Research Article

Synthesis and Biological Evaluation of Isatin incorporated Quinoxalines as Anti-Tubercular Agents

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ABSTRACT

Heterocyclic compounds are very much used as therapeutic agents. Indole, an important class of nitrogen, containing heterocyclic with wide variety of biological activities. Isatin is a derivative of indole which is indole-2, 3 Dione. Isatin is reported for anti-tubercular activity. Quinoxaline is also reported for various biological activities. So, a scheme was designed and isatin incorporated quinoxaline were prepared to improve biological activity. In the present research isatin incorporated quinoxaline (1, 1A, 1B and 1C) were prepared, and were characterized by using TLC, IR, NMR and MASS spectral data. They were evaluated for anti-tubercular activity. Among those derivatives, compound 1 showed good activity.

Keywords: Isatin, tubercular activity, Quinoxaline.

INTRODUCTION

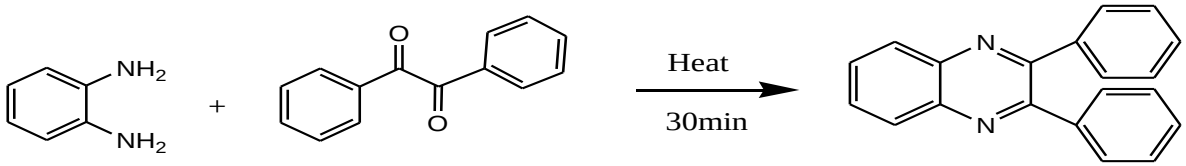
Heterocyclic compounds play a major role in medicinal chemistry and synthesis of drugs. Modification of the structure of heterocyclic compounds through reactions and development of the compound facilitate the production of new compounds. Some heterocyclic compounds are modified and developed to show anti-tubercular activity. [1] A quinoxaline, also called a benzopyrazine, in organic chemistry is a heterocyclic compound containing a ring complex made up of benzene ring and a pyrazine ring. It is isomeric with other naphthyridines including quinazoline, phthalazine and cinnoline. Quinoxalines are used as dyes, pharmaceuticals and antibiotics such as echinomycin, levomycin and actinoleutin. Studies were carried out in order to explore the antitumoral properties of quinoxaline compounds. Recently, quinoxalines and its analogues have been investigated as "catalysts". [3]

Isatin is an indole derivative, its chemical name is 1-H indole-2, 3-Dione. In 1841 this compound was prepared by Erdman and Laurent by performing the oxidation of Indigo dye by chromic acid and nitric acid. This compound is also a part of some plants like Corupita guianensis, Isatis tinctoria and Calanthe discolor. It has also been found as a component of the secretion from the parotid gland of Bufo frogs, and in humans as it is a metabolic derivative of adrenaline Isatin belongs to the class of organic compounds known as Indolines. Substituted isatins are also found in some plants like Melosatin alkaloids (methoxy phenylpentyl isatins) obtained from the Ca- ribbean tumorigenic plant Melochia tomentosa. This compound contains the indole moiety which is having pyrrolidine ring fused to benzene to form 2, 3-dihydro indole. It is a monoamino oxidase inhibitor and its high levels have been found in the urine of Parkinsonism patients. [4] Quinoxaline and isatin are also reported for anti-tubercular activity. So the present work is aimed to incorporate isatin in quinoxaline to get the synergic effect.

MATERIALS AND METHODS

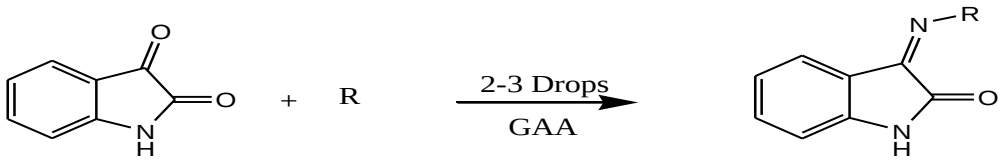
Preparation of 2, 3-Biphenyl Quinoxaline

To a warm solution of 2.1 gm. of Benzil in 8ml of rectified sprit, add a solution of 1.1gm of O-Phenylene diamine in 8ml of rectified spirit. Warm in water bath for 30 minutes and add water until a slight cloudiness persists and allow to cool. Filter and recrystallize from aqueous ethanol.



General procedure for Preparation of Isatin – 3- schiff's Bases

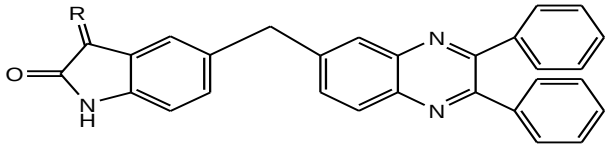
Equimolar quantities of isatin (0.03mole) and various substitution bases (like phenyl hydrazine , Semi carbazide and Hydroxyl amine) 0.03mole were added into 10ml of absolute ethanol (95%) containing 2-3 drops of glacial acetic acid in 100ml round bottomed flask. The reaction mixture was refluxed for half an hour at the refluxing temperature. Then cooled in an ice bath and the products obtained were recrystallized from ethanol.



General Procedure for Condensation of Isatin and Quinoxaline

Take equimolar concentrations of Isatin – 3- schiff's Bases and quinoxaline were dissolved in suitable solvents with 35parts of formaldehyde solution and 35% HCl solution. Stirring for 4hrs at 70°C using magnetic stirrer. Solution was made alkaline using NH_3 solution. Filter the products and recrystallize from aqueous ethanol.

Physical and spectral data of synthesized compounds were given in tables 1,2and 3.



General structure of isatin incorporated quinoxalines

Table 1: Physical characterization data of synthesized compounds

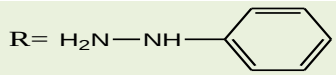
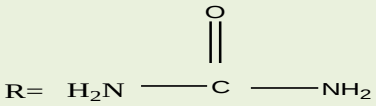
S. No.	Alkyl group(R)	Molecular formula	Molecular weight	% Yield	R_f values
1	R= O	$C_{29}H_{19}N_3O_2$	441	84	0.867
1A		$C_{35}H_{25}N_5O$	531	78	0.756
1B		$C_{30}H_{21}N_5O_2$	483	80	0.890
1C	R= NH_2OH	$C_{29}H_{20}N_4O_2$	456	79	0.852

Table 2: Spectral data of synthesized compounds

Compounds	Nature of the compound	Frequency(cm-1)
1	(C=C)aromatic (C-H)aromatic (C-N) (C=O) ketone (N-H)	1467cm-1 728.8cm-1 1345.09cm-1 1669.89cm-1 3236.85cm-1
1A	(C=C)aromatic (C-H)aromatic (C-N) aromatic amine (C=N) (N-H) (C=O) (N-H)amide	1458.81cm-1 3165.21cm-1 1345.12cm-1 1699.19cm-1 3345.27cm-1 1713.90cm-1 3502.19cm-1
1B	(C=C)aromatic (C-H)aromatic (C-N)aromatic amine (C=N) (N-H)	1493.65cm-1 3165.21cm-1 1344.55cm-1 1652.75cm-1 3308.22cm-1
1C	(C=C)aromatic (C-H)aromatic (C-N)aromatic amine (C=N) (N-H))	1507.37cm-1 799.14cm-1 1344.20cm-1 1662.69cm-1 3242.95 cm-1

Table 3: NMR Spectral Data of synthesized compounds

Compound code	Chemical shift	Proton nature/nor protons
1B	7.032-8.174 11.041 2.546 12.760	17(Ar-H) 1H(N-H) 2^0 amine 2H(Alkyl) 2H(Amide)
1C	7.364-8.798 12.039 2.564	16H(Ar-H) 1OH(Oxime) 2H(Alkyl)

Pharmacological Evaluation

Anti-TB activity using Alamar Blue Dye

Procedure

1) The anti-mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA).
2) This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.
3) Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.

4) The 96 wells plate received 100 µl of the Middle brooks 7H9 broth and serial dilution of compounds were made directly on plate.

5) The final drug concentrations tested were 100 to 0.2 µg/ml.

6) Plates were covered and sealed with parafilm and incubated at 37ºC for five days.

7) After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.

8) A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.

9) The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. And the results were represented in table:4

RESULTS

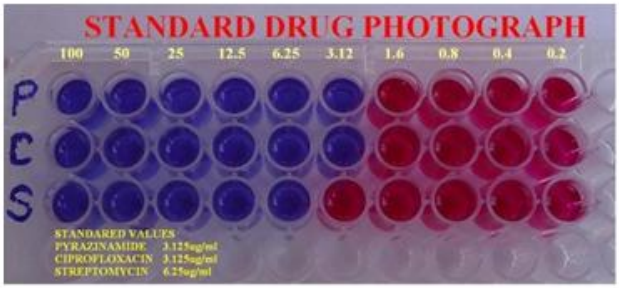


Figure 1 Assay results of the standard drug

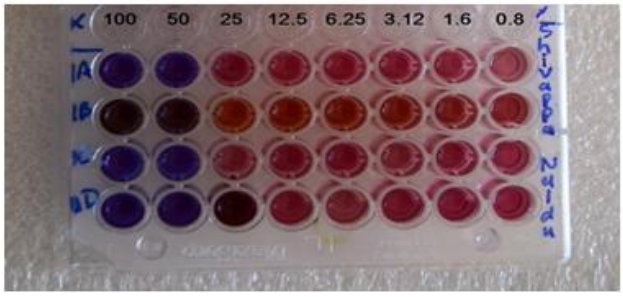


Figure 2 Assay results of Test dugs

Table 4: anti tubercular activity of isatin incorporated quinoxalines

S. No	Samples	100µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	A	S	S	R	R	R	R	R	R
2	B	S	S	R	R	R	R	R	R
3	C	S	S	R	R	R	R	R	R
4	D	S	S	S	R	R	R	R	R

NOTE: S – Sensitive R – Resistant, Strain used: ***M tuberculosis*** (H37 RV strain): ATCC No- 27294.

Here are the ***standard values*** for the Anti-Tb test which was performed.

Pyrazinamide- 3.125µg/ml, Streptomycin- 6.25µg/ml, Ciprofloxacin-3.125µg/ml

CONCLUSION

New drugs for Tuberculosis are urgently needed. Unfortunately there are few new drugs in the pipeline, Isatin and Quinoxaline are versatile lead molecules for potential bio active agents and its derivatives are reported to possess anti-tubercular activity. So research will hopefully continue to shed light on ways to increase the therapeutic efficacy.

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