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Research Article

Synthesis of Novel 1H- Indole-2, 3-Dione Derivatives as potent Antimycobacterial Agents.

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ABSTRACT

In the present study, isatin (Indole-2,3- dione) was reacted with benzyl chloride to form N-Benzyl isatin **i,** which upon reaction with *p*-phenylenediamine to yield N-benzyl imesatin **ii.** The compound **ii** was subjected to react with various substituted aromatic aldehydes in presence of ethanol as a solvent to form Schiff bases **A_1-A_8 (**scheme 1). Similarly, 4-substituted aniline **i** was treated with chloral hydrate in presence of hydroxylamine hydrochloride and sodium sulphate to form 5-subsituted-isonitroso acetanilide **ii**, which undergoes cyclization in presence of sulphuric acid to form 5-substituted isatin **iii** which further undergoes mannich reaction in the presence of ciprofloxacin to give corresponding mannich bases **A_9-A_{12}** (Scheme 2). The structures of the compounds were confirmed by IR, 1HNMR and Mass spectral analysis. All the compounds have been evaluated for their antitubercular properties by Microplate Alamar Blue assay (MABA). Most of the compounds have shown promising biological activity.

Keywords: Isatin, Schiff bases, Mannich bases, Microplate alamar blue assay, Tuberculosis.

INTRODUCTION

Tuberculosis (TB) remains a global health threat. It is generally accepted that TB results from intensive cross-talk between the host and the pathogen *Mycobacterium tuberculosis*. Every year, half a million multidrug resistance tuberculosis (MDR-TB) cases emerge and more than 1,30,000 people die of MDR-TB. One-third of the world's population is currently infected and more than 5000 people die from TB everyday[1]. Mycobacterium tuberculosis (MTB) causes more human deaths than any other single infectious organism with an estimated eight million new TB cases and 2 million fatalities each year[2]. MTB has two features that render the deadliest infectious disease to date. Its high infectivity or virulence and its ability to enter latency for subsequent reactivation, phenomenon that leads to the deadliest synergy with Acquired Immune Deficiency Syndrome (AIDS). As a result TB is also the leading cause of death for AIDS patients. Moreover the emergence of multi-drug resistance tuberculosis (MDR-TB) is severely hampering TB treatment and therefore there is an urgent need to develop more new drugs. In recent years, there has been increased interest in thechemistry of 1H-Indole-2,3-Dione derivatives since these have been known as promising class of biologically active compounds. Many of them have been reported to possess antimicrobial, anti inflammatory, anti tubercular, analgesic and antiproliferative properties. Since Indole-2,3-Diones are excellent reservoirs of bioactive substances and the stability of the indole nucleus has inspired medicinal chemists to introduce many bioactive molecular scaffolds into this nucleus and synthesize new potential medicinal agents. During our research we have found that incorporation of flouroquinolone and N-Benzyl isatin scaffolds are reported as potent anti-mycobacterial agents[3]. Incorporation of flouroquinolone scaffolds into the isatin nucleus i.e. bioreversible form will increase the lipophilicity of the compounds. In view of the above facts, we report the synthesis of title compounds comprising of N-Benzyl isatin derivatives and flouroquinolone substituted isatin derivatives.

Materials and methods:

Melting points of the synthesised compounds were determined by using Veego melting point apparatus and are uncorrected. The IR spectra of the compounds were recorded using potassium bromide pellet method in the range of 4000-500

cm^{-1} on ABB Bomem FT-IR spectrometer and the frequencies were recorded in wavenumbers. The ^{1}H-NMR and ^{13}C-NMR spectra of the synthesized compounds were recorded on a JOEL GSX 400 NMR spectrometer using deuterated chloroform as solvent. Chemical shifts were reported in parts per million (ppm).Tetra methyl silane was used as internal reference. Mass spectra were recorded on Shimadzu GCMS QP 5000. The purity of the compounds was checked by TLC on precoated SiO_2 gel (HF_{254} 200 mesh) aluminium plates (E-Merck) using ethyl acetate: n-hexane as mobile phase and visualized in UV- chamber.

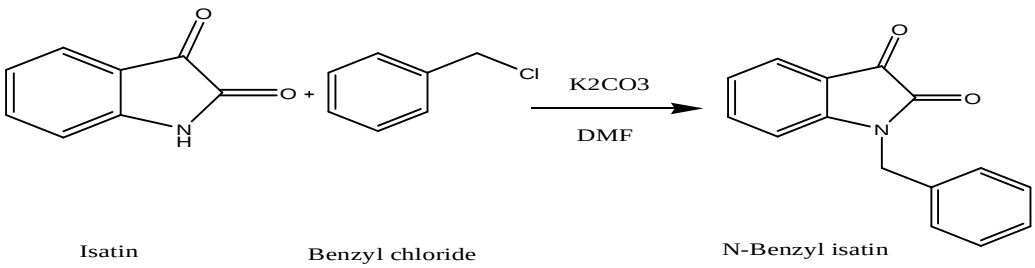
Preparation of n-benzyl isatin[4]:
Isatin (0.42g, 0.0029 mol) and benzyl chloride (0.36, 0.029mol) were mixed with DMF (10ml) in a round bottomed flask, potassium carbonate (0.96g, 0.007mol) was added to the mixture and the contents of the flask were refluxed for about 2hr, cooled and poured into 50ml of cold water. The resultant orange precipitate was collected, washed with water, dried and recrystallised from acetonitrile.

Preparation of n-benzyl imesatin:
A 0.01mol of N-benzyl isatin, 0.02mole of amine and 30ml of absolute alcohol are heated under reflux on the waterbath for an hour, cooled and allowed to room temperature then filter the N-benzyl imesatin crystals through the buchner funnel and dried.

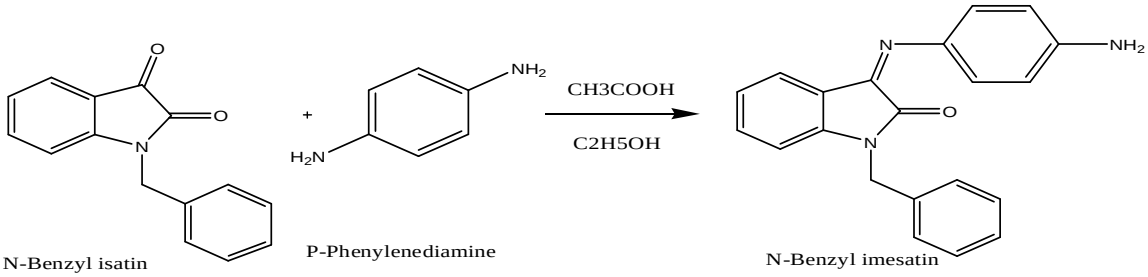
Preparation of Schiff bases (A_1-A_8): Schiff bases were prepared by taking a mixture of N-benzyl imesatin (0.01mol) and 0.01mol of various aromatic aldehydes were dissolved in 70ml of ethanol then refluxed for 1-2hrs and kept aside. After completion of the reaction mixture was poured over crushed ice with stirring. The product obtained was filtered, dried and recrystallised from ethanol.

Preparation of 5-substituted isonitrosoacetanilide:
In a round bottomed flask 18g (0.108moles) of chloralhydrate in 260ml of water was placed. To this, 26g of sodium sulphate, a solution of 0.1 of para-substituted aniline (*p*-chloroaniline-12.7g, *p*-methoxyaniline-12.63g, *p*-flouroaniline-1.11g) in 60ml of water containing 8.42 (8.6ml,0.104moles) of con. Sulphuric acid to dissolve the amine and finally a solution of 22g (0.316mole) of hydroxylamine hydrochloride in 50ml of water were added. The reaction mixture was heated to boiling over wire gauze so that vigorous boiling begins in about 40-45 min after 1-2 min of vigorous boiling the reaction was completed. During the heating period some crystals of *p*-substituted isonitrosoacetanilide separates out. On cooling the solution in running water the remainder crystallizes, was filtered with suction, and air-dried.

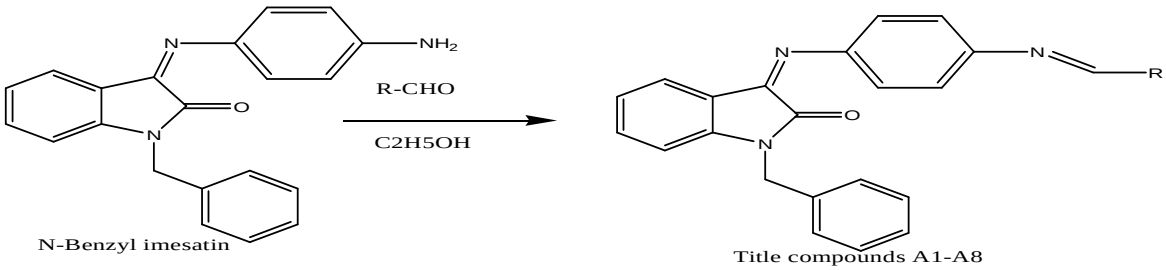
Preparation of 5-substituted isatin[5]:
21g of the concentrated sulphuric acid was warmed to 50^0C in a one liter round bottomed flask fitted with an efficient mechanical stirrer, to this added 7.5g (0.046 moles) of *p*- substituted isonitrosoacetanilide at such a rate as to keep the temperature between 60^0 and 70^0C but not higher. External cooling should be applied at this stage so that the reaction can be carried out more rapidly. After the addition of isonitrosoacetanide was finished, the solution was heated to 80^0C and kept at this temperature for about 10 min to complete the reaction. Then the reaction mixture is cooled to room temperature and poured upon ten to twelve times its volume of cracked ice. After standing for about half an hour, the orange colored isatin is filtered with suction, washed several times with cold water to remove the sulphuric acid, and the dried in the air.



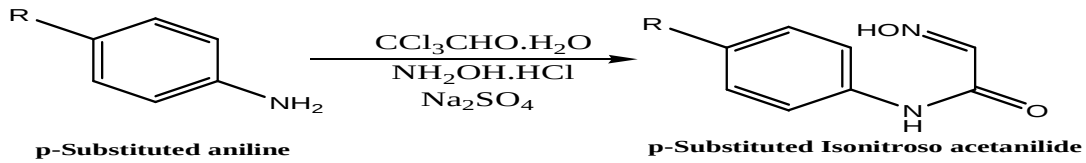
Preparation of n-benzyl isatin



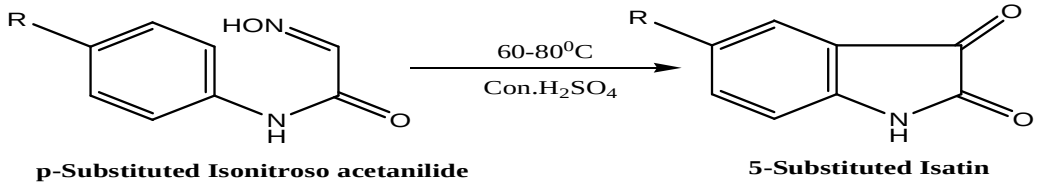
Preparation of n-benzyl imesatin



Preparation of Schiff bases (A_1-A_8)



Preparation of 5-substituted isonitrosoacetanilide



Preparation of 5-substituted isatin

Preparation of mannich bases (A_9-A_{12}):

Mannich bases were prepared by taking a solution of 1-cyclopropyl-6-flouro-1,4-dihydro-7-piperazin-1-yl-4-oxo quinoline-3-carboxylic acid (ciprofloxacin, 0.02mole) in glacial acetic acid (50ml), was added substituted isatins (0.02moles) and 37% formaldehyde (1ml). The reaction mixture was refluxed of over water bath for 1-3hrs. The mixture was concentrated to approximately half of its initial volume and the resulting precipitate

obtained was filtered dried, recrystallised from a mixture of DMF and water.

Analytical information of synthesized compounds:

The purity of the compounds was checked by TLC and elemental analysis, and synthesized compounds were assigned by spectral data. In general, IR spectra showed C=N (azomethine) peak at 1635cm^{-1} and –CH_2- (mannich methylene) peak at 2855 and 2845Cm^{-1}. In the ^{1}H NMR spectra, the signals of the respective protons of the prepared derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants.

The spectra of all the compounds **A_9-A_{12}** showed a singlet at 4.9 ppm corresponding to –NCH_2N- group. A singlet was observed at 4.22 ppm and 8.09 corresponding to N-CH_2- and -N=CH- groups respectively. Phenolic-OH group was identified at 5.03 ppm as a singlet peak the proton peak for –OCH_3 group observed at 3.71 ppm as a singlet. Methyl protons had show a peak at 2.84 ppm as a singlet. Lipophilicity of the synthesized derivatives **A_1-A_{12}** is expressed in terms of their log *P* values (Table:1).These values were computed using Chem office 2004 software.

Antimycobacterial activity:

The anti mycobacterial activity was evaluated by Microplate Alamar Blue Assay (MABA). Bacterial strains. M. tuberculosis H_{37}Rv ATCC 27294 (American Type Culture Collection), H_{37}Rv inoculate was grown in 100 ml of Middle brook 7H9 broth (Difco, Detroit, Mich.) supplemented with 0.2% v/v glycerol (Sigma Chemical Co., Saint Louis, Mo.), 10% (v/v) OADC (oleic acid, albumin, dextrose, catalase; Difco), and 0.05% (v/v), tween 80 (Sigma). The complete medium was referred to as 7H9GC-T80. Rifampicin (RMP) was a positive control (Sigma). MIC (Minimum Inhibition concentration) was determined after incubated 7 days at 37°C.

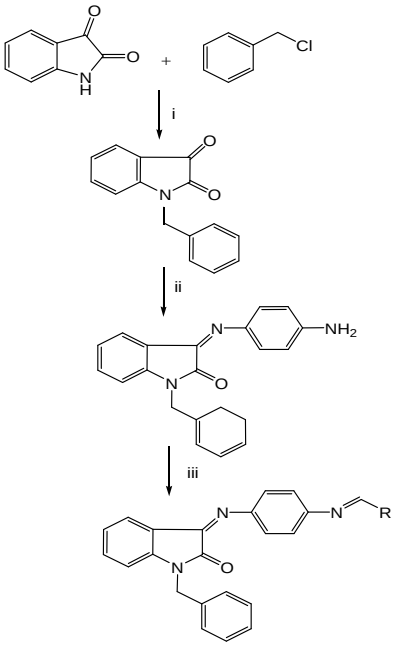
Microplate Alamar blue Assay (MABA)[6]:

Anti-TB susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, Conn.) in order to minimize background fluorescence. Initial drug dilutions were prepared in dimethyl sulfoxide, and subsequent two fold dilutions were performed in 0.1 ml of 7H12 media in the microplates. The H37 Rv were diluted in 7H9 media to reach approximately 2*10 ^{5} cfu/ml, and 0.1 ml was added to wells. Wells containing compounds only were used to detect autofluorescence of compounds. Plates were incubated at 37°C. At day 7 of incubation, 20 µl of Alamar Blue solution (Trek Diagnostic Systems, Cleveland, Ohio) and 12.5 ml of 20% Tween 80 were added to all the wells, and plates were reincubated at 37°C for 24 h. Fluorescence was measured in a Victor II multilabel fluorimeter (Perkin Elmer Life Sciences Inc., Boston, MA) at 530 nm and 590 nm. For fluorometric MICs (Minimum Inhibition Concentration) were determined after incubated 7 days at 37°C. Percent inhibition was defined as 1 - (test well FU/mean FU of triplicate B wells) * 100. The lowest drug concentration effecting an inhibition of ≥90% was considered the MIC. Rifampicin was used as positive control. The MIC of rifampicin was 0.0047 - 0.0095 µg/ml.

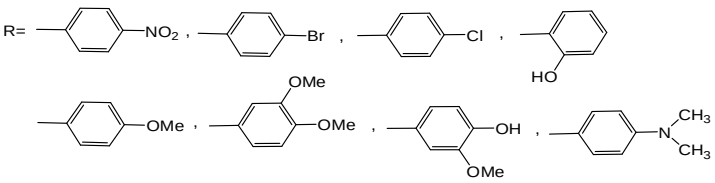
RESULTS AND DISCUSSION:

The synthesized compounds **A_1-A_{12}** was tested for their antimycobacterial activity *invitro* against *Mycobacterium tuberculosis H37RV* using the Micro Plate Alamar Blue Assay (MABA) in duplicate and MICs of the compounds were reported (Table 1). MIC is defined as the minimum concentration of compound required to give 90% inhibition of bacterial growth. Rapid glance at the obtained results shown that all the compounds exhibited good antimycobacterial activity with percentage inhibition ranging from 94-100%. Some of the compounds (A_9-A_{12}) which exhibited good percentage inhibition were further examined for minimum inhibitory concentration (MIC).This research work reveals that Indole-2,3-diones Schiff and mannich bases possess potent anti mycobacterial activities.

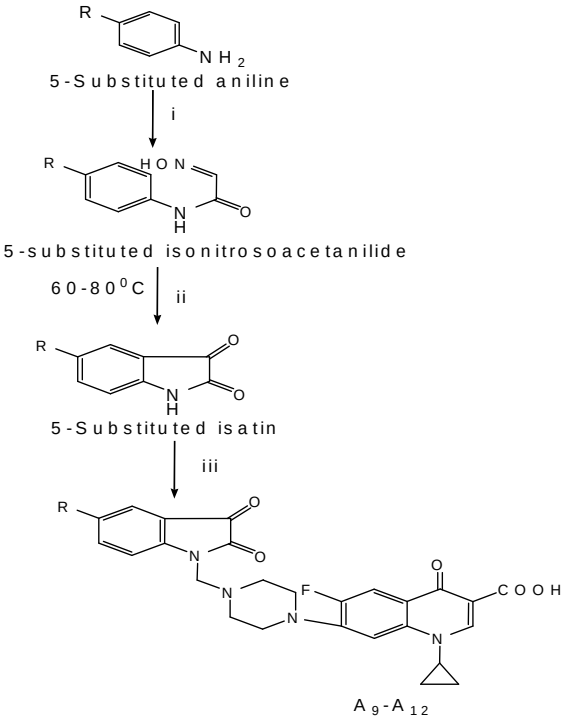
Scheme 1: Synthesis for N-benzyl isatin derivatives:



i) Dimethyl formamide, K_2CO_3, ii) *p*-phenylenediamine, iii) R-CHO, Ethanol 2h reflux



Scheme 2: Preparation of 5-substituted isatin derivatives



i) $NH_2OH.HCl$, Na_2SO_4, Chloral hydrate, ii) Con. = Con.H_2SO_4, iii) Ciprofloxacin / Ethanol, R = -Br, -F, -NO_2, -I

Table 1: **Physical data and antimycobacterial activity of synthesized compounds**

SAMPLES	MOLECULAR FORMULA	MOLECULAR WEIGHT	m.p (^{0}C)	YIELD (%)	logP[a]	MIC[b]	%Inhibition
A_1	$C_{28}H_{20}N_4O_3$	460.48	219-223	58	4.43	2.35	65
A_2	$C_{28}H_{20}BrN_3O$	494.38	230-234	65	7.13	1.53	69
A_3	$C_{29}H_{20}ClN_3O$	449.93	245-249	68	6.86	1.98	77
A_4	$C_{28}H_{21}N_3O_2$	431.49	207-209	49	5.91	1.37	69
A_5	$C_{25}H_{23}N_3O_2$	445.51	259-263	78	6.17	1.28	71
A_6	$C_{30}H_{25}N_3O_3$	475.54	239-243	72	6.04	1.73	73
A_7	$C_{29}H_{23}N_3O_3$	461.51	269-273	60	5.78	1.50	62
A_8	$C_{30}H_{26}N_4O$	458.21	277-281	45	6.58	1.89	79
A_9	$C_{26}H_{22}BrFN_4O_5$	569.38	185-189	72	3.14	1.1	100
A_{10}	$C_{26}H_{22}BrF_2N_4O_5$	508.47	165-169	78	2.47	0.6	101
A_{11}	$C_{26}H_{22}FN_5O_7$	535.48	195-199	67	NT	0.7	100
A_{12}	$C_{26}H_{22}FIN_4O_5$	616.38	213-217	79	3.67	1.6	100
Rifampicin	-	-	-	-	-	0.06	100
Isoniazid	-	-	-	-	-	0.74	100

MIC-Minimum inhibitory concentration, log P - partition coefficient.

Conclusion:

The lipophilicity of the synthesized compounds increased remarkably by pharmacophores like –Br, -F, -I, -NO_2 this may render them more capable of penetrating into the biological membranes and inhibiting the mycolic acid synthesis. In conclusion, it has been shown that the potency of the synthesized compounds could serve as lead molecule for beneficial structural modification. Further studies on structure activity relationships are processing in our laboratory.

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