Research Article

Synthesis, Anti TB, Antioxidant, Antimicrobial Activity of some Isatin-3hydrazone derivatives

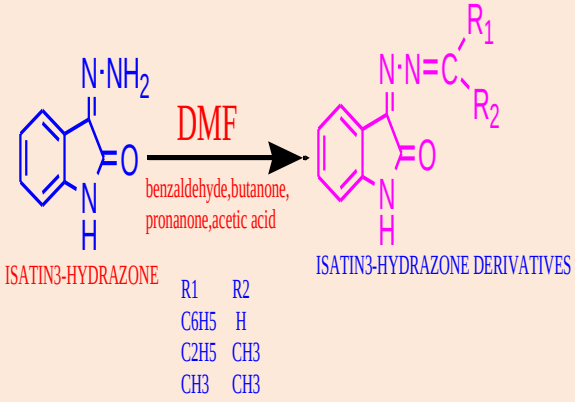
Arti saxena[1]*, Dr. Ratnesh das[2], and shweta saxena[3]

Department of Chemistry, Dr.Hari Singh Gour (Central) University, Sagar (M.P.) 470003 India

Abstract

Some new isatin3hydrazone derivatives that are Isatin3-(benzylidene)hydrazone, Isatin3-(methyl ethylide)hydrazone, isatin 3-(isoproplylidene)hydrazone have been synthesized from the reaction of isatin3-hydrazone and different aldehydes and ketones. Thestructure of synthesized compound is assigned on the basis of, IR, 1HNMR, 13CNMRdata. The anti microbial activity was done by disk diffusion method, all compound tested against three bacteria and two fungi.All compounds also screen for their antioxidant activity, among the synthesized compound, isatin3-(isoproplylidene)hydrazone exhibited excellent antioxidant activity and Antitubercular activity was done by disk diffusion method, result show that compound Isatin3-(benzylidene)hydrazone is inactive against TB bacteria and Isatin3-(methyl ethylide)hydrazone, isatin 3-(isoproplylidene)hydrazone show good activity against TB bacteria.

Keywords: isatin-3hydrazone and its derivatives, anti fungal, anti bacterial and antioxidant activity.



***Correspondence**
Author: Arti saxena
Email: artisaxena21@rediffmail.com

Introduction

Isatin are synthetically resourceful substrates, where they can be used for the synthesis of a large variety of heterocyclic compounds, such as indole and quinoline and work as raw material for drug synthesis. It is evident from literature that isatin derivatives are known to be associated with broad spectrum of biological activity like antibacterial [1], anti-inflammatory [2], analgesic [3], anti-viral [4], antifungal [5], antitubercular [6], and also anticonvulsant activity(7) . In view of these facts and as a continuation of our work in the laboratory [8] prompted us to synthesize some new isatin3-hydrazone derivatives. All the synthesized compounds were screened for their *in vitro* anti-bacterial and antifungal activity. Isatin is an endogenous compound identified in humans, and its effect has been studied in a variety of systems. Isatin moiety having both the keto and lactam form has aroused tremendous curiosity due to its diverse biological and pharmacological studies. From literature survey it is well known that isatin heterocycles show large importance in the field of medicinal chemistry as a potent chemotherapeutic agent. Biological properties of isatin include a range of actions in the brain and offer protection against certain types of infections. Indole is an aromatic heterocyclic compound that has a bicyclic structure. It is an accepted constituent of fragrances and the precursor to many pharmaceuticals. One of the oldest and most reliable methods for synthesizing substituted indole is the Fischer indole synthesis.

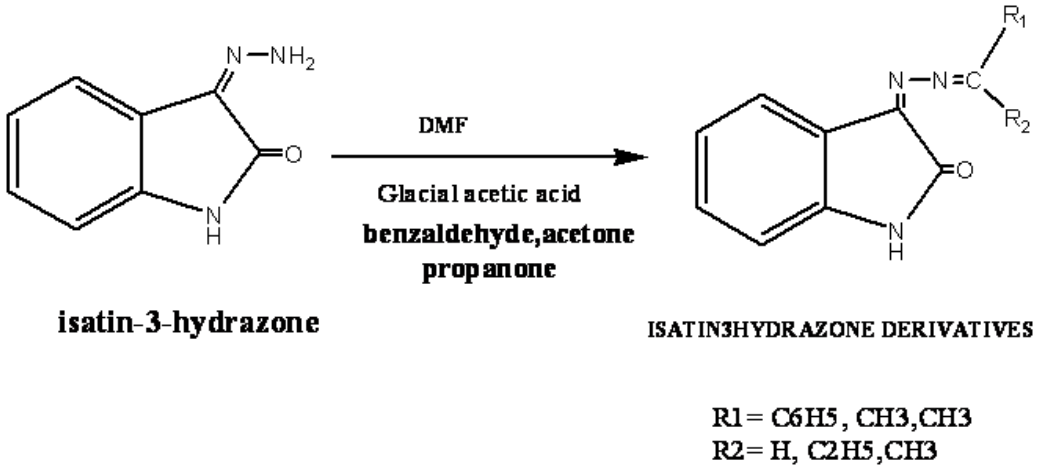
Materials and Methods

Drugs & Chemical

A general synthetic strategy employed to find the main compound in good yield is show in **Scheme 1**.In the present investigation, isatin-3hydrazone were obtained from reaction of isatin and hydrazine hydrate in alcoholic medium and

subjected to substitution with differentaldehydes and ketones. Aldehydes which is used in the substitution; benzaldehyd, and ketones; acetone, butanone. All reagents were of the highest purity commercially available.

Scheme 1



isatin-3-hydrazone

ISATIN3HYDRAZONE DERIVATIVES

R1= C6H5, CH3,CH3
R2= H, C2H5,CH3

Experimental

All reagents were analytically pure. Isatin and different aldehydes and ketones were bought from the Sigma Aldrich ltd.Reaction are monitored by thin-layer chromatography (TLC) on silica gel 60 F^{254} aluminium sheet .The mobile phase was benzene: chloroform: methanol (27: 9: 4) and detection was made using iodine chamber. The infrared (IR) spectra were recorded on a FTIR-8310 Shimadzu spectrometer using potassium bromide pellets. 1H NMR spectra were recorded on a JEOL AL3OO FTNMR , CHEMISTRY DEPARTMENT Banaras Hindu University, Varanasi-221005 in CDCl3 or DMSO-d6 with TMS as the internal reference The chemical shifts are expressed in ppm downfield from the internal standard; the coupling constants are in Hz, and signals are quoted as*s*(singlet), *d*(doublet), *t* (triplet), *q* (quartet), or *m* (multiplet). Melting points were determined using open capillary tube in Toshniwal Melting point apparatus and are presented without any correction. UV-Visible spectra were recorded anti oxidant activity on systronics 2201 double beam UV-Visible spectrophotometer.

Synthesis and Reaction Scheme

Isatin has been show anti microbial agent in the field of pharmacology. Indole 2,3 dione react with hydrazine hydrate in the presence of ethylalcohol formed isatin-3hydrazone. This is parental compound for forming isatin-3hydrazone derivatives.

Synthesis of isatin-3htdrazone-0.01 mol ofisatin was dissolved in 20ml ethylalcohol and added 0.015mol of hydrazine hydrate with shaking. The reaction mixture was stirred well, warmed on a water-bath for 10 min and left in the refrigerator for 3 hr. The resultant yellow crystalline solid was filtered, washed repeatedly with small portions of cold water and finally with a small quantity of cold alcohol. The product was dried and recrystalization done from ethylalcohol. M.P; 220°C, Yield; 74.5%

Synthesis of isatin-3hydrazone derivatives-0.1 mol ofIsatin3-hydrazone and 0.1 mol of appropriate aldehydes or ketones were dissolved in 30ml of DMFand add 10ml of protonating agent i.e. glacial acetic acid were keptat 60°C on water bath for half an hr with vigorous stirring. The reaction mixture was poured into water (300ml) and re-crystallized from ethanol solvent.

Spectral Data

Isatin-3-(benzylidene)hydrazone:FTIR (KBr) (ν cm^{-1}): 2983 (C-H), 1688 (C=O), 1615 (C=C), 1275 (C-O), 1180 (C-N). ^{1}HNMR (400 MH_Z, $CDCl_3$, TMS, δppm): 3.32 (s, 1H, =CH), 7.3 (9H, H_{Ar}), 9.2 (d, 1H, NH), 10.8 (s, 1H, H_{enolic}), 2.13 (t, 3H, CH_3),; ^{13}CNMR (300 MH_Z, DMSO, δppm) δ_c: 165, 122, 118, 41.

Isatin -3-(methyl ethylide)-hydrazone: *(*FTIR (KBr) (ν_{max} cm^{-1}): 3473 (N-H), 1669(C=O), 1591 (C=C), 1222 (C-O), 3316 (N-N) 1342 (C-N), ^{1}HNMR (300 MH_Z, DMSO, TMS, δppm): 7 (m, 8H, H_{Ar}), 9.3 (s, 1H, NH), 31 (s, 3H, CH_3), 10.5 (s, 1H, H_{enolic}); ^{13}CNMR (400 MH_Z, $CDCl_3$, δppm) δ_c: 42, 112, 122,164.

isatin -3-(isoproplylidene) hydrazone: (TIR (KBr) (ν_{max} cm^{-1}): 3471 (N-H), 1676(C=O), 15910 (C=C), 1225 (C-O), 3314 (N-N) 1347 (C-N),1103 (C-C-C) ^{1}HNMR (300 MH_Z, DMSO, TMS, δppm): 7.2 (m, 8H, H_{Ar}), 9.2 (d, 1H, NH), 3.1 (s, 3H, CH_3), 10.5 (s, 1H, H_{enolic}); ^{13}CNMR (400 MH_Z, $CDCl_3$, δppm) δ_c: 41, 105, 112,154.

In vitro antimicrobial activity

Evaluation of antibacterial (3-bacteria) and antifungal (2-fungi) activities was done by the disk diffusion technique. The tested compounds solutions were prepared in DMF and evaluated for their *in vitro* antibacterial andantifungal activities against*Bacillus pumillus*, *E.coli*, *Pseudomonas aureuginosa*, *Aspergillus niger* and*Candida albicance*.All bacteria were grown on Mueller-Hintonagar (Hi-Media) plates (37°C, 24 h) and fungi were grown on Potato dextrose agar (Hi-Media) plates (26°C, 48-72 h).The results were established by the presence of clear zone of inhibition around the active compounds. Referencecompoundsfor antibacterial activity is Streptomycin and for antifungal activity is Gentamycin.

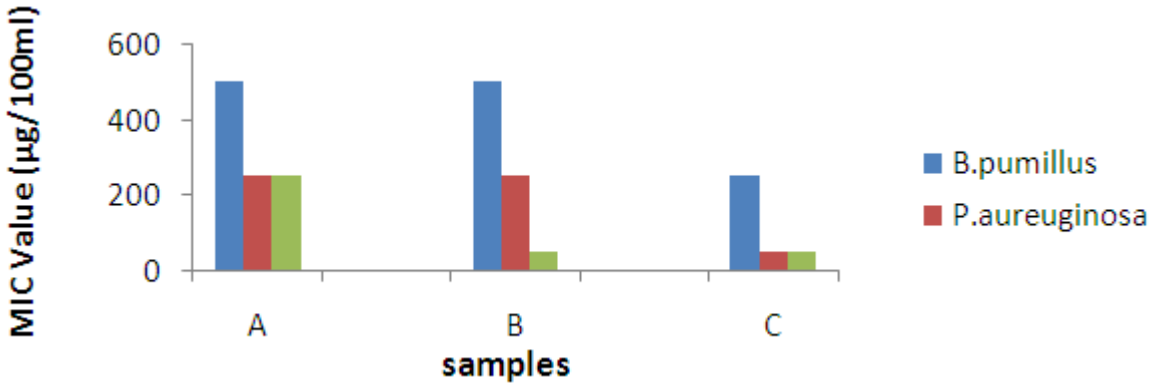


Figure 1 Comparative antibacterial activity of synthesized compound (A-C)

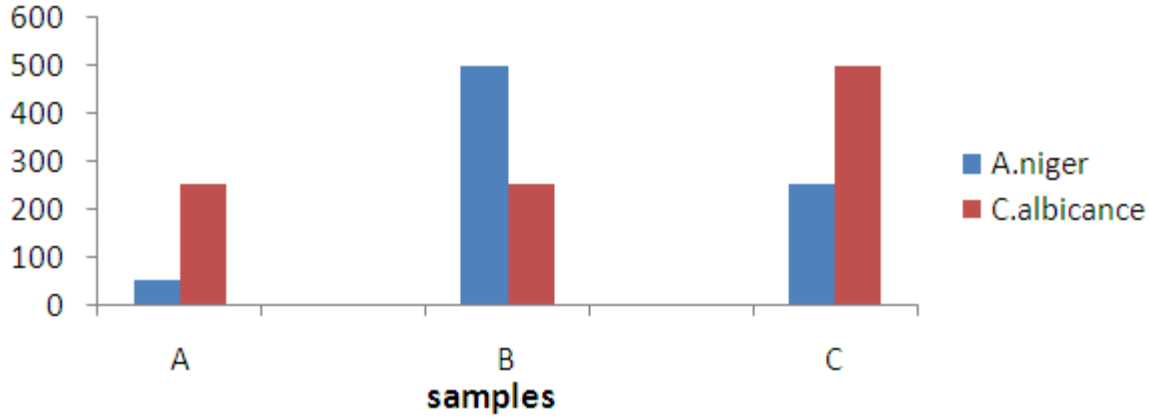


Figure 2 Comparativeantifungal activity of synthesized compound (A-C)

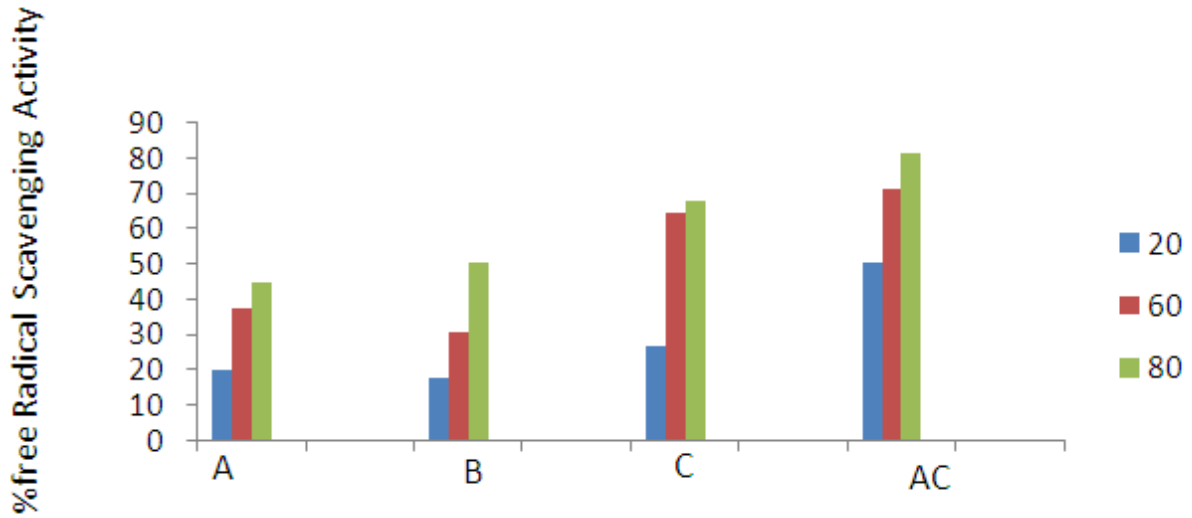
Table 1 Parameters related to Anti microbial activity of compounds 2 (A-C)

S.No	compound	substitution		MIC Value (µg/100ml)				
				Anti bacterial			Anti fungal	
		R_1	R_2	B.pumillus	P. aureuginos	E.coli	A.niger	C.albicance
1	A	C_6H_5	H	500	250	250	50	250
2	B	C_2H_5	CH_3	500	250	50	500	250
3	C	CH_3	CH_3	250	50	50	250	500

Antioxidant activities-(free radical scavenging activity)

The synthesized compound was screen for free radical scavenging activity by DPPH method [9]. The sample were prepared at concentrations of 20, 60, 80 µg/100µml, and ascorbic acid (AC) is taken as standard. Sample isatin-3hydrazone derivatives;isatin-3-(isoproplylidene) hydrazones(C)has very good scavenging activity because both methyl substituent increase the activity,Isatin-3-(benzylidene)-hydrazone (A) exhibit least activity with standard and Isatin3-(methyl-ethylide)-hydrazone(B)has shown moderate activity.bar chart representation of percentage of free radical scavenging activity is shown in **Figure 3**.

Figure 3 Free radical scavenging activity of synthesized compounds (A-C)



Antitubercular activity

Antitubercular activity was carried out by disc diffusion susceptibility method (Lawrence et al., 1972). Mycobacterium tuberculosis (MTCC, 300) purchased from Institute of Microbial Technology (MTCC), Chandigarh (India) were cultured in blood nutrient agar medium in late logarithmic (A_{600} nm = 1) fashion. Bacterial strains were cultured in the same media. It was shortly followed by streaking of 0.5 µl bacterial spread on LB agar plates (25 ml agar medium ± 90 µg/ ml Isoniazid discs over 9 cm Petri plates) as control. Filter discs (5mm diameter) of Whatman range were treated with 5µL of compound solutions including reference (**Tables 2 and 3**). After this, discs were air-dried for 7-10 minutes and kept over plates. These plates were incubated at 37 ^{0}C for about 48 h in a humid chamber. Following this bacterial zone-inhibition diameters were observed and measured carefully.

As is evident from Tables 3 and 4 compound A is anti TB inactive and compound B, C, have shown better inhibition in samples concentration ranging from 80-800 µg/ml in reference to isoniazid taken as standard against mycobacterium tuberculosis. In these compound C=N carbon atom substitute with different group. When C=N carbon atom attached with phenyl ring then it became TB inactive and when it attach with electron releasing group then activity increase.

Table 2 parameters related to anti TB activity of synthesized compound 2(A-C)

Compound	Molecular weight	Zone of Inhibition (mm)*	Minimum Inhibitory Concentration (ug/mL)
A	249	–	–
B	215	7.10±0.2	>800
C	189	9.10±0.5	>800
Isoniazid (standard)	137.14	25.70±0.67	10

Table 3 parameters related to anti TB activity of synthesized compound 2(A-C)

S.No.	Concentration (ug/mL)	Absorbance of compounds			
		Benzaldehyde	Butanone	Propanone	Standard
1	5	NA	1.682	1.602	0.882
2	10	NA	1.472	1.51	0.809
3	20	NA	1.39	1.409	0.742
4	30	NA	1.122	1.289	0.651
5	40	NA	1.067	1.09	0.608
6	80	NA	0.9	1.002	0.513
7	100	NA	0.869	0.906	0.484
8	160	NA	0.772	0.798	0.326
9	200	NA	0.641	0.721	0.25
10	320	NA	0.401	0.458	0
11	400	NA	0.271	0.272	0
12	640	NA	0.118	0.249	0
13	800	NA	0.103	0.131	0
14	MIC		>800	>800	320

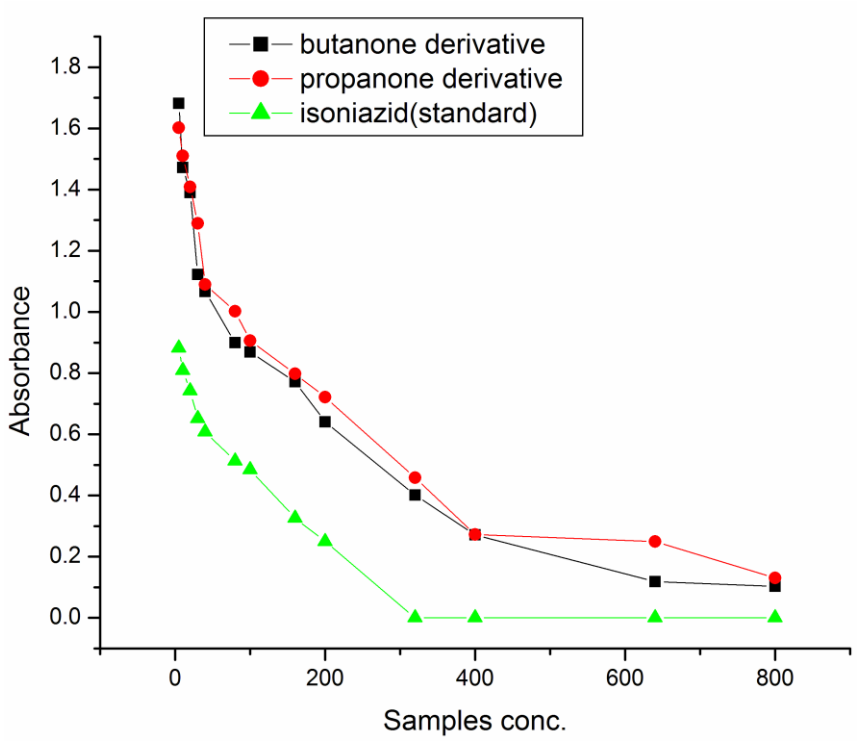


Figure 4 graph between Absorbance and samples concentration.

Results and Discussion

Azines are prepared by reaction of isatin3-hydrazone with different aldehydes and ketones. The details of some of the representative compounds formula, molecular weight, melting point % yield of each compound is given in the **Table 4**. All the synthesized compounds were tested for *in vitro* antimicrobial, antioxidant activity and anti TB activity. The tested compounds exhibit antimicrobial activity against all five microbes. The result of antimicrobial activity show that compound A. show better activity against all fungi and all bacteria. The result of antioxidant activity show that compound C exhibit better free radical scavenging activity then compounds A and B. The result of Antitubercular

activity of all compound reveal that compound A is inactive against TB bacteria and compound C exhibit better antitubercular activity compare to compound B.

Table 4 physical and elemental data analysis of compounds 2(A-C)

S.No	Compound	Substituent	Molecular formula	Melting point	Molecular weight	Yield %
1	A	C_6H_5CHO	$C_{15}H_{11}N_3O$	207-209	249	90.52
2	B	$CH_3COC_2H_5$	$C_{12}H_{13}N_3O$	178	215	89.3
3	C	CH_3COCH_3	$C_{11}H_{11}N_3O$	199-200	189	90.3

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