPN	16.5	16.2	×	-	-	0.2	0.2	-	-	-
CLOE	-	-	16.8	17.2	16.7	-	-	0.4	0.2	0.4

Values are given an average mean, (*n = 3*). Anti-microbial activity synthesized chalcones 6a-j (30 µg D disc) and standard antibiotics (10 µg D disc).
K A - Klebsiella aerogenes, E C- Escherichia coli, A N- Aspergillus niger, C A-Candida albicans, P C- Penicillium Citrinum, CIPN- Ciprofloxacin, CLOE- Clotrimazole.

Table 3. In vitro anthelminitic activity of the synthesized benzimidazolyl chalcones (6a-j)

Groups	Time taken for paralysis (P)			Time taken for death (D)		
	10 mg/group	30 mg/group	50 mg/group	10 mg/group	30 mg/group	50 mg/group
6a	29.30±1.24*	18.05±0.53**	16.15±0.46***	45.37±0.50***	33.05±0.61***	24.45±0.18***
6b	30.22±1.01**	20.05±0.58***	18.56±0.21***	51.25±0.65***	38.25±0.58***	29.07±0.16***
6c	32.10±1.17***	19.45±0.57***	18.09±0.47***	51.18±0.62***	37.44±0.64***	27.48±0.31***
6d	30.02±0.97**	18.38±0.51***	17.50±0.26***	48.02±0.78***	35.06±0.52***	25.12±0.26***
6e	29.56±0.80**	20.40±0.38***	20.25±0.27***	53.02±0.72***	39.47±0.63***	30.22±0.33***
6f	28.58±0.99*	17.22±0.59*	14.29±0.35**	40.26±0.66*	29.03±0.57*	21.05±0.15***
6g	30.43±1.33**	19.12±0.53***	19.05±0.29***	51.40±0.52***	37.20±0.59***	27.25±0.19***
6h	30.06±1.11**	18.55±0.59***	18.22±0.27***	49.44±0.70***	35.43±0.57***	25.38±0.20***
6i	29.57±1.18*	18.21±0.57**	17.32±0.34***	46.19±0.51***	33.40±0.40***	24.10±0.21***
6j	29.07±0.90*	17.25±0.53*	15.19±0.36***	42.27±0.64***	30.25±0.60***	22.18±0.24***
ALZ	23.51±0.91	14.55±0.62	12.33±0.21	37.24±0.33	26.28±0.26	19.12±0.07

Each average value represents the mean ± SEM (n=5). Significance levels *P<0.5, **P<0.01 and ***P<0.001 as compared with the respective standard drug (ALZ- Albendazole).

compounds 6f and 6j exhibited higher potency than other tested benzimidazoles chalcone while the compounds 6b and 6e were registered less activity. The structure activity relationship studies revealed that polar electron donating substituent's like OH and OCH_3 group is found to increase the activity, where as non polar electron donating substituent -N–$(CH_3)_2$ group found to reduce activity. It has been observed that unsubstituted compounds (6b, 6c) marked more effective activity than N–$(CH_3)_2$ substituted compound (6e). The compounds with OCH_3 substituent at the para position (6a) led to a enhance the activity than meta position substituted compound (6j). Moreover, the para OH substituted compound (6j) has more potent activity than ortho OH substituted compound (6i). The para F substituted compound (6f) has excellent potency than ortho Cl substituted compound (6d). The bulky electron withdrawing NO_2 group shows less potency than F and Cl groups. Amoung this, para NO_2 substituted compound (6h) produced more activity than that of ortho NO_2 substituted compound (6g) and the extended conjugated compound (6c) displayed better activity than parent benzimidazole (6b).

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