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RESEARCH ARTICLE

SYNTHESIS, CHARACTERIZATION AND ANTI-FUNGAL ACTIVITY OF ISATIN SCHIFF BASE DERIVATIVES

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ABSTRACT

Isat-2-one was reacted with 4-amino acetanilide in the presence of glacial acetic acid and ethanol. Water molecule was eliminated to form Isatin Schiff base. The obtained Isatin Schiff bases, on reaction with multiple benzaldehyde derivatives in the presence of ethanol and potassium hydroxide formed various products of chalcone derivatives. The obtained compounds (C1-C7) were analysed using H+ NMR, IR, Mass spectroscopy and elemental analysis data and were found to match with the given structures. The synthesized compounds were evaluated for their in vitro anti-fungi activity by using agar disc diffusion method. Was carried out against 2 fungi at concentration 24, 50 and 100 µg/ml.C1 and C7were found to be the most potent against the fungi.

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INTRODUCTION

Isatin is a di-one indole derivatives. Erdman and Laurent were the first to acquire Isatin in 1841 through the oxidation of indigo by nitric acid and chromic acids. In nature, plants of the genus Isatis (*Calanthe discolor* LINDL and in *Couroupitaguianensis* Aubl) were found to contain Isatin. Also in animal like Bufo frogs, isatin is found in its secretion from the parotid gland. The vast recent research and exploitation of Isatin and its derivatives as a synthesized product or substrate is as a result of the presence of numbers of functionali sable groups and its broad range of biological and pharmacological properties like –antibacterial (Erdmann, 1840), anti HIV (Alshehri et al., 2010), antitubercular (Ozlen et al., 2008), antitumor (Hoyun, 2009), anti-inflammatory (Gummadi et al., 2010), antioxidant (Shibinskya et al., 2010), antiviral (Shibinskya et al., 2010), anticonvulsant (Prince et al., 2009) and CNS depressant activities (Zapata-Sudo et al., 2007). Infections caused by fungi are called mycoses and they can be divided into superficial infections (affecting skin, nails, scalp or mucous membranes) and systemic infections (affecting deeper tissues and organs). Systemic mycoses are difficult to treat and are often life threatening. The common systemic fungal disease is systemic candidiasis caused by a yeast like organism. Other more infections are cryptococcal meningitis or endocarditis, pulmonary aspergillosis and rhinocerebralmucormycosis. Invasive pulmonary aspergillosis is a leading cause of death in recipients of bone marrow transplants.

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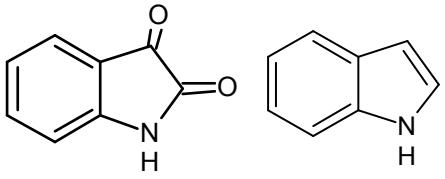
Bronchopulmonary aspergillosis is caused by colonisation of lungs of patients with asthma or cystic fibrosis. Superficial fungal infections can be classified into dermatomycoses and candidiasis. Dermatomycoses are infections of the skin, hair, and nails caused by species such as Trichophyton, Microsporum and Epidermophyton. These cause various types of ring worm or Tinea. Tinea capitis affects the scalp, Tinea curis - the grain, Tinea pedis - the eet (causing athletes foot) and Tinea corposis - the body. In superficial candidiasis, the yeast-li ke organism infects the mucous membranes of the mouth, vagina and skin. Individuals suffering from malignancy, diabetes mellitus, those on corticosteroids and immuno- compromised subjects are more prone to develop fungal infections. Infections caused by Fungi affect different parts and system of the human and can be very difficult to eradicate. Hence there is great necessity for the development and discovery of new compounds with anti-fungal properties which could become a potential lead molecule for control and management of fungal infection

ISATIN



Isatin (1) Indole (2)

The objective of the proposed study is to synthesize and evaluate the anti-fungal activity of the compound with the aim to:

- To Synthesize Schiff base from isatin derivative.
- To incorporate the benzaldehyde derivatives to the synthesized Schiff base of isatin to form various chalcones.
- To characterize the new chalcone derivatives by IR, NMR, Mass spectroscopy and elemental analysis and to evaluate their anti-fungal activity (*in vitro*)

MATERIALS AND METHODS

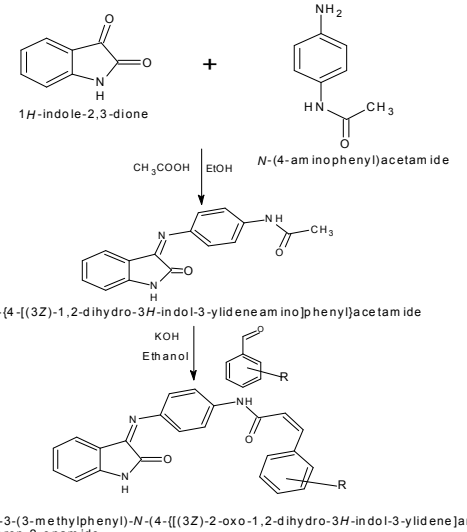
Melting point (mp) were determined in open capillary tubes and was uncorrected. The IR spectra were recorded in potassium bromide disks using IR Affinity-1 SHIMADZU spectrometer. The ^{1}H-NMR spectra were recorded on BRUKER (^{1}H NMR IN $CDCl_3$). The Chemical shift were recorded in parts per million (ppm) relative to TMS as an internal reference. Mass spectra were recorded on a LCMS-2010A DATA REPORT SHIMADZU instrument using fast atom bombardment (FAB Positive). The progress of all the reactions were monitored by readymade silica gel plates (Merck). Iodine and UV lamp were used as a developing agent. Spectral data (IR, 'HNMR and Mass spectra) were confirmed the structures of the synthesized compounds and the purity of these compounds were ascertained by microanalysis.

General procedure

Synthesis of Isatin Schiff's bases: 0.01 molof Isatin and 4-amino acetanilide were added into 20 mil of ethanol the presence of 2-4 drops of glacial acetic acid in RBF. The product formed was recrystallized using ethanol. The product (Isatin Schiff base) was obtained by distilling off the solvent and was recrystallized using ethanol.

Synthesis of Chalcone derivative. C1-C7: 0.1 mol of Isatin and 0.1 mol of substituted benzaldehyde derivatives were added to 30ml ethanol in RBF placed in an ice bath. To the mixture, 60% of 10ml KOH solution was added with constant stirring for 2-3h at Room Temperature and the kept still overnight at. It was then diluted using 40ml ice cold diluted water. It was washed with cold water, filtered and dried. Then recrystallized using rectified methanol to form synthesized compounds (C1-C7)

Scheme



Antifungal Activities: The standard strains of fungi were procured from the CSIR Instituted of Microbial Technology, Chandigarh, India. Synthesized compounds (C1-C7) were examined for anti-fungal activity using Agar disc diffusion method. The antifungal activity of the compounds were evident against two fungi (*Aspergillus niger* ATCC 9029 and *Aspergillus flavus* ATCC 10124). The fungi yeast was cultured over night at 30 °C in nutrient agar medium for anti-fungal activity tests. Fluconazole was used as standard drug for anti-fungal activities. Sabouraud dextrose agar medium (Hi-Media Laboratories, India) was used as the medium for the study of anti-fungal activity. The discs measurements were 6.25 millimeter in diameter and obtained from What man filter paper. The Stock of synthesized compounds (C1-C7)were diluted in 1% dimethyl sulphoxide to give 25 µg/ml, 50 µg/ml and 100 µg/ml as final concentration. A standard reference solution for fungi was made by dissolving weighed amount of a fluconazole (50µg/ml) in sterile distilled water, separately. It was then incubated at 37°C for 24h. 0.1 mL of dimethyl sulfoxide was used to maintain the control and showed no inhibition. The zone of inhibition revealed by each compound was measured in millimeter.

RESULTS

Physical data: The physical data and solubility data of all the title compounds are presented in the Table 2 and 3 respectively.

Spectral Data

(2*Z*)-3-(4-methoxyphenyl)-*N*-{4-[(*Z*)-(2-oxo-1,2- dihydro-3*H*-indol-3-ylidene) amino]phenyl}prop-2-enamide (C1): IR (KBr) cm-^{1}:1570 bending (N-H), 3270 stretching (N-H), 3060 (Ar-CH), 1320 (C-N), 1710 (C=O), 2150 (N-C=O) & 950 (OCH_3). ^{1}HNMR (CDCl3), δ (ppm): 6.8-8.3: 12H, Ar-R. 4.8-5.0: 1H, CH=CH. 1.6-2.0: 1H, CH-C=O. 2.0-2.4: 2H, N-H. 3.2-3.5: 3H, RO-CH. Mass spectrum : 397(M^+.+1) m/z = 379.426.

(2*Z*)-*N*-{4-[(*Z*)-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene) amino] phenyl}-3-phenylprop-2-enamide (C2): IR (KBr) cm-^{1}:1560 bending (N-H), 3290 stretching (N-H), 3050 (Ar-CH), 1310 (C-N), 1720 (C=O) & 2160 (N-C=O). ^{1}HNMR (CDCl3), δ (ppm): 6.7-8.3: 13H, Ar-R. 4.9-5.1: 1H, CH=CH. 1.8-2.0: 1H, CH-C=O. 2.1-2.4: 2H, N-H. 3.2-3.5: 3H, RO-CH. Mass spectrum: 368(M^+.+1) m/z = 367.399.

(2*Z*)-3-(3-bromophenyl)-*N*-{4-[(*Z*)-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)amino]phenyl}prop-2-enamide (C3): IR (KBr) cm-^{1}:1600 bending (N-H), 3250 stretching (N-H), 3060 (Ar-CH), 1300 (C-N), 1720 (C=O) , 630 (C-Br), & 2220 (N-C=O). ^{1}HNMR (CDCl3), δ (ppm): 6.6-8.1: 12H, Ar-R. 4.8-5.0: 1H, CH=CH. 1.6-2.0: 1H, CH-C=O. 2.0-2.4: 2H, N-H. Mass spectrum: 447(M^+.+1) m/z = 446.296.

(2*Z*)-3-(4-chlorophenyl)-*N*-{4-[(*Z*)-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene) amino]phenyl}prop-2-enamide (C4): IR (KBr) cm-^{1}:1610 bending (N-H), 3240 stretching (N-H), 3020 (Ar-CH), 1340 (C-N), 1730 (C=O) , 740 (C-Cl), & 2170 (N-C=O). ^{1}HNMR (CDCl3), δ (ppm): 6.6-8.2: 12H, Ar-R. 4.8-5.2: 1H, CH=CH. 1.9-2.2: 1H, CH-C=O. 2.0-2.4: 2H, N-H. Mass spectrum : 402(M^+.+1) m/z = 401.845.

Table 1. Derivatives of synthesized compounds

COMPOUND		R
1.	4-methoxybenzaldehyde	4-OCH_3
2.	Benzaldehyde	H
3.	3-bromobenzaldehyde	3-Br
4.	4-chlorobenzaldehyde	4-Cl
5.	4-hydroxy,3-methoxy benzaldehyde	4-OH, 3-OCH_3
6.	3-nitrobenzaldehyde	3-NO_2
7.	2-hydroxylbenzaldehyde	2-OH

Table 2. Physical data of the title compounds C1-C7

COMPOUND CODE	MOLECULAR FORMULA	MOLECULAR WEIGHT	M.P. (°C)	Rf Value	YIELD (%)
C1	$C_{24}H_{19}N_3O_3$	397	228-231	0.41	81
C2	$C_{23}H_{17}N_3O_2$	367	210-212	0.35	85
C3	$C_{23}H_{16}BrN_3O_2$	446	233-235	0.37	79
C4	$C_{23}H_{16}ClN_3O_2$	402	225-227	0.42	82
C5	$C_{24}H_{19}N_3O_4$	413	241-243	0.45	81
C6	$C_{23}H_{16}N_4O_4$	412	217-219	0.49	86
C7	$C_{23}H_{17}N_3O_3$	383	191-193	0.44	78

Table 3. Solubility data of the title compounds C1-C7

S.NO	*SOLVENT*	*C1*	*C2*	*C3*	*C4*	*C5*	*C6*	*C7*
1.	DMSO	+++	+++	+++	+++	+++	+++	+++
2.	Acetic acid	+++	+++	+++	+++	+++	+++	+++
3.	Ether	-	-	-	-	-	-	-
4.	Toluene	++	++	++	++	++	++	++
5.	Cyclohexane	++	++	++	++	++	++	++
6.	Methanol	++	++	++	++	++	++	++
7.	Ethanol	++	++	++	++	++	++	++
8.	DMF	+++	+++	+++	+++	+++	+++	+++
9.	Water	-	-	-	-	-	-	-
10.	Dichloromethane	+	+	+	+	+	+	+
11.	Ethyl acetate	++	++	++	++	++	++	++

+++ = freely soluble, ++ = sparingly soluble, + = slightly soluble, - = Insoluble

Table 4. Anti-fungal activity (zone of inhibition in mm) of title compounds C1-C7

Microorganism	Conc. (µg/ml)	C1	C2	C3	C4	C5	C6	C7	Con	Std
Aspergillus niger	25	8	6	6	6	4	4	7	-	20
	50	13	10	9	9	9	7	10	-	
	100	15	13	11	12	11	11	16	-	
Aspergillus flavus	25	5	6	6	8	2	8	7	-	22
	50	12	10	10	11	7	7	12	-	
	100	17	16	14	14	9	12	18	-	

Con : Control (DMSO), Std : Standard drug (Fluconazole for fungi)

(2*Z*)-3-(4-hydroxy-3-methoxyphenyl)-*N*-{4-[(*Z*)-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)amino]phenyl}prop-2-enamide (C5): IR (KBr) cm-^{1}:1680 bending (N-H), 3290 stretching (N-H), 3100 (Ar-CH), 1190 (C-N), 1750 (C=O) , 3300 (C-OH), 1100 (OCH_3) & 2280 (N-C=O). ^{1}HNMR (CDCl3), δ (ppm): 6.5-8.1: 12H, Ar-R. 5.0-5.3: 1H, CH=CH. 2.0-2.2: 1H, CH-C=O. 2.0-2.5: 2H, N-H, 3.2-3.4: 3H, RO-CH, 3.5-3.7: 1H, R-OH. Mass spectrum: 414 (M^+.+1) m/z = 413.425.

(2*Z*)-3-(3-nitrophenyl)-*N*-{4-[(*Z*)-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)amino]phenyl}prop-2-enamide (C6): IR (KBr) cm-^{1}:1680 bending (N-H), 3250 stretching (N-H), 3100 (Ar-CH), 1220 (C-N), 1720 (C=O) , 1550 (C-NO) & 2300 (N-C=O). ^{1}HNMR (CDCl3), δ (ppm): 6.7-8.3: 12H, Ar-R. 5.0-5.2: 1H, CH=CH. 2.0-2.3: 1H, CH-C=O. 2.3-2.8: 2H, N-H. Mass spectrum: 413 (M^+.+1) m/z = 412.397.

(2*Z*)-3-(2-hydroxyphenyl)-*N*-{4-[(*Z*)-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)amino]phenyl}prop-2-enamide (C7): IR (KBr) cm-^{1}:1620 bending (N-H), 3210 stretching (N-H), 3090 (Ar-CH), 1210 (C-N), 1770 (C=O) , 3550 (C-OH) & 2290 (N-C=O). ^{1}HNMR (CDCl3), δ (ppm): 6.6-8.2: 12H, Ar-R. 4.8-5.2: 1H, CH=CH. 1.9-2.2: 1H, CH-C=O. 2.4-2.6: 2H, N-H. 3.2-3.5: 1H, R-OH. Mass spectrum: 384 (M^+.+1) m/z = 383.399.

Anti-fungal Activity: Table (4) represents the comparison of anti-fungal activity of the standard drugs and synthesized compounds.

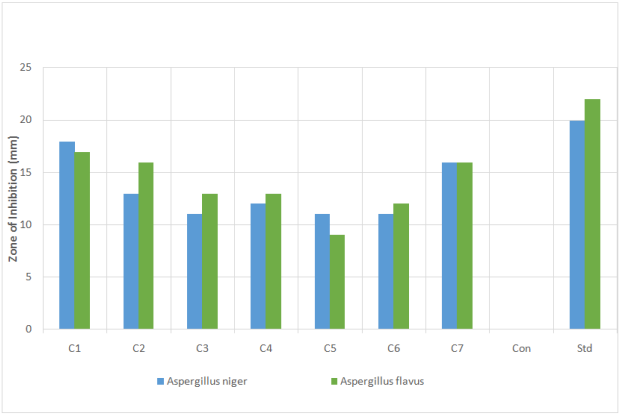


Figure 2. Anti-fungal activity of title compounds C1-C7 at 100 µg/ml concentrations

All the compounds were screened for *in-vitro* for antifungal activity against *Aspergillusflavus* and *Aspergillus niger* by using agar disc diffusion method, by measuring the zone ofinhibition in mm. In anti-fungal activity, it was found that mostpotent anti-fungal activity was exhibited by compounds (C1 and C7) possessing electron donating substituent like methoxy and hydroxyl. The zone of inhibition was observed and measured & it is in the order of (**C1>C7**). Fluconazole was used as a standard and also screened under similar conditions for comparison.

Conclusion

It was found that mostpotent anti-fungal activity was exhibited by compounds C1 and C7 possessing electrondonating substituent like methoxy and hydroxyl respectively groups on phenyl ring attached to propenamidering. While other compounds containing electron withdrawing substituents suchas bromine, chlorine and nitro group (C3, C4 and C6) exhibit weaker *in-vitro* antifungal activity. The chemical structure and anti-fungal relationship of the synthesized compounds revealed that the compounds having electron donating moiety exhibited better activity than compounds having electron withdrawing moieties. the compound C1 (2*Z*)-3-(4-methoxyphenyl)-*N*-{4-[(*Z*)-(2-oxo-1,2- dihydro-3*H*-indol-3-ylidene)amino]phenyl}prop-2-enamide) exhibit better activity while comparing with the other synthesized compounds, which may due to presence of electron donating group. Hence, this molecule may be considered as a lead molecule for antifungal activity and further studies may be needed by substituting with other electron donating groups.

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