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Synthesis and Biological Evaluation of some Isatin derivatives for Antimicrobial Properties

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ABSTRACT

A series of (2a to 2l) some isatin derivatives have been synthesized using Schiff's and Mannich reactions. The structure of the synthesized compounds were elucidated by spectral analysis (IR and ^{1}HNMR). Investigation of antimicrobial properties were done against *S.aureus, B.subtilis, S.typhi E.coli, A.niger* and *C.albicans* by cup-plate method using amoxacillin and fluconazole as standard drugs. All the tested compounds shown moderate activity.
Keywords: isatin, formaldehyde, Mannich, Schiff's and antimaicrobial.

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INTRODUCTION

Isatin is chemically 1H-indole-2,3-dione and is a versatile lead molecule for potential bioactive agents and its derivatives were reported to posses wide spectrum of activity like antibacterial[1], antifungal[2], anticonvulsant[3], antiHIV[4], antidepressant[5] and anti-inflammatory[6] etc., in view of these facts we hereby report the synthesis of some isatin derivatives using Schiff's and Mannich reaction[7]. The present study deals with the synthesis of isatin Schiff's bases (1a to1e) by reacting isatin with substituted aromatic amines (*p*- bromo aniline, *p*- nitro aniline, *p*- methyl amiline , *p*- methoxy aniline, *p*-chloro aniline, *o*- nitro, *p*-methoxy aniline) and N-Mannich bases of these Schiff's bases by condensing acidic amino group of isatin with formaldehyde and secondary amines (morpholine, NMP, piperidine, N,N'-diethylamine) scheme1. The chemical structures of the synthesized compounds were confirmed by means of their IR and 1HNMR spectral data. The synthesized compounds were tested for their antimicrobial activity by cup-plate method.

MATERIALS AND METHODS

Melting points of all the synthesized compounds were determined in open capillary tubes and are uncorrected. The purity of the compounds was checked by TLC using silica gel G as stationary phase (Table.1). The structure of the synthesized compounds was elucidated using FTIR 8400 spectrophotometer in KBr disc and ^{1}HNMR spectra was taken on a Bruker AMX (400MHz) FT-NMR (Table 2).

Synthesis of isatin Schiff's bases (1a to1f) general procedure

Equimolar quantity of isatin (0.005mol) and substituted aromatic amines (0.005mol) were added into 30ml of absolute ethanol containing 2-3 drops of glacial acetic acid in 100ml round bottomed flask. The reaction mixture was refluxed for half an hour at the refluxing temperature. The solvent was then distilled off and the product obtained was recrystallized from chloroform.

Synthesis of N-Mannich bases of isatin (2a to 2l) general procedure.

N-Mannich bases were synthesized by stirring equimolar proportions of (0.01mol) isatin Schiff's bases (1a to 1e) secondary amine (morpholine, NMP, piperidine, diethylamine) and formaldehyde in 40ml of ethanol at room temperature followed by refrigeration for 2days. The product thus obtained were separated by suction filteration and recrystallised from ethanol.

Antimicrobial Activity

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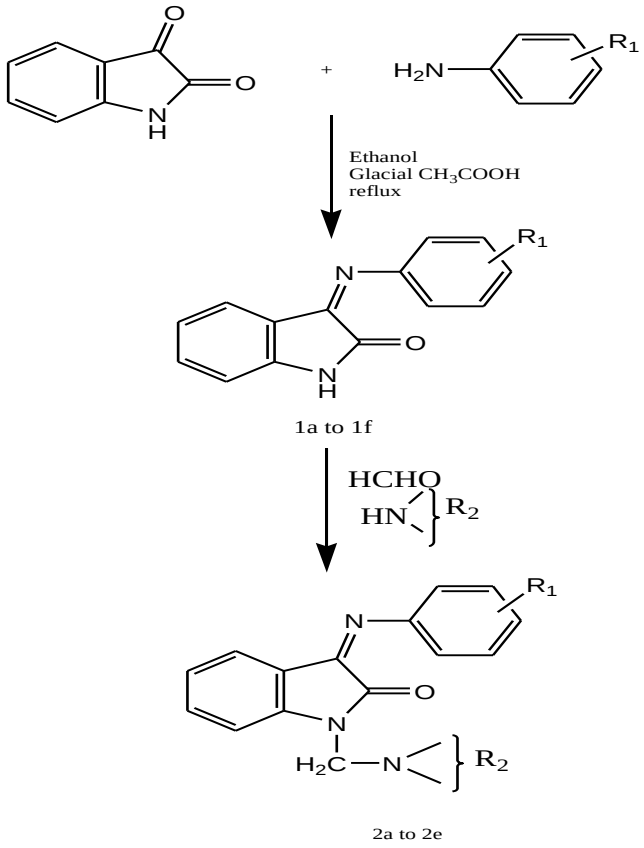
Antimicrobial activities of all the synthesized compounds were done by cup-plate agar diffusion method [8]. The compounds were prepared in DMF and evaluated them for their in-vitro antibacterial and antifungal activities against *B.subtilis, S.aureus, S.typhi, E.coli, A.niger* and *C.albicans* respectively.

All the bacterial strains were grown on the Mueller-Hinton (37^0C, 24h) the fungi were grown on Sabouraud dextrose agar plates (260C, 48-72h). The results were noted by the presence of clear zone of inhibition around the active compounds. (Table 3)



Scheme 1

Where, R_1= *p*-Br in 1a, R_1= *p*-OCH_3 in 1b, R_1= *p*-NO_2 in 1c, R_1= *p*-CH_3 in 1d R_1= *p*-OCH_3, o-NO_2 in 1e, R_1= *p*-Cl in 1f

RESULTS AND DISCUSSION

The spectral data reveals that the structures of all the synthesised compounds are in good agreement with the proposed ones. In IR spectra nothyworthy peaks are only indicated and the appearance of δ peak at 4.0-3.9 in ^{1}HNMR spectra of compounds reveals the formation of CH_2 bridge between isatin Schiff's bases and secondary amines which supports the formation of N-Mannich bases of isatin Schiff's bases. All the synthesized compounds were tested for in-vitro antibacterial activity by cup-plate agar diffusion method against the reference compound

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amoxicillin. It has been observed that all the compounds tested showed mild to moderate activity against tested bacteria.

The antifungal activity of the compounds was studied with two pathogenic fungi. The results are summarized in Table 3. Fluconazole has been used as reference for inhibitory activity against fungi and all the tested compounds showed lesser activity to standard against the tested fungi.

Table 1: physical data of the synthesized compounds (2a to 2l).

Compound	R	R_1	m.p	mol.formula	mol.wt	% yield	R_f value
2a	4-Br	Diethyl amine	139	$C_{19}H_{20}N_3OBr$	386	63	0.60
2b	4-Br	Morpholine	229	$C_{19}H_{18}N_3OBr$	384	71	0.52
2c	4-Br	NMP	245	$C_{20}H_{21}N_4OBr$	413	52	0.45
2d	4-OCH_3	Piperidine	176	$C_{21}H_{23}N_3O_2$	349	76	0.49
2e	4-OCH_3	NMP	155	$C_{21}H_{24}N_4O_2$	364	46	0.73
2f	4-NO_2	Morpholine	211	$C_{19}H_{18}N_4O_4$	366	68	0.68
2g	4-NO_2	Piperidine	263	$C_{20}H_{20}N_4O_3$	364	60	0.63
2h	4-CH_3	NMP	198	$C_{21}H_{24}N_4O$	380	59	0.78
2i	4-CH_3	Morpholine	220	$C_{20}H_{21}N_3O_2$	335	74	0.81
2j	4-Cl	Morpholine	272	$C_{19}H_{18}N_3O_2Cl$	355	42	0.39
2k	4-Cl	Piperidine	256	$C_{20}H_{20}N_3O_4$	353	79	0.36
2l	4-OCH_3, 2-NO_2	NMP	298	$C_{21}H_{23}N_5O_4$	409	56	0.3

Table 2: Spectral data of Isatin derivatives (2a to 2l).

Compound	IR (KBr, cm^{-1})	^{1}HNMR (δ, ppm)
2a	3030(CH-Ar), 1604(C=O), 1398(CH-aliphatic), 1110(Ar-Br).	7.7-7.0(m, 8H, Ar-H), 4.0(s, 2H, methylene), 2.4-2.3(q, 4H, -CH_2 aliphatic), 1.0-0.8(t, 6H, -CH_3 aliphatic)
2b	3030(CH-Ar), 1660(C=O), 1115(Ar-Br), 1091(C-N-C of morpholine),	7.7-7.0(m, 8H, Ar-H), 4.0-3.9(s, 2H, methylene), 3.6-3.5(t, 4H, -CH_2α morpholine), 2.5-2.2(t, 4H, -CH_2β morpholine)
2c	3030(CH-Ar), 1671(C=O), 1010(C-N-C of NMP), 1110(Ar-Br).	7.8-7.0(m, 8H, Ar-H), 4.0-3.9(s, 2H, methylene), 2.5-2.4(s, 8H, -CH_2 α, β NMP), 2.2-2.1(s,3H, -NCH_3).
2d	3029(CH-Ar), 1691(C=O), 1373(CH-aliphatic), 1120(ether).	7.8-6.7(m, 8H, Ar-H), 4.0(s, 2H, methylene), 2.2-2.0(t, 4H, -CH_2 piperidine), 1.5-1.4(t, 6H, -CH_2 piperidine)
2e	3030(CH-Ar), 1669(C=O), 1373(CH-aliphatic), 1010(C-N-C of NMP), 1121(ether).	7.7-6.6(m, 8H, Ar-H), 4.0-3.9(s, 2H, methylene), 2.5-2.4(s, 8H, -CH_2 α, β NMP), 2.2-2.1(s,3H, -NCH_3).
2f	3038(CH-Ar), 1710(C=O), 1510(Ar-NO_2), 1090(C-N-C of morpholine).	8.3-7.0(m, 8H, Ar-H), 4.0(s, 2H, methylene), 3.6-3.5(t, 4H, -CH_2α morpholine), 2.5-2.2(t, 4H, -CH_2β morpholine)
2g	3029(CH-Ar), 1691(C=O), 1509(Ar-NO_2), 1373(CH-aliphatic).	8.3-7.0(m, 8H, Ar-H), 4.0(s, 2H, methylene), 2.2-2.0(t, 4H, -CH_2 piperidine), 1.5-1.4(t, 6H, -CH_2 piperidine)
2h	3030(CH-Ar), 1670(C=O), 1371(CH-aliphatic), 1010(C-N-C of NMP).	7.7-7.0(m, 8H, Ar-H), 4.0-3.9(s, 2H, methylene), 2.5-2.4(s, 8H, -CH_2 α, β NMP), 2.3(s, 3H, Ar-CH_3), 2.1-2.0(s,3H, -NCH_3).
2i	3030(CH_Ar), 1671(C=O), 1373(CH-aliphatic), 1091(C-N-C of morpholine).	7.7-7.0(m, 8H, Ar-H), 4.0-3.9(s, 2H, methylene), 3.6-3.5(t, 4H, -CH_2 α morpholine), 2.4-2.2(t, 4H, -CH_2 β morpholine), 2.3(s,3H, Ar-CH_3).
2j	3029(CH-Ar), 1669(C=O), 1373(CH-aliphatic),	7.7-7.1(m, 8H, Ar-H), 4.0(s, 2H, methylene), 3.6-3.5(t, 4H,

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	1090(C-N-C of morpholine), 1111(Ar-Cl).	-CH_2 α morpholine), 2.3-2.2(t, 4H, -CH_2 β morpholine).
2k	3030(CH-Ar), 1661(C=O), 1373(CH-aliphatic), 1111(Ar-Cl).	7.7-7.1(m, 8H, Ar-H), 4.0(s, 2H, methylene), 2.1-2.0(t, 4H, -CH_2 piperidine), 1.4(t, 6H, -CH_2 piperidine).
2l	3030(CH-Ar),1660(C=O), 1373(CH-aliphatic), 1010(C-N-C of NMP).	7.7-7.1(m, 8H, Ar-H), 4.0(s, 2H, methylene), 3.8-3.7(s,3H, Ar-OCH_3), 2.5-2.4(s, 8H, -CH_2 α, β NMP), 2.2-2.1(s,3H, -NCH_3).

NMP= N-methyl piperazine

Table 3 Antimicrobial Activity of Compounds (2a To 2l)

Compound	Zone of Inhibition (mm)					
	Bacterial strain			Fungal strain		
	S. aureus	*B. sublitilis*	*S. typhai*	*E. coli*	*C. albicans*	*A. niger*
2a	12	16	09	07	10	08
2b	11	12	08	09	07	10
2c	09	10	10	11	09	12
2d	12	09	12	10	11	14
2e	15	10	11	10	09	12
2f	10	12	19	11	08	10
2g	08	14	07	09	06	10
2h	13	11	10	09	08	12
2i	10	09	11	10	09	11
2j	12	12	10	09	09	11
2k	11	13	10	08	09	13
2l	10	12	09	11	08	11
amoxicillin	20	23	19	18	-	-
fluconazole	-	-	-		25	21

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