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Syntheses and Pharmacological Activities of Isatin Derivatives

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ABSTRACT

Twenty derivatives of isatin (1H-indole-2, 3-Dione) were synthesized by the schematic route as per as scheme. These were further characterized by spectroscopic analysis. All the novel synthesized derivatives of isatin were screened for their antimicrobial activity against Gram-positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumonia*; Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and fungal strains: *Candida albicans*, *Aspergillus niger, Penicillium chrysogenum, Rhizopus oryzae* by cup-plate method. Results of antimicrobial screening showed that compound ID5, ID9, ID15 and ID18 possess potent antibacterial activity. Whereas ID1, ID3, ID4, ID7 and ID20 possess antifungal activity comparable to standard drugs.

Keywords: Isatin; Antimicrobial; Antibacterial; Antifungal.

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1. INTRODUCTION

There are several heterocyclic compounds but five and six membered ring compounds have gained a special place among different classes of organic compounds for their versatile and diversified biological activities[1]. Among all the variety of heterocycles that have been identified for producing pharmaceutically valuable molecules, indoles and oxadiazines have shown a remarkable role in pharmaceutical chemistry[2].

Isatin is a heterocyclic compound having indole ring system. Isatin mainly reacts at three different sites, namely aromatic substitution at C-5, *N*-alkylation, and carbonyl reactions at C-3. If the system carries electron-withdrawing groups in the benzene ring or at the nitrogen, attacks at C-2 can also occur[3]. Nitration of isatin yields 5-nitroisatin, where the reaction proceeds smoothly at 0°C but the temperature needs to be controlled precisely or else the nitration will give rise to several nitrated products[4-6]. Nevertheless, 5, 7-dinitroisatin can be synthesized by merely heating 3,3,5,7-tetranitrooxindole, which in turn can be obtained from the nitration of oxindole[7]. When bromine is added to a solution of isatin in ethanol it yields dibrominated product 5,7-dibromoisatin[8]. Nevertheless mono bromination at C-5 could be achieved by treatment of isatin with *N*-bromosuccinimide, while 5-chloroisatin by using *N*-chlorosuccinimide[9]. A few years ago, mono iodination of isatin yielding 5-iodoisatin was achieved by using an aqueous potassium dichloroiodate ($KICl_2$) as the iodinating agent[10].

Commonly, N-alkylation of isatin proceeds *via* the sodium salt of isatin, which reacts with appropriate alkyl halide or alkyl sulfonate. Methylation can also be achieved with other reagents, for example potassium *tert*-butoxide and dimethyl oxalate (60% yield)[11]. However, normal alkylation conditions such as dimethyl sulfate in ethanolic potassium hydroxide (80% yield) or benzylation with sodium hydride and benzyl bromide (90% yield) give better yields[12-13].

N-aceylation can be acheived by heating isatin in acetic anhydride for a couple of hours, although a modified procedure (sodium acetate and isatin heated shortly in acetic anhydride) has been published[14-15]. Protecting amines as *N*-carbamates is a commonly adopted strategy, which has also been applied to isatin. N-Boc isatin and N-CBz isatin have been synthesized in 89% and 85% yield, respectively[16-17].

All ketones, as well as, the C-3 carbonyl group of isatin were susceptible towards nucleophiles. Ketalisation serves this perfectly, as a good example of nucleophilic attack on the carbonyl functionality. Thus, employing ethylene glycol, 1, 2-ethanedithiol or 2-mercapto ethanol on isatin yields different spiro ketals of oxindole[18-20]. Though, the example above considers heteroatom dinucleophiles, carbon nucleophiles

do also react at C-3. Grignard reagents also attack at C-3 and yield the 3-hydroxy-3-substituted oxindoles, which readily can be reduced to 3-substituted indoles[21].

On the basis of above literature survey, we have planned the present scheme for the synthesis of different isatin derivatives with different pharmacological activities.

2. MATERIALS AND METHODS

All the chemicals used in the synthesis of isatin derivatives were of synthetic grade and procured locally. Thin layer chromatography was used for monitoring the progress of reaction and product formation. The thin layer chromatography of the synthesized compounds was done on precoated TLC (silica gel) plates by using different solvent medium. Identification of spots was done under UV lamp and in iodine chamber. Detection of spots under UV lamp was done at both short and long wavelength. Melting points of the synthesized compounds were determined on melting point apparatus.

Experimental

Step 1-General procedure for synthesis of Intermediates

Equimolar quantities (0.008 mol) of 5-bromo isatin and *p*-bromo aniline were taken in 250ml round bottom flask, containing 1 ml of glacial acetic acid and these were dissolved in 20 ml of ethanol. It was refluxed for 2 hrs. After standing for approximately 24 hours at room temperature, the product was separated by filtration, washed with sodium bisulfite solution to remove excess of aldehyde, vacuum dried and recrystallized with ethanol.

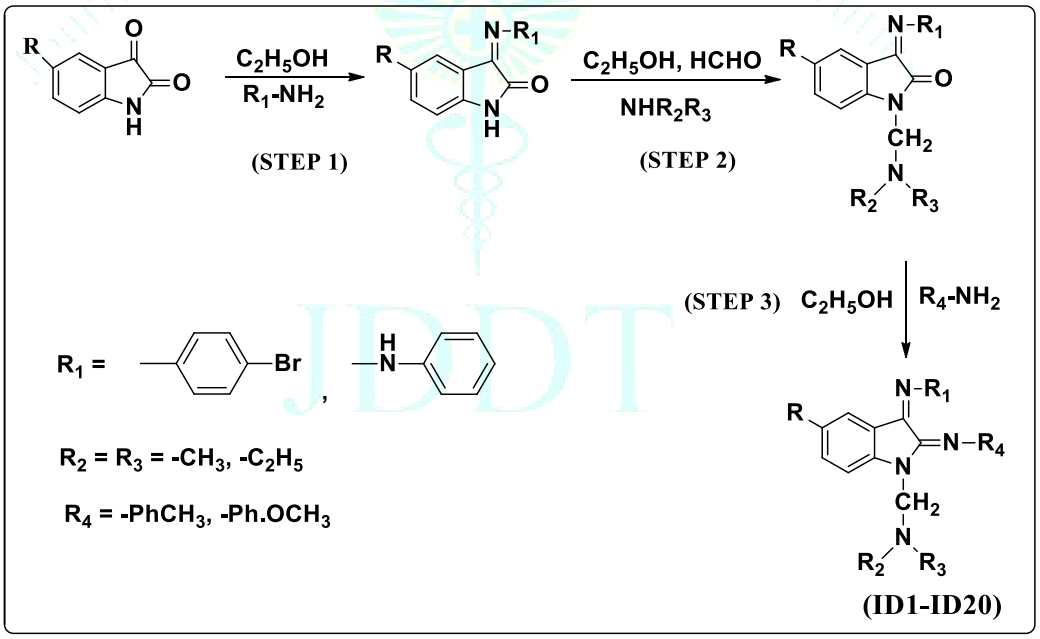
Step 2-General procedure for synthesis of Mannich base derivatives

Equimolar quantities (0.004 mol) of diphenylamine in 10 ml ethanol was taken in a 250ml round bottom flask and to this added a slurry containing appropriate compound obtained from step 1 and 0.3ml of formaldehyde solution (37%v/v) in 10ml of ethanol. The mixture was stirred for 2 hrs at room temperature and refrigerated for 48 hours. The product was separated by filtration and vacuum dried. Recrystallization was done with ethanol.

Step 3-General procedure for synthesis of isatin derivatives (ID1-ID20)

Equimolar quantities (0.008 mol) of p-methyl aniline and step 2 product were taken in 250ml round bottom flask, containing 1 ml of glacial acetic acid and these were dissolved in 20 ml of ethanol. Refluxed it for 2 hrs. After standing for approximately 24 hours at room temperature, the product was separated by filtration, washed with sodium bisulfite solution to remove excess of aldehyde, vacuum dried and recrystallized with ethanol.

Scheme-1 Synthesis of Istain derivatives (ID1-ID20)



Physicochemical property prediction of synthesized derivatives (ID1-ID20)

A variety of molecular properties like Log P, molar refractivity and topological polar surface area were calculated for both standard drugs and target compounds by using Chem 3D Ultra version 12.0.2, software program. The observations are as follows in **Table 1**.

Table 1: Molecular properties of the synthesized isatin derivatives (ID1-ID20)

Compound Code	Molecular Formula	MW[x]	MR[y]	tPSA[z]	Log P
ID1	$C_{26}H_{27}N_5O_2$	441.52	NC	83.01	4.00
ID2	$C_{25}H_{21}BrN_4O_4$	521.36	131.36	105.80	5.64
ID3	$C_{26}H_{27}N_4F$	414.52	125.97	31.20	7.02
ID4	$C_{26}H_{27}N_4Br$	475.42	133.26	31.20	7.69
ID5	$C_{33}H_{23}N_4Br_2F$	654.37	164.11	31.20	11.01
ID6	$C_{21}H_{15}N_3OBr_2$	485.17	114.44	45.98	6.41
ID7	$C_{21}H_{13}N_4O_4Br$	465.26	NC	125.86	4.17
ID8	$C_{21}H_{13}N_3O_2Cl_2$	410.25	107.83	74.05	5.56
ID9	$C_{21}H_{15}N_3ClOF$	379.81	104.06	45.98	5.47
ID10	$C_{22}H_{17}N_3Br_2$	483.20	117.69	36.75	7.44
ID11	$C_{18}H_{18}N_3Br$	356.26	95.51	36.75	5.37
ID12	$C_{21}H_{16}N_3F$	329.37	98.11	36.75	5.53
ID13	$C_{34}H_{26}N_4Br_2$	650.41	169.60	31.20	11.34
ID14	$C_{28}H_{31}N_5O_2$	469.58	NC	83.01	4.91
ID15	$C_{24}H_{20}N_4O_2BrCl$	511.80	129.18	68.50	6.64
ID16	$C_{26}H_{27}N_4Cl$	430.97	130.17	31.20	7.42
ID17	$C_{28}H_{31}N_4Br$	503.48	142.46	31.20	8.53
ID18	$C_{27}H_{28}N_4BrCl$	523.90	142.46	31.20	8.67
ID19	$C_{37}H_{33}N_4Cl$	569.14	173.92	31.20	11.56
ID20	$C_{33}H_{23}N_4Br_2Cl$	670.82	168.31	31.20	11.41
Ciprofloxacin	$C_{17}H_{18}N_3FO_3$	331.34	89.39	72.88	1.32
Fluconazole	$C_{13}H_{12}N_6F_2O$	306.27	78.46	76.15	0.99

Abbreviations: x-Molecular weight, y-Molar refractivity, z-Topological polar surface area, NC-Not calculated

***In vitro* antimicrobial activity**

The antimicrobial assay is based upon a comparison of the inhibition of growth of micro-organism by measured concentrations of the antimicrobial agents to be examined with that procedure by known concentration of standard preparation of the antibiotics having a known activity[22].

Six strains of bacteria and four strains of fungi were taken as the test organism. In order to evaluate the antibacterial spectrum of the prepared compounds, strains of *Bacillus subtillis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* MTCC (1573), *Staphylococcus aureus* (MTCC1430), *Streptococcus pneumoniae* (MTCC 655), and *Klebsiella pneumoniae* (MTCC 618), were procured as pure culture from Institute of Microbial Technology, Chandigarh. For antifungal spectrum of the prepared compounds, strains of *Aspergillus niger* (MTCC 2546), *Rhizopus oryzae* (MTCC 2775), *Candida albicans* (MTCC 183) *and Penicillium chrysogenum* (MTCC 161), were procured as pure culture from Institute of Microbial Technology, Chandigarh. Growth media were prepared in accordance with the direction laid down in the package insert received from MTCC, Institute of Microbial Technology, Chandigarh.

Evaluation of Antimicrobial Activity

The test organisms were inoculated in nutrient broth. A definite volume of this suspension was mixed with nutrient agar (cooled to 40°C). Nutrient agar was poured into petridishes to obtain a uniform thickness. The surface of agar plates was pierced using a sterile cork borer. The prepared wells were filled with equal volumes of a solution of antimicrobial agents ID1-ID20. After a period of pre incubation diffusion, the plates were incubated face up for a definite time and at the specific temperature for that strain. The diameters of zones of inhibition were measured and are reported in **Table 2** and **Table 3**.

Table 2: Observation of Synthesized Isatin Derivatives for Antibacterial Activity.

Compound code	Conc. (µg/ml)	Observation of Antibacterial activity					
		Gram –ve			Gram +ve		
		E.coli	*P.aeruginosa*	*K.pneumoniae*	*B.subtilis*	*S.aureus*	*S.pneumoniae*
ID1	200	++	++	-	-	-	-
ID2	200	+	+	++	+	-	-
ID3	200	++	++	+	-	-	+
ID4	200	++	-	++	-	++	-
ID5	200	+++	-	-	++	-	-
ID6	200	+	-	-	-	-	+
ID7	200	++	++	-	-	-	-
ID8	200	+	-	++	-	++	-
ID9	200	+++	-	-	++	-	-
ID10	200	+	-	+	-	-	++
ID11	200	++	-	-	+	-	-
ID12	200	++	++	-	-	++	-
ID13	200	+	-	++	-	-	++
ID14	200	+	-	+	-	+	-
ID15	200	+++	-	-	++	++	-
ID16	200	++	-	-	-	-	+
ID17	200	+	++	+	-	++	-
ID18	200	+++	-	++	++	-	-
ID19	200	+	+	-	-	+	+
ID20	200	++	++	+	+	-	-
Ciprofloxacin		+++	+++	+++	+++	+++	+++
DMF		—	—	—	—	—	—

Zones of inhibition for bacteria

- = < 6 mm (inactive); + = 6-9 mm (slightly active); ++ = 9-12 mm (moderately active); +++ = 12-15 mm (highly active); **Standard** - Ciprofloxacin, **Control** – DMF

Table 3: Observation of Synthesized Isatin Derivatives for Antifungal activity

Compound code	Conc. (µg/ml)	Observation of Antifungal activity			
		A. niger	*R. oryzae*	*C. albicans*	*P. chrysogenum*
ID1	200	++	-	-	-
ID2	200	-	+	-	+
ID3	200	++	-	-	-
ID4	200	++	-	+	-
ID5	200	-	-	-	-
ID6	200	+	-	-	-
ID7	200	++	+	-	-
ID8	200	-	-	-	-
ID9	200	++	-	-	+
ID10	200	-	-	+	-
ID11	200	++	-	-	+
ID12	200	-	+	-	-
ID13	200	+	-	-	-
ID14	200	+	-	+	-
ID15	200	-	-	-	+
ID16	200	++	-	-	-
ID17	200	-	+	+	-
ID18	200	++	-	-	+
ID19	200	-	+	-	-
ID20	200	++	+	+	+
Fluconazole		+++	+++	++	+++
DMF		—	—	—	—

Zones of inhibition for fungi

- = < 6 mm (inactive); + = 6-9 mm (slightly active); ++ = 9-12 mm (moderately active); +++ = 12-15 mm (highly active);

Standard - Fluconazole, **Control** – DMF **(+) represents the activity while (-) represent do not active.**

3. RESULTS AND DISCUSSION

Synthesis of isatin derivatives **(ID1-20**) has shown in general pathway outlined in the Scheme-1. All of the derivatives were synthesized in good yield. The structures of newly synthesized derivatives were analysed on the basis of IR, Mass and ^{1}H NMR (data not shown). The physicochemical properties of synthesized derivatives are calculated and tabulated in **Table 1**. The purity of synthesized derivatives were checked and monitored by TLC.

Most of the synthesized derivatives have molar refractivity under 150. Topological polar surface area (tPSA) values for the synthesized derivatives were found within 31.20-125.86, which is a surface sum over all polar atoms, primarily oxygen and nitrogen, also their attachment to the hydrogen atoms that helps the compound permeation through the cell. Compounds having a polar surface area of greater than 140 angstroms squared show poor permeating behaviour through cell membranes[23]. The Log P value of synthesized derivatives were 4.00-11.56 within the range of standard drugs which shows that a positive value for Log P indicates a higher concentration in the lipid phase. This indicates that the compounds potentially cross the blood brain barrier. The synthesized compounds were tested for their antibacterial and antifungal activity against different bacteria and fungi. The results got for the same are shown in **Table 2** and **Table 3**. It is evident from the results of synthesized derivatives containing halogen atoms like chloro, bromo and fluoro were showing significant antibacterial and antifungal activity, which depicts that halogen atoms have an important role in antimicrobial activity. On the other hand remaining synthesized compounds revealed the variable antibacterial and antifungal activity. Among all, synthesized derivatives ID5, ID9, ID15 and ID18 possess potent antibacterial activity. Whereas ID1, ID3, ID4, ID7 and ID20 possess antifungal activity.

4. CONCLUSION

In present study, we synthesized the isatin derivatives with range of pharmacological activities as antimicrobial agents. It has been found that most of the synthesized compounds were more potent as compared to the standard, which can be used as lead for novel antimicrobial agents.

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