PROJECT REPORT

(UGC Major Research Project)

Synthesis, characterization and anti-tubercular evaluation of novel Isatin derivatives

Submitted to

University Grants Commission (UGC)
Bahadur Shah Zafar Marg, New Delhi
Pin: 110 002

by

Principal Investigator: Dr. Mahesh Kumar
Assistant Professor
Department of Pharmaceutical Sciences
Maharshi Dayanand University
Rohtak, Haryana

-

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Final Report Assessment / Evaluation Certificate

(Two Members Expert Committee Not Belonging to the Institute of Principal Investigator)

(To be submitted with the final reports)

It is certified that the final report of Major Research Project entitled **"SYNTHESIS, CHARACTERIZATION AND ANTI-TUBERCULAR EVALUATION OF NOVEL ISATIN DERIVATIVES"** by **Dr. MAHESH KUMAR** at Dept. of **PHARMACEUTICAL SCIENCES, MAHARSHI DAYANAND UNJIVERSITY, ROHTAK** has been assessed by the committee consisting the following members for the submission of the report to the UGC, New Delhi under the scheme of Major Research Project.

Comments/Suggestions of the Experts Committee:

(1) The work has been done in accordance with aims & objectives of the project and the work done by the candidate is satisfactory

Sandeep Jain 08/9/22

Name & Signature of Experts with Date

Prof. SANDEEP JAIN, Dept. of Pharm. Sci, GJU&ST, Hisar HR.

(2) The workdone by the PI is satisfactory and as per the objective of the title of the Project.

Prof. Neelum Jain 08/9/2022

Name & Signature of Experts with Date

Deptt. of Pharm. Sci, BPSMV, Khanpur, Sonepat, HR.

Name of Expert University/College name:

Signature with Date

It is certified that final report Executive summary of the report Research documents monograph academic papers provided under **Major Research Project** have been posted on the website of the university/College.

(Registrar/Principal)

08/09/2022

Dr. Mahesh Kumar
Assistant Professor
Deptt. of Pharm. Sci.

Annexure-IX

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1.	**TITLE OF THE PROJECT**	**Synthesis, Characterization, and Anti-tubercular Evaluation of Novel Isatin Derivatives**
2.	**NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR**	**Dr. Mahesh Kumar** **Department of Pharmaceutical Sciences** **Maharshi Dayanand University** **Rohtak-124001 (HR)**
3.	**NAME AND ADDRESS OF THE INSTITUTION**	**Department of Pharmaceutical Sciences** **Maharshi Dayanand University** **Rohtak-124001 (HR)**
4.	**UGC APPROVAL LETTER NO. AND DATE**	**F.NO.-43-501/2014(SR), 30-10-2015**
5.	**DATE OF IMPLEMENTATION**	**01-07-2015**
6.	**TENURE OF THE PROJECT**	**3 YEARS FROM** **01-07-2015 TO 30-06-2018**
7.	**TOTAL GRANT ALLOCATED**	**Rs. 14,15,000**
8.	**TOTAL GRANT RECEIVED**	**Rs. 13,15,000**
9.	**FINAL EXPENDITURE**	**Rs. 11,98,120**
10.	**OBJECTIVES OF THE PROJECT**	**ATTACHMENT**
11.	**WHETHER OBJECTIVES WERE ACHIEVED**	
12.	**ACHIEVEMENTS FROM THE PROJECT**	
13.	**SUMMARY OF THE FINDINGS**	
14.	**CONTRIBUTION TO THE SOCIETY**	
15.	**WHETHER ANY PH.D. ENROLLED/**	**No**

	PRODUCED OUT OF THE PROJECT	
16.	**NO. OF PUBLICATIONS OUT OF THE PROJECT**	**One Research Article Published**

PRINCIPAL INVESTIGATOR

REGISTRAR/PRINCIPAL
(Seal)

HOD

1. OBJECTIVES OF THE PROJECT

For the synthesis, characterization and evaluation of novel isatin derivatives following protocol has been adopted:

1. Synthesis of novel Isatin derivatives by using following experimental procedure

2. Characterization of the synthesized derivatives of Isatin by using IR, NMR and MASS spectroscopy.

3. All the synthesized and characterized Isatin derivatives were tested for Anti-tubercular activity.

2. WHETHER OBJECTIVES WERE ACHIEVED

The achievements from the project are:-

The Pyrimidine derivatives of Bromoisatin were showed good results than the Bromoisatin derivatives due the significant medicinal effect of attached Pyrimidine ring which may have enhance the liphophilicity and metabolic resistance as well. Two compounds 32 and 33 could kill the 50% of MTB even at concentration lower than 20μM. The study led to the knowledge that the isatin has antitubercular value. The Isatin scaffold may be utilized as template for further modification and development of drug like candidate.

3. ACHIEVEMENT FROM THE PROJECT

The Isatin scaffold may be utilized as template for further modification and development of drug like candidate. 01 paper has been published in UGC approved journal.

4. SUMMARY OF THE FINDINGS

Since the main antitubercular drugs have developed resistant to the large proportion of TB patients. Bacteria's are showing tolerances for more of antibiotics. The long treatment due to resistance causing heptotoxicity, nephrotoxicity etc. So there

the Isatin was selected as template for modification and development of a compound library because anti-tubercular potential of novel isatin derivatives like the first antitubercular potential of Isatin was confirmed by Erdman and Laurent in 1841. Isatin has medicinal potential to be used as drug template for identifying the novel drug candidates which can eliminate these treatments associated problems and can work potentially over first line drug resistant.

1. The synthesized derivatives were potentially characterized for their purity through the chromatography and spectroscopic techniques.
2. The confirmed derivatives were tested, the results showed like the few compounds from Bromoisatin derivatives found to show 50% MTB inhibition at concentration between 20µM to 30µM. The compounds with hydrocarbon attached substituents could show little good activity that may be due the little enhanced liphophilicity of the compounds which made the entry of the compounds easy into the MTB.
3. The Pyrimidine derivatives of Bromoisatin were showed good results than the Bromoisatin derivatives due the significant medicinal effect of attached Pyrimidine ring which may have enhance the liphophilicity and metabolic resistance as well. Due that two compounds 32 and 33 could kill the 50% of MTB even at concentration lower than 20µM.
4. The study led to the knowledge that the isatin has antitubercular value. The Isatin scaffold may be utilized as template for further modification and development of drug like candidate.

FD Diary No. - 8049
Dated - 29.09.2015

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ज्ञान-विज्ञान विमुक्तये

UNIVERSITY GRANTS COMMISSION
BAHADURSHAH ZAFAR MARG
NEW DELHI-110002

F. No. - 43-501/2014(SR)

Dated :- Oct, 2015

30 OCT 2015

MRP-MAJOR-PHAR-2013-34662
(SC)

The Under Secretary (FDIII),
University Grants Commission,
Bahadur Shah Zafar Marg,
New Delhi-110002.

Sub.:- Release of Grants-in-aid to **Maharshi Dayanand University, Rohtak-PIN-124001** for the year **2015-16** under Plan in respect of Major Research Project entitled **"Synthesis, Characterization and anti-tubercular evaluation of novel isatin derivatives."** awarded to **Dr. Mahesh Kumar , Department of Pharmaceutical Sciences** , Tenure of project for 3 year(s) w.e.f. **01/07/2015** .

Sir/Madam,

I am directed to convey the approval sanction of the University Grants Commision for payment of grant of **Rs. 13,15,000/-** (Rupees: **THIRTEEN LAKHS FIFTEEN THOUSAND ONLY**) as **1st instalment** for the years **2015-16** towards Major Research Project to the **REGISTER , Maharshi Dayanand University, Rohtak-PIN-124001** for the Plan expenditure to be incurred during **2015-16** .

S. No.	Items	Head of Account	Amount Approved(Rs.)	Grant being Released as 1st Installment(Rs.)	Grant Already Released(Rs.)	Total Grant(Rs.)
A.	**Non-Recurring**	3(B).35				
1.	Books & Journals		Rs. 0/-	Rs. 0/-	-	Rs. 0/-
2.	Equipment		Rs. 12,00,000/-	Rs. 12,00,000/-	-	Rs. 12,00,000/-
B.	**Recurring**	3(B).31				
1.	Honorium to Retd. Teacher @ Rs. 18,000/- p.m.		Rs. 0/-	Rs. 0/-	-	Rs. 0/-
2.	**a.** Project Fellow (Non-Gate/Non NET) @ Rs. 14,000/- p.m. **b.** Project Fellow (Gate/NET/GPAT) @ Rs. 16,000/- p.m. Tenure - 3 year(s)		Rs. 0/-	Rs. 0/-	-	Rs. 0/-
3.	Chemical/Glassware/Consumable (Raw Material & Packaging Material etc.)		Rs. 50,000/-	Rs. 25,000/-		Rs. 25,000/-
4.	Contingency		Rs. 50,000/-	Rs. 25,000/-	-	Rs. 25,000/-
5.	Hiring Services		Rs. 50,000/-	Rs. 25,000/-	-	Rs. 25,000/-
6.	Travel / Field Work		Rs. 50,000/-	Rs. 25,000/-		Rs. 25,000/-
7.	Any Other		Rs. 0/-	Rs. 0/-		Rs. 0/-
8.	Overhead Charges 10% of approved recurring Grant (Except Travel & Field Work)		Rs. 15,000/-	Rs. 15,000/-		Rs. 15,000/-
	Total (A + B)		**Rs. 14,15,000/-**	**Rs. 13,15,000/-**		**Rs. 13,15,000/-**

ISSUED

G. S. AULAKH
Under Secretary
University Grants Commission
Ministry of Human Resource Development
Govt. of India
New Delhi-110002

1. The sanctioned amount is debitable to the Major **Head 3(B).31 Rs. 1,15,000/- & Head 3(B).35 Rs. 12,00,000/-** and is valid for payment during financial year **2015-16** .

2. The amount of the Grant shall be drawn by the Under Secretary (Drawing and Distributing Officer), University Grants Commission on the Grants-in-aid Bill and shall be disbursed to and credited to the **REGISTER , Maharshi Dayanand University, Rohtak-PIN-124001** through Electronic mode as per the following details.

	Payment Details	
(a)	Bank Name & Address of Branch	**State Bank of India, MDU, Rohtak Branch University Campus, MDU Rohtak, Haryana**
(b)	Account No.	**10222112164**
(c)	Type of Account (SB/Current/Cash Credit)	**SAVING**
(d)	IFSC Code	**SBIN0004734**
(e)	MICR Code of Branch	**124002008**
(f)	Whether Bank Branch is RTGS or NEFT enabled? :	**Yes (RTGS/NEFT/Both)**
(g)	Name & Address of Account Holder	**The Registrar, Maharshi Dayanand University, Rohtak, Haryana**

3. The Grant is subject to the adjustment of the basis of Utilization Certificate in the prescribed performa submitted by the University/Colleges/Institution.

4. The University/College/Institution shall maintain proper accounts of the expenditure out of the grants which shall be utilized only on approved items of expenditure.

5. The University/Institution may follow the General Financial Rules, 2005 and take Urgent necessary action to amend their manuals of financial procedures to bring them in conformity with GFRs, 2005 and those don't have their own approved manuals on financial procedures may adopt the provisions of GFR's 2005 and instructions/guideline there under from time to time.

6. The Utilization Certificate to the effect that the grant has been utilized for the purpose for which it has been sanctioned shall be furnished to the University Grants Commission as early as possible after the close of the current financial year.

7. The assets acquired wholly or substantially out of University Grant Commission's grant shall not be disposed or encumbered of utilized for the purposes other than those for which the grant was given, without proper sanctioned of the University Grants Commission and should, at any time the College/University ceased in function such assets shall revert to the University Grants Commission.

8. A register of assets acquired wholly or substantially out of the grant shall be maintained by the University/College in the prescribed proforma.

9. The grantee institution shall ensure the utilization of grant-in-aid for which it is being **sanction/paid**. In case non-utilization/part utilization, thereof simple interest @ 10% per annum as amended from time to time on unutilized amount from the date of drawl to the date of refund as per provisions contained in General Financial Rules of Govt. of India will be charged.

10. The University/College/Institute shall follow strictly the Government of India / University Grants Commission guidelines regarding implementation of the reservation policy **[both vertical (for SC, ST & OBC) and horizontal (for persons with disability etc.)]** in teaching and non-teaching posts.

11. The University/College shall fully implement the Official Language Policy of Union Govt. and comply with the Official Language Act, 1963 and Official Languages (Use for Official purposes of the Union) Rules, 1976 etc.

12. The sanction is issued in exercise of the delegation of powers vide University Grants Commission Office Order No. **69/2014 F.No.10-11/12 (Admn. IA & B) dated 26/03/2014** .

13. The University/Institution shall strictly follow the University Grants Commission Regulations on curbing the menace of Ragging in Higher Educational Institutions, 2009.

14. The University/Institution shall take immediate action for its accreditation by National Assessment & Accreditation Council (NAAC).

15. The accounts of the University/Institution will be open for audit by the Comptroller & Auditor General of India in accordance with the provisions of General Financial Rules, 2005.

16. The annual accounts i.e. balance sheet, income and expenditure statement and statement of receipts and

Government.

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It is certified from the B.C.R. that the funds are available under the scheme. Entered in BCR at S.No. - P. No. 79.

The funds to the extent of Rs. _____ Crores are available under the scheme or BE/RE of the year **2015-16**.

19. This issue with the concurrence of IFD Vide No. Diary No. **10946** Dated, **10.03.2015** .

20. This issue with the approval of the **Chairman, (UGC)** Vide Diary No. **28731** Dated **30.04.2015** .

Yours faithfully,

(G. S. AULAKH)
Under Secretary

जी. एस. औलख
G. S. AULAKH
अवर सचिव/Under Secretary
विश्वविद्यालय अनुदान आयोग
University Grants Commission
मानव संसाधन विकास मंत्रालय
Min. of Human Resource Development
भारत सरकार /Govt. of India
नई दिल्ली /New Delhi-110002

Copy forwarded for information and necessary action to :-

1. The **REGISTER , Maharshi Dayanand University, Rohtak-PIN-124001 .**
2. Office of the Director General of Audit, Central Revenues, A.G.C.R. Building, I.P. Estate, New Delhi.
3. Accountant General, Govt. of State, **Haryana .**
4. **Dr. Dr. Mahesh Kumar** , Principal Investigator, **Department of Pharmaceutical Sciences , Maharshi Dayanand University, Rohtak-PIN-124001** .

(ARUN KUMAR SINHA)
SECTION OFFICER

Ph. SC-733
23/06/2021

MAHARSHI DAYANAND UNIVERSITY ROHTAK
(A State University established under Haryana Act No. XXV of 1975)
'A++' Grade University accredited by NAAC

No.FO/UGC/21/ 744-45
Date 8/6/21

To

Under Secretary,
University Grants Commission,
Bahadurshah Zafar Marg,
New Delhi- 110002.

Sub: Statement of Expenditure & Utilization Certificate in respect of UGC MRP assigned to Dr. Mahesh Kumar, Department of Pharmaceutical Science.

Ref. No. F.43-501/2014 (SR).

Sir/Madam,

Please find enclosed herewith the Statement of Expenditure and Utilization Certificate in respect of the UGC Major Research Project assigned to Dr. Mahesh Kumar in the Department of Pharmaceutical Sciences, duly signed by the Finance Officer, Registrar and Joint Director (Audit) of this University.

This is for your kind consideration.

Yours faithfully,

08/06/21
Superintendent (Research)
for Director (Research)

Encl: As above.

Copy to:

1. **Head, Department of Pharmaceutical Sciences, M.D. University, Rohtak.**

Annexure-III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR RESEARCH PROJECT

1. Name of Principal Investigator : **Dr. MAHESH KUMAR**
2. Deptt. of Principal Investigator : **DEPT OF PHARMACEUTICAL SCIENCES**
3. University/College: **MAHARSHI DAYANAND UNIVERSITY, ROHTAK**
4. UGC approval Letter No. and Date: **F.43-501/2014(SR), 30-10-2015**
5. Title of the Research Project: **SYNTHESIS, CHARACTERISATION AND ANTITUBERCULAR EVALUATION OF NOVEL ISATIN DERIVATIVES**
6. Effective date of starting the project: **01-07-2015**
7. a. Period of Expenditure: From: **01-07-2015 to 28-02-2017**

   b. Details of Expenditure:

S.No.	Item	Amount Approved (Rs.)	Grant Released (Rs.)	Expenditure Incurred (Rs.)	Balance (Rs.)
i.	Equipment	12,00,000	12,00,000	11,98,120	1880
ii.	Contingency	50,000	25,000	Nil	25,000
iii.	Chemicals & Glassware	50,000	25,000	Nil	25,000
iv	Hiring Service	50,000	25,000	Nil	25,000
v	Travel/Field Work	50,000	25,000	Nil	25,000
Vi	Overhead Charges	15,000	15,000	15,000	Nil
	TOTAL	14,15,000	**13,15,000**	**12,13,120**	1,01,880

   c. Staff: Nil Project Fellow: NIL

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.
2. If as a result of check or audit objection some irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.
3. Payment @ revised rates shall be made with arrears on the availability of additional funds.

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4. It is certified that the out of total grant of **Rs.13,15,000 (Rupees Thirteen Lakhs Fifteen Thousand Only)** received from the University Grants Commission under the scheme of support for Major Research Project entitled SYNTHESIS, CHARACTERIZATION AND ANTITUBERCULAR EVALUATION OF NOVEL ISATIN DERIVATIVES vide UGC letter No. F. 43-501/2014(SR) dated 30-10-2015, an amount of **Rs. 12,13,120 (Rupees Twelve Lakhs Thirteen Thousand One Hundred Twenty Only)** has been utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

Note: Unspent balance of Rs. 101880/- has been refund to the Secretary, UGC-New Delhi, via UTR No. SBIN120275308852 in UGC bank account no. 8627101002122 on dated 01-10-2020.

17/03/2021

Principal Investigator

17/3/21

Finance Officer
M.D. University
ROHTAK

17/03/21

Registrar
M.D. University, Rohtak

Joint Director (Audit)

Resident Senior Auditor,
Local Audit ... Haryana,
M.D.U., Rohtak.

05/4/21

e-mail verified from Rs. 12/3/2021

Annexure-V

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

Utilization Certificate

Certified that an amount of **Rs. 12,13,120 (Rupees Twelve Lakhs Thirteen Thousand One Hundred Twenty Only)** out of **Rs.13,15,000** received from the University Grants Commission under the scheme of support for Major Research Project entitled SYNTHESIS, CHARACTERIZATION AND ANTITUBERCULAR EVALUATION OF NOVEL ISATIN DERIVATIVES vide UGC letter No. F. No.-43-501/2014(SR), dated 30-10-2015 has been utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University

Grants Commission.

If as a result of check or audit objection some irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.

Unspent balance of **Rs. 101880/-** has been refund to the Secretary, UGC-New Delhi, via UTR No. SBIN120275308852 in UGC bank account No. 8627101002122 on dated 01-10-2020.

10/03/2021

Principal Investigator

12/3/21

Finance Officer
M.D. University
ROHTAK

17/03/21

Registrar
M.D. University, Rohtak

05/4/21

Joint Director (Audit)
Local Audit Haryana,
M.D.U., Rohtak.

05/4/21 05/04/21

1. Introduction

Tuberculosis is caused by a bacterium called *Mycobacterium tuberculosis*. This bacterium typically attacks the lungs but may also attack other parts of the body such as the kidney, spine, and brain. Tuberculosis may also be linked to certain risk factors, including alcoholism, IV drug abuse, and homelessness. Infection with *Tubercle bacillus* (most often *M. tuberculosis*) is characterized by the formation of tubercles, hard nodules in the lungs that are the result of interaction between the bacteria and the host‘s immune system. The infected macrophages result in an inflammatory response (heat, swelling, dilated capillaries) which attracts more macrophages until the site of infection is completely surrounded by many of these compressed phagocytic cells. Inflammation triggers other cells within the host to essentially quarantine the area by depositing collagen fibers around the packed macrophages, forming an enclosed infection within the lung called a tubercle. The cells at the center of the tubercle may eventually die, producing either an area of necrosis or an actual cavity. Tuberculosis usually attacks the lungs (pulmonary tuberculosis) but it also affects the central nervous system, the lymphatic system, the circulatory system, the genitourinary system, the gastrointestinal system, bones, joints, and even the skin **(Wehenkel, *et al.* 2008).**

1.1 Types of tuberculosis:

Primary TB: - Primary tuberculosis refers to the infection process which eventually eliminates the pathogen or results in a stalemate between the *Mycobacteria* and the immune system. With most TB infections, the immune system is able to contain, although not eliminate, the *Mycobacteria* within the tubercle, preventing the spread of bacteria and progression of the disease. *M. tuberculosis* can remain in this impasse of dormant infection for many years.

Secondary Reactivated TB:- The infection can become reactivated if the *Mycobacteria* are able to rupture the tubercle and spread through the lungs. This reactivation typically happens to those with a weakened or suppressed immune system.

Disseminated TB:- The spread of the disease within the body may result if infected macrophages moving through the blood and lymph transport the bacteria to other sites. Once infected, symptoms of disseminated TB correspond to the locations infected. The antiquated term "consumption" arose from the myriad of symptoms associated with disseminated tuberculosis, when those infected seemed to slowly waste away.

Dissemination of tuberculosis outside of lungs can lead to the appearance of a number of uncommon findings with characteristic patterns:

Skeletal Tuberculosis:Tuberculous osteomyelitis involves mainly the thoracic and lumbar vertebrae (known as Pott's disease) followed by knee and hip. There is extensive necrosis and bony destruction with compressed fractures (with kyphosis) and extension to soft tissues, including psoas "cold" abscess.

Genital Tract Tuberculosis:Tuberculous salpingitis and endometritis result from dissemination of tuberculosis to the fallopian tube that leads to granulomatous salpingitis, which can drain into the endometrial cavity and cause a granulomatous endometritis with irregular menstrual bleeding and infertility. In the male, tuberculosis involves prostate and epididymis most often with non-tender induration and infertility.

Urinary Tract Tuberculosis:A "sterile pyuria" with WBC's present in urine but a negative routine bacterial culture may suggest the diagnosis of renal tuberculosis. Progressive destruction of renal parenchyma occurs if not treated. Drainage to the ureters can lead to inflammation with ureteral stricture.

CNS Tuberculosis:A meningeal pattern of spread can occur, and the cerebrospinal fluid typically shows a high protein, low glucose, and lymphocytosis. The base of the brain is often involved, so that various cranial nerve signs may be present. Rarely, a solitary granuloma, or "tuberculoma", may form and manifest with seizures.

Gastrointestinal Tuberculosis:This is uncommon today because routine pasteurization of milk has

eliminated Mycobacterium bovis infections. However, *M. tuberculosis* organisms coughed up in sputum may be swallowed into the GI tract. The classic lesions are circumferential ulcerations with stricture of the small intestine. There is a predilection for ileocecal involvement because of the abundant lymphoid tissue and slower rate of passage of lumenal contents.

Adrenal Tuberculosis:Spread of tuberculosis to adrenals is usually bilateral, so that both adrenals are markedly enlarged. Destruction of cortex leads to Addison's disease.

Scrofula:Tuberculous lymphadenitis of the cervical nodes may produce a mass of firm, matted nodes just under the mandible. There can be chronic draining fistulous tracts to overlying skin. This complication may appear in children, and *Mycobacterium scrofulaceum* may be cultured.

Cardiac Tuberculosis:The pericardium is the usual site for tuberculous infection of heart. The result is a granulomatous pericarditis that can be hemorrhagic. If extensive and chronic, there can be fibrosis with calcification, leading to a constrictive pericarditis.

1.2 Origin of the research problem:

Multidrug-resistant TB

Resistance is growing for standard anti-tubercular drugs have been used frequently. Disease strains that are resistant to a single anti-TB drug have been documented in every country surveyed. Multidrug-resistant tuberculosis (MDR-TB) is a form of TB caused by bacteria that do not respond to, at least, isoniazid, ethambutol and rifampicin, the two most powerful, first-line (or standard) anti-TB drugs. About 450 000 people developed MDR-TB in the world in 2012. More than half of these cases were in India, China and the Russian Federation. It is estimated that about 9.6% of MDR-TB cases had XDR-TB. **(WHO Fact sheet 2019, *http://www.who.int/mediacentre/factsheets/fs104/en/*).**

Disease caused by resistant bacteria fails to respond to conventional, first-line treatment. MDR-TB is being treated by using second-line drugs but there is no satisfactory results have been found. Howeversecond-line treatment options are limited and recommended medicines are not

always available. The extensive chemotherapy required (up to two years of treatment) is more costly and can produce severe adverse drug reactions in patients.

Isoniazid, rifampicin and ethambutol main first line antitubercular drugs have been found not effective for resistant mycobacterium tuberculosis individually or not in combination therapy. Isoniazid has been experimentally identified resistant towards tuberculosis. Isoniazid is also responsible for heptotoxicity at the cost of long term treatment.

Rifampicin is also a first line drug that has been found not effective against mutated mycobacterium tuberculosis (MTB) in combination drug therapy. Rifampicin has become resistant to kill MTB pathogen.

Isoniazid

Because INH is the most commonly used antitubercular drug, resistance to INH occurs more frequently among clinical isolates than resistance to any other agent **(Karakousis, 2009).** Mutations have been commonly detected in the *katG*gene in INH-resistant clinical isolates occurring in 50–80% of cases. Mutations in *katG* gene reduce or affect the ability of the catalase-peroxidase to activate the INH pro-drug. Commonly point mutations in *katG*are observed and a single point mutation which is responsible for substitution of threonine for serine at residue 315 (S315T) accounts for the majority of INH resistance among clinical isolates **(Marttila, *et al.* 1998; Abate, *et al.* 2001).** INH resistance has also been found to arise from mutations in *inhA* that can also result in reduced affinity of the enzyme for NADH without affecting enoyl reductase activity of NADH **(Basso, *et al.* 1998).**Mutations in *inhA*also have been found to cause resistance to the structurally related second-line drug ethionamide.

Rifampicin

The rifamycins were first isolated in 1957 from *Amycolatopsismediterranei*as part of an Italian antibiotic screening program **(Sensi, 1983).** While INH resistance alone is more common in *M. tuberculosis* than resistance to rifampin alone and more than 90% of rifampin-

resistant isolates has been found also resistant to INH. Therefore, rifampin resistance isolates has been used as a substitute marker for MDR-TB. Resistance to rifampin in *M. tuberculosis* is caused most commonly as single point mutations in the *rpoB*gene, which encodes the RNA polymerase **(Telenti, *et al.* 1993).** Point mutations cluster in an 81-base pair "hot-spot" region between codons 507 and 533 of the *rpoB*gene, with mutations in codons 531 encodes Serine and codons 526 encodes Histidine predominatly in More than 90% of rifampin-resistant clinical isolates **(Ramaswamy and Musser 1998).**

Pyrazinamide

Duration of treatment vital to achieve acceptable relapse rates has been reduced to six months from 9–12 months since the discovery of pyrazinamide (PZA) **(Steele and Des Prez 1988).** PZA resistance has been recognized primarily due to mutations in the *pncA*gene which encodsPZase**(Scorpio and Zhang 1996).** Most of mutations found are due to point mutations, deletions, and insertions which have been reported in a 561-bp region of the open reading frame or in an 82-bp region of its putative promoter **(Scorpio, *et al.* 1997; Jureen, *et al.* 2008).** A small percentage of isolates with high-level PZA resistance contain no mutations in *pncA*or its promoter that suggests about alternative mechanisms of resistance such as deficient uptake and enhanced efflux and altered *pncA*regulation **(Raynaud, *et al.* 1999).**

Ethambutol

Resistance to ethambutol in *M. tuberculosis* is commonly found to be caused due to point mutations in the *embCAB*operon **(Belanger, *et al.* 1996).** The *EmbA* and *EmbB* proteins are found to involve in the formation of the proper terminal hexaarabinofuranoside motif during arabinogalactan synthesis **(Escuyer, *et al.* 2001)** where *EmbC* is found to involve in lipoarabinomannan synthesis **(Zhang, *et al.* 2003).***EmbB* is considered to be the main target of ethambutol because more percentage of EMB-resistant clinical isolates found to have mutations in the *embB*gene **(Sreevatsan, *et al.* 1997; Telenti, *et al.* 1997; Ramaswamy, *et al.* 2000).**

1.3 Antibiotic resistance

Antibiotic tolerance is the capability of non replicating bacteria to get resistant againt particular antibiotic i.e the bacteria resist killing by cell wall-active antibiotics **(Tomasz, *et al.*1970).** This occurrence of tolerance is distinct from drug resistance as that can be intrinsic or acquired tolerance since it is not attributable to genetic mutations, and the organisms regain susceptibility to these antibiotics once the stress conditions have been removed and bacterial growth resumes. The prolonged treatment with antibiotics required to eradicate TB is supposed to alter the physiological state of persistent bacilli which have developed tolerance to standard anti-tuberculosis drugs, particularly to isoniazid, which inhibits mycolic acid synthesis **(Karakousis, *et al.* 2008; Adhikari, 2010).**

1.4 National Status

In India situation is more complicated because TB disproportionately affects the young India, with its population of over 1000 million, is estimated to account for nearly 15- 25 per cent of the global tuberculosis burden. Tuberculosis (TB) continues to be a major health problem in India because of its high mortality and morbidity. The National Tuberculosis Control Programmes (NTP) was implemented in 1962. However, when reviewed in 1992, after three decades of implementation, the NTP was shown to have made no epidemiological impact, mainly due to poor case finding and low treatment completion rates .Each year 1.8 million cases of new tuberculosis occurring in India, of which 0.8 million of pulmonary tuberculosis are new smear positive cases **(Baldeviano-Vidalon, *et al.* 2005).**

1.5 International status

Tuberculosis (TB) is second greatest killer after HIV/AIDS worldwide due to a single infectious agent. In 2012, 8.6 million people fell ill with TB and 1.3 million died from TB.

Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top three causes of death for women aged 15 to 44. In 2012, an estimated 530 000 children became ill with

TB and 74 000 HIV-negative children died of TB. Multi-drug resistant TB (MDR-TB) is present in virtually all countries surveyed. The estimated number of people falling ill with tuberculosis each year is declining, although very slowly, which means that the world is on track to achieve the Millennium Development Goal to reverse the spread of TB by 2015. **(WHOFactsheet2013, *http://www.who.int/mediacentre/factsheets/fs104/en/*).**

This is deplorable when one considers that various cost-effective tools that can cure tuberculosis have existed since the 1960s. The global community woke up to this disease in 1993 when the WHO declared TB as a global emergency.

TB occurs in every part of the world. TB has the dubious distinction of being the most persistent scourge of humankind worldwide statistics are staggering: in 2001, the WHO estimated that 1.86 billion persons were infected with tuberculosis. Each year, 8.74 million develop tuberculosis and nearly 2 million die. This means that someone somewhere contracts TB every four seconds and one of them dies every 10 seconds **(Baldeviano-Vidalon, *et al.* 2005).**

In 2012, the largest number of new TB cases occurred in Asia, accounting for 60% of new cases globally however 55 cases p, sub-Saharan Africa carried the greatest proportion of new cases per population with over 2er 100 000 population. In 2012, about 80% of reported TB cases occurred in 22 countries. Some countries are experiencing a major decline in cases, while cases are dropping very slowly in others. Brazil and China for example, are among the 22 countries that showed a sustained decline in TB cases over the past 20 years. In the last decade, the TB prevalence in Cambodia fell by almost 45% **(WHO Fact sheet 2013, *http://www.who.int/mediacentre/factsheets/fs104/en/*).**

1.6 Interdisciplinary relevance

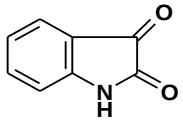
This research project having the correlation with other pharmaceutical sciences/applied sciences subject like; pharmacology, microbiology and applied chemistry. In the pharmacology we will perform the animal studies and its toxicity studies and under microbiology we will

perform the anti-tubercular evaluation. In the chemistry section we will perform synthesis of targeted compounds.

1.7 Review of Research and Development in the Subject:

Since more of the main antitubercular drugs such as isoniazid, rifampicin, pyrazinamide and ethambutol have been found resistant to the large proportion of TB patients. Bacteria's are showing tolerances for more of antibiotics. Because of these reasons treatment is getting longer in order to kill persistent mycobacteria which leads to heptotoxicity, nephrotoxicity etc. So there is erge of some new potential drug candidates which can eliminate these treatments associated problems and can work potentially over first line drug resistant.

In light of above in the present study we will perform synthesis and evaluation of the anti-tubercular potential of novel isatin derivatives. Isatin (1H-indole-2,3-dione) was first obtained by Erdman and Laurent in 1841 as a product from the oxidation of indigo by nitric and chromic acids. In nature, isatin is found in plants of the genus Isatis, in Calanthe discolor and in Couroupitaguianensis, and 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. Substituted isatins are also found in plants, for example the melosatin alkaloids (methoxy phenylpentylisatins) obtained from the Caribbean tumorigenic plant Melochia tomentosa as well as from fungi: 6-(3'-methylbuten-2'-yl)isatin was isolated from Streptomyces albus and 5-(3'-methylbuten-2'-yl)isatin from Chaetomium globosum**(da Silva, *et al.* 2001).**



Isatin

Isatin, possessing an indole nucleus having both the keto and lactam moiety has aroused tremendous curiosity due to its diverse biological and pharmacological studies. The biological importance of isatin is summarized in Table 1.

Table. 1: The biological importance of isatin

S.No.	Isatin derivative	Activity reported	References
1	Balofloxacin ethylene	Antitubercular	Feng *et al.*
2	Dispiro-oxindolylpyrrolothiazoles	Antitubercular	Maheswari*et al.*
3	Thiolactone–isatin hybrids	Antitubercular	Hans *et al.*
4	Schiff bases by indoline-2,3-dione	Antitubercular	Fadl*et al.*
5	1-methyl-3,5-bis[(E)-arylmethylidene]-tetrahydro-4(1*H*)-pyridinones	Antitubercular	Karthikeyan *et al.*
6	Series of 2-(arylmethylene)-2,3-dihydro-1*H*-inden-1-ones	Antitubercular	Prasanna *et al.*
7	Dispiropyrrolidines derivatives	Antitubercular	Kumar *et al*.
8	5-substituted-3-[{5-(6-methyl-2-oxo/thioxo-4-phenyl-1,2,3,4 tetrahydro pyrimidin-5-yl)-1,3,4-thiadiazol-2-yl}imino]-1,3-dihydro-2*H*-indol-2-one	Antitubercular	Akhaja*et al*.
9	7-substituted ciprofloxacin derivatives	Antitubercular	Sriram *et al.*
10	7-substituted gatifloxacin derivatives	Antitubercular	Sriram *et al.*
11	Series of lamivudine prodrugs involving N^4- substitution with isatin derivatives	antiretroviral activities	Sriram *et al.*
12	Isatin b-thiosemicarbazone derivatives	anti-HIV activity	Bal *et al.*
13	Dispirooxindolopyrrolizidine derivatives	Antimicrobial	Periyasami*et al*
14	2'-(indol-3-yl)-2-oxospiro (indoline-3,4'-pyran) derivatives	Antimicrobial	Nandakumar *et al.*
15	Aminopyrimidiniminoisatin lead compound	*HIV, HCV, Mycobacterium tuberculosis*	Sriram *et al.*

16	5-substituted-1-(arylmethyl/alkylmethyl)-1*H*-indole-2,3-dione-3-(*N*-hydroxy/methoxy thiosemicarbazone) analogues	anti-HIVactivity and anti-tubercular activity	Banerjee *et al.*
17	4-[(1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)amino]-*N*-(4,6-dimethyl-2-pyrimidin-2-yl)benzenesulphonamide and derivatives	Antiviral against influenza *A (H1N1, H3N2, and H5N1*) and *B* viruses	Selvam *et al.*
18	*N*-benzylated isatin oximes	mitogen-activated kinase, JNK3	Cao *et al*.
19	4-aminoquinoline derivatives	malaria parasite *Plasmodium falciparum*	Chiyanzu*et al*.
20	3,3-diindolyl oxyindoles	Anticancer	Kamal *et al*.

Significance of study:

At present a number of frontline drugs are available for the treatment of tubercular infection but most of them are becoming ineffective due to resistance acquired by the bacteria. This initiated a real need for the discovery of new chemical entitites [NCE] endowed with antitubercular activity. The proposed work will provide new chemical entities for the treatment of *Mycobacterium tuberculosis* infections.

ANNEXURE-1

2. Objective of the Project

2.1 Synthesis of novel Isatin derivatives will be done.

2.2 Characterization of the synthesized derivatives of Isatin will be done.

2.3 All the synthesized and characterized Isatin derivatives will be tested for Anti-tubercular activity.

3. Methodology

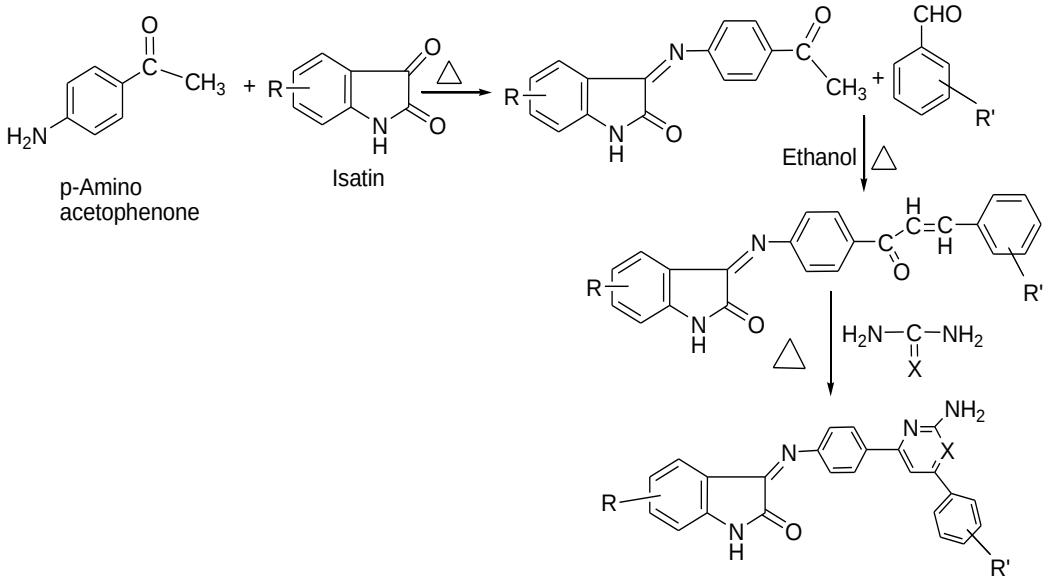
3.1 Synthesis of Isatin derivatives

The Isatin derivatives will be synthesized based on the following synthetic scheme. Attempts will be made to modify the Isatin molecule using following synthetic steps

(i) In the first step, modification of 3rd position of the Isatin will be done by treating it with p-amino acetophenone.

(ii) In the second step, intermediate chalcone derivatives of Isatin will be synthesized by the reaction of modified Isatin (first step) and substituted aldehydes.

(iii) In the last step, novel Isatin derivatives will be synthesized by reacting chalcone derivatives of Isatin (second step) with compounds such as guanidine.



3.2 Characterization of synthesized compounds

The synthesized compounds were characterized by determination of physicochemical characteristics like melting point, boiling point and Rf value. Further, these were characterized spectroscopically by recording IR, ^{1}HNMR, ^{13}C NMR and MASS

spectra.Reaction steps forward was checked by thin layer chromatography (TLC) using ethyl acetate as mobile phase. Te scheme was drawn via ChemDraw 8.03. Melting point of synthesized derivatives was determined by open capillary tube technique. An infrared (IR) spectrum was recorded on Bruker 12060280, Software: OPUS 7.2.139.1294 spectrometer using ATR and results were in cm−1. Bruker Avance III 600 NMR spectrometer was utilized for 1 H/13C-NMR (DMSO-d6, δ ppm). WatersMicromassQ-ToF Micro instrument was used for mass spectra. Elemental analysis was performed on PerkinElmer 2400 C, H and N analyzer and all synthesized compounds gave C, H and N analysis within±0.4% of the theoretical results.

3.3 Evaluation of anti-tubercular activity

Alamar blue susceptibility test (MABA): Antimicrobial susceptibility testing will be performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, Conn.) in order to minimize background fluorescence. Outer perimeter wells will be filled with sterile water to prevent dehydration in experimental wells. **(Collins and Franzblau 1997).**The M. tuberculosis (RCMB 010126) strain was used for anti tubercular activity and the reference drugs Isoniazide and pyrazinamide were used. There the anti MTB activity was of the derivatives was done based on the Alamar blue assay (MABA). Assay was done in black, clear-bottomed, 96 well microplates. The serial dilutions of the each derivative were made for the testing. The plates containing MTB and test compounds were incubated at 37 °C. The compounds were tested in triplicates. The IC_{50} of the derivatives was calculated.

4. Results

4.1 Compound library enumeration and Drug likeness prediction

The compound library developed was screened for their druglikeness using the online available tool DataWarriar. The compounds were filtered through Lipinski rule of five which decides the physicochemical properties of the compounds needed for being a druglike compound.

Table.1: Physicochemical parameters of compounds as perLipisnki rule of five.

S.No	HB A	HBD	cLogP	MW	PSA	LRV	M	C	RE
3a	4	1	4.028	431.288	58.53	0	False	False	False
3b	5	1	3.958	461.314	67.76	0	False	False	False
3c	5	1	3.958	461.314	67.76	0	False	False	**low**
3d	5	2	3.6823	447.287	78.76	0	False	False	False
3e	4	1	4.634	465.733	58.53	0	False	False	False
3f	4	1	4.7532	510.184	58.53	**1**	False	False	False
3g	7	1	3.1064	476.285	104.35	0	False	False	False
3h	7	1	3.1064	476.285	104.35	0	False	False	False
3i	4	1	5.24	500.178	58.53	**2**	False	False	False
3j	5	1	3.9244	474.357	61.77	0	**High**	**High**	**low**
3k	4	1	4.634	465.733	58.53	0	False	False	False
3l	4	1	4.7532	510.184	58.53	**1**	False	False	False
3m	7	1	3.1064	476.285	104.35	0	False	False	False
3n	6	1	3.888	491.340	76.99	0	False	False	False
3o	6	2	3.61235	477.313	87.99	0	False	False	False

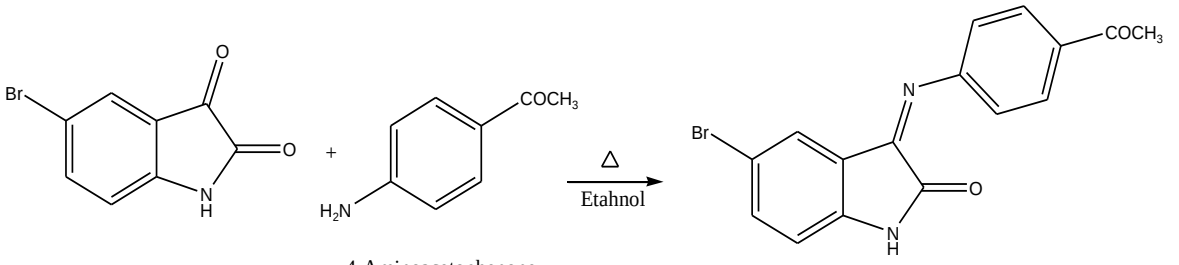
HBA: Hydrogen bond acceptor; **HBD:** Hydrogen bond donor; **MW:** Molecular weight; **PSA:** Polar surface area; **LRV:** Lipinski rule violations; **M:** Mutagenic; **C:** Carcinogenic; **RE:** Reproductive effects.

4.2 Synthesis of compounds and Anti tubercular activity

4.2.1 Synthesis of Schiff-Base of 5-Bromoisatin:

3-(4-Acetyl-phenylimino)-5-bromo-1, 3-dihydro-indol-2-one (a schiff base) was prepared by refluxing 5-bromoisatin with 4-aminoacetophenone for 1 h in ethanol using catalytic

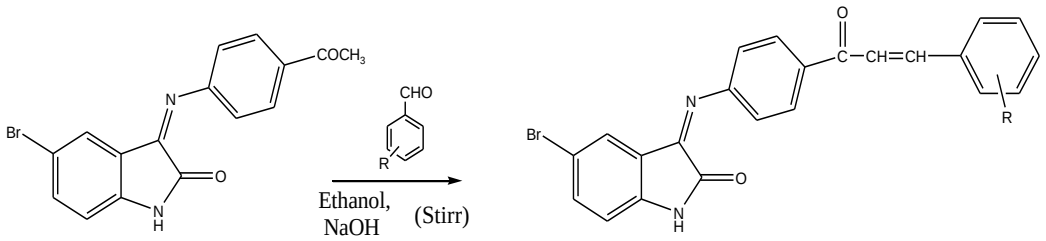
amount of acetic acid. The content was put undisturbed for 24h. The solid was filtered and recrystallised using ethanol.



5-Bromoisatin 4-Aminoacetophenone 3-(4-Acetyl-phenylimino)-5-bromo-1,3-dihydro-indol-2-one

4.2.2 Synthesis of chalcone derivatives of 5-Bromoisatin:

A mixture of **3-(4-acetylphenyl)-5-bromo-1, 3-dihydro-indol-2-one** (0.01mol) and substituted **aromatic aldehyde** (0.01mol), in ethanol, was taken in RBF. A solution of 10 ml of NaOH was added dropwise to the mixture. The mixture was stirred for 2-3h till it become thick. The solid was filtered and recrystallised with ethanol. The progress of reaction was monitored by TLC



3-(4-Acetyl-phenylimino)-5-bromo-1,3-dihydro-indol-2-one

5-Bromo-3-[4-(3-phenyl-acryloyl)-phenylimino]-1,3-dihydro-indol-2-one
Chalcone derivative
(3a-3o)

Table.2: The substituent (R) groups for the compound designing

Substituent (R)			
3a	H	**3i**	2,4-Cl
3b	2-OCH_3	**3j**	P-Dimethylamino
3c	4-OCH_3	**3k**	2-Cl
3d	4-OH	**3l**	2-Br
3e	4-Cl	**3m**	3-NO_2
3f	4-Br	**3n**	3,4-OCH_3
3g	2-NO_2	**3o**	4-OH, 3OCH_3
3h	4-NO_2		

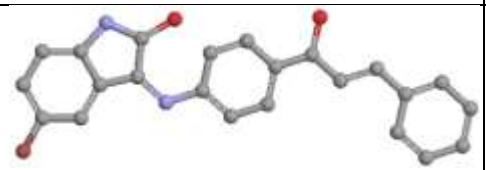
Table.3: Physicochemical properties of synthesized compounds from (3a-3o).

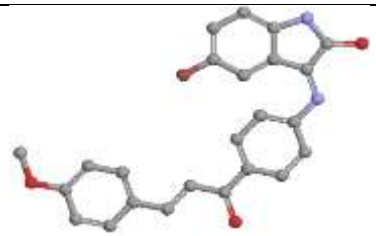
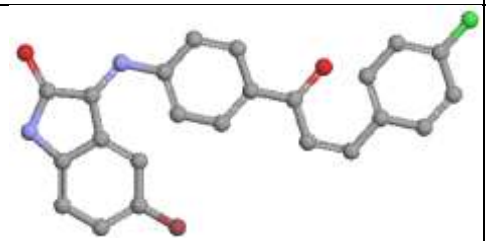
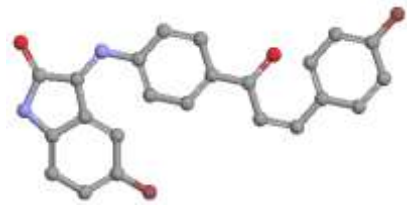
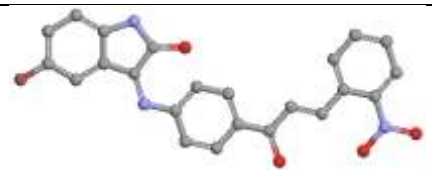
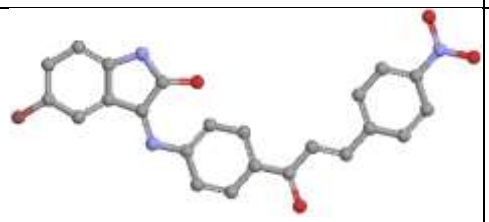
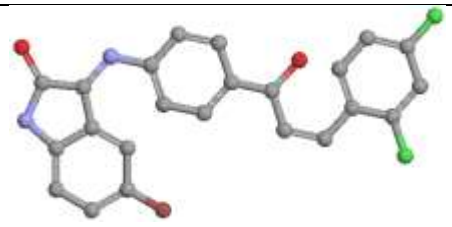
S.no	Compounds	MW	MP	Rf value	%Yield
1.	**3a**	431.288	190-193	0.68	70.0
2.	**3b**	461.314	212-214	0.71	68.0
3.	**3c**	461.314	206-209	0.76	65.0
4.	**3d**	447.287	196-199	0.65	71.0
5.	**3e**	465.733	230-233	0.69	76.0
6.	**3f**	510.184	224-226	0.73	71.0
7.	**3g**	476.285	228-230	0.77	64.0
8.	**3h**	476.285	226-228	0.75	69.0
9.	**3i**	500.178	218-221	0.65	61.0
10.	**3j**	474.357	202-205	0.78	74.0
11.	**3k**	465.733	226-229	0.67	70.0
12.	**3l**	510.184	228-230	0.72	75.0
13.	**3m**	476.285	222-224	0.74	68.0
14.	**3n**	491.340	237-239	0.60	78.0
15.	**3o**	477.313	234-236	0.64	80.0

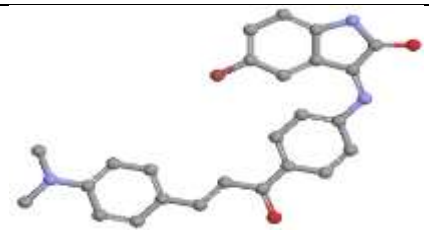
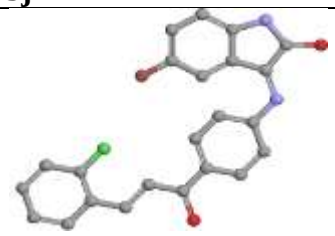
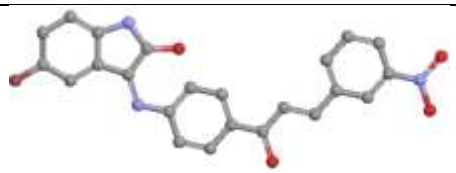
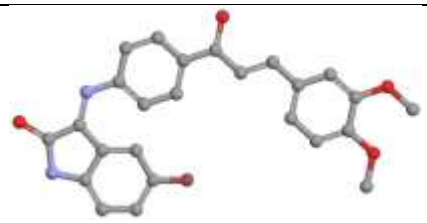
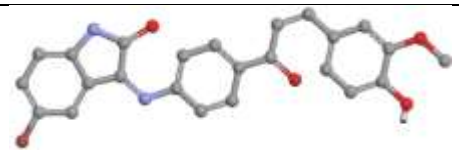
MW: Molecular weight; MP: Melting point.
Solvent front:- Chloroform: Benzene: Acetic acid

A series isatin derivative was synthesized. A significant number of derivatives showed potential anti-TB activities with IC_{50} in a range of 20.2 to 58.0 µM concentration. The IC50 was found very high than the references drugs Pyrazinamide and INH (IC50: 0.083µg/mL and 0.105µg/mL). The derivatives containing methoxy substuents showed the better inhibition (50% inhibition) of MTB growth below 30 µM.

Table.4: 50% inhibition of MTB parasite at incubation with the synthesized compounds.

S.No.	Structure	IUPAC Name	IC_{50} (µM)
1.	 **3a**	(3*Z*)-5-bromo-3-({4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one	32.1
2.	 **3b**	(3*Z*)-5-bromo-3-({4-[(2*E*)-3-(2-methoxyphenyl)prop-2-enoyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one	20.2

3.	 **3c**	(3*Z*)-5-bromo-3-({4-[(2*E*)-3-(4-methoxyphenyl)prop-2-enoyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one	22.1
4.	 **3d**	(3*Z*)-5-bromo-3-({4-[(2*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one	35.3
5.	 **3e**	(3*Z*)-5-bromo-3-({4-[(2*E*)-3-(4-chlorophenyl)prop-2-enoyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one	46.2
6.	 **3f**	(3*Z*)-5-bromo-3-({4-[(2*E*)-3-(4-bromophenyl)prop-2-enoyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one	58.0
7.	 **3g**	(3*Z*)-5-bromo-3-({4-[(2*E*)-3-(2-nitrophenyl)prop-2-enoyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one	28.7
8.	 **3h**	(3*Z*)-5-bromo-3-({4-[(2*E*)-3-(4-nitrophenyl)prop-2-enoyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one	30.2
9.	 **3i**	(3*Z*)-5-bromo-3-({4-[(2*E*)-3-(2,4-dichlorophenyl)prop-2-enoyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one	49.7

10.	 **3j**	CN(C)c1ccc(cc1)/C=C/C(=O)c1ccc(cc1)/N=C1/c2cc(Br)ccc2NC1=O	34.1
11.	 **3k**	(3*Z*)-5-bromo-3-({4-[(2*E*)-3-(2-chlorophenyl)prop-2-enoyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one	51.2
12.	 **3l**	(3*Z*)-5-bromo-3-({4-[(2*E*)-3-(4-bromophenyl)prop-2-enoyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one	28.6
13.	 **3m**	(3*Z*)-5-bromo-3-({4-[(2*E*)-3-(3-nitrophenyl)prop-2-enoyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one	36.7
14.	 **3n**	COc1ccc(cc1OC)/C=C/C(=O)c1ccc(cc1)/N=C1/c2cc(Br)ccc2NC1=O	32.6
15.	 **3o**	(3*Z*)-5-bromo-3-({4-[(2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one	25.3
16.	**Isoniazide**	Reference drug	0.105 µg/mL
17.	**Pyrazinamide**	Reference drug	0.83 µg/mL

4.2.3 Synthesis of Pyrimidine derivative of 5-Bromoisatin (Series-III):

In a 250 ml RBF (0.01 mol) of chalcone derivatives (3a-3o) and 0.01 mol of guanidine hydrochloride were taken in ethanol. A solution of KOH was added to

mixture. The mixture was refluxed for 10 hr. The content was then cooled and poured in crushed ice. The product was then filtered, washed with water and recrystallised using ethanol [Padarthi, 2013; Kachroo, 2014].

The Pyrimidine derivatives showed little better antitubercular activity. There derivatives 32 and 33 were found to show the 50% inhibition of MTB bacteria at the concentration below the 20 micromolar of concentration. The other twelve compound derivatives also showed the IC_{50} below the 50µM except 36th compound. The activity of the derivatives was found very far higher than the reference drugs.

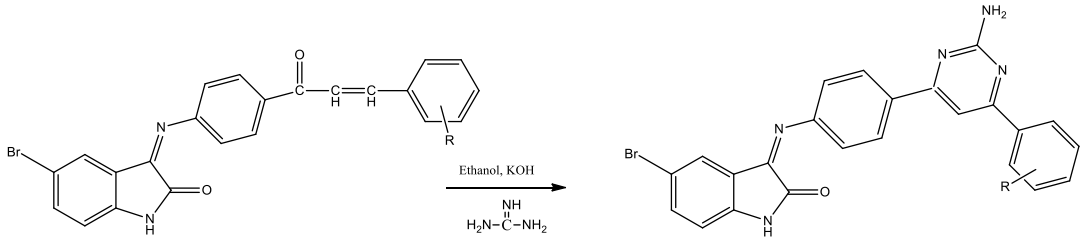


Table.5: The substituent (R) groups for the compound designing

Compounds	-R	Compounds	-R
31	-H	39	2,4-Cl
32	2-OCH_3	40	*p*-Dimethylamino
33	4-OCH_3	41	2-Cl
34	4-OH	42	2-Br
35	4-Cl	43	3-NO_2
36	4-Br	44	3,4-OCH_3
37	2-NO_2	45	4-OH,3-OCH_3
38	4-NO_2		

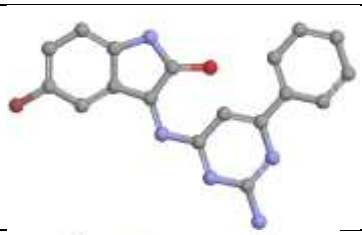
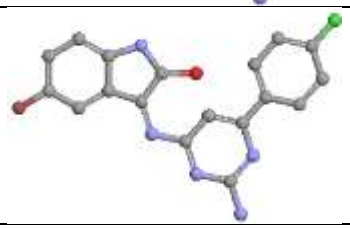
Table.6: Physicochemical properties of synthesized compounds (31-45).

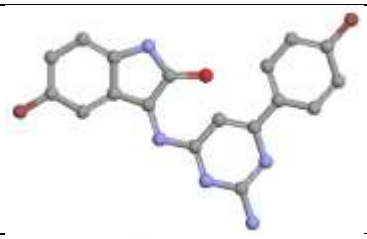
Compounds	Molecular Formula	Mol. Weight	Melting Point (^{o}c)	Rf value	% Yield
31	$C_{18}H_{12}BrN_5O$	394.22	190-193	0.68	70.0
32	$C_{19}H_{14}BrN_5O_2$	424.25	212-214	0.71	68.0
33	$C_{19}H_{14}BrN_5O_2$	424.25	206-209	0.76	65.0
34	$C_{18}H_{12}BrN_5O_2$	410.22	196-199	0.65	71.0
35	$C_{18}H_{11}BrClN_5O$	428.67	230-233	0.69	76.0

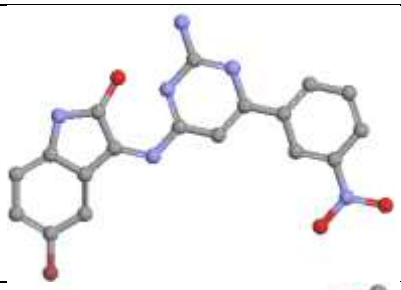
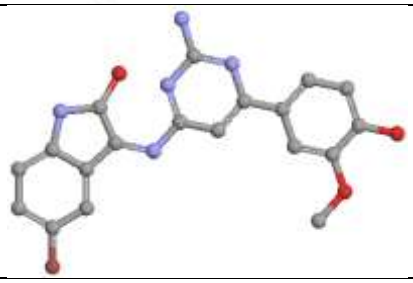
36	$C_{18}H_{11}Br_2N_5O$	473.12	224-226	0.73	71.0
37	$C_{18}H_{11}BrN_6O_3$	439.22	228-230	0.77	64.0
38	$C_{18}H_{11}BrN_6O_3$	439.22	226-228	0.75	69.0
39	$C_{18}H_{10}BrCl_2N_5O$	463.11	218-221	0.65	61.0
40	$C_{20}H_{17}BrN_6O$	437.27	202-205	0.78	74.0
41	$C_{18}H_{11}BrClN_5O$	428.67	226-229	0.67	70.0
42	$C_{18}H_{11}Br2N_5O$	473.12	228-230	0.72	75.0
43	$C_{18}H_{11}BrN_6O_4$	455.22	222-224	0.74	68.0
44	$C_{20}H_{16}BrN_5O_3$	454.28	237-239	0.60	78.0
45	$C_{19}H_{14}BrN_5O_3$	440.25	234-236	0.64	80.0

(Solvent front: chloroform: benzene: acetic acid)

Table.7: 50% inhibition of MTB parasite at incubation with the synthesized compounds.

S.No.	Structure	IUPAC Name	IC_{50} (μM)
1		(3*Z*)-3-[(2-amino-6-phenylpyrimidin-4-yl)imino]-5-bromo-1,3-dihydro-2*H*-indol-2-one	22.3
2		(3*Z*)-3-{[2-amino-6-(2-methoxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	12.4
3		(3*Z*)-3-{[2-amino-6-(4-methoxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	16.7
4		(3*Z*)-3-{[2-amino-6-(4-hydroxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	31.5
5		(3*Z*)-3-{[2-amino-6-(4-chlorophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	41.1

6		(3*Z*)-3-{[2-amino-6-(4-bromophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	51.6
7		(3*Z*)-3-{[2-amino-6-(2-nitrophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	20.9
8		(3*Z*)-3-{[2-amino-6-(4-nitrophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	20.9
9		(3*Z*)-3-{[2-amino-6-(2,4-dichlorophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	41.2
10		(3*Z*)-3-({2-amino-6-[4-(dimethylamino)phenyl]pyrimidin-4-yl}imino)-5-bromo-1,3-dihydro-2*H*-indol-2-one	32.4
11		(3*Z*)-3-{[2-amino-6-(2-chlorophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	46.1
12		(3*Z*)-3-{[2-amino-6-(2-bromophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	22.5

13		(3*Z*)-3-{[2-amino-6-(3-nitrophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	33.6
14		(3*Z*)-3-{[2-amino-6-(3,4-dimethoxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	24.7
15		(3*Z*)-3-{[2-amino-6-(4-hydroxy-3-methoxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	22.8

ANNEXURE-2

5. Summary

Since the main antitubercular drugs have developed resistant to the large proportion of TB patients. Bacteria's are showing tolerances for more of antibiotics. The long treatment due to resistance causing heptotoxicity, nephrotoxicity etc. So there the Isatinwas selected as template for modification and development of a compound librarybecause anti-tubercular potential of novel isatin derivatives like the first antitubercular potential of Isatin was confirmed by Erdman and Laurent in 1841. Isatin has medicinal potential to be used as drug template for identifying the novel drug candidates which can eliminate these treatments associated problems and can work potentially over first line drug resistant.

5.1 The synthesized derivatives were potentially characterized for their purity through the chromatography and spectroscopic techniques.

5.2 The confirmed derivatives were tested, the results showed like the few compounds from Bromoisatin derivatives found to show 50% MTB inhibition at concentration between 20µM to 30µM. The compounds with hydrocarbon attached substituents could show little good activity that may be due the little enhanced liphophilicity of the compounds which made the entry of the compounds easy into the MTB.

5.3 The Pyrimidine derivatives of Bromoisatin were showed good results than the Bromoisatin derivatives due the significant medicinal effect of attached Pyrimidine ring which may have enhance the liphophilicity and metabolic resistance as well. Due those two compounds 32 and 33 could kill the 50% of MTB even at concentration lower than 20µM.

5.4 The study led to the knowledge that the isatin has antitubercular value. The Isatin scaffold may be utilized as template for further modification and development of drug like candidate.

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Acknowledgement

We are thankful to University Grant Commission, for providing the UGC-MRP project(43-[501/2014(SR)] funding. We are highly thankful to Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak for allowing the use offacilities and resources for research work. We are also highly thankful to JALMA for providing *M. tuberculosis* (RCMB 010126) strain to carry out *In-vitro* experiments and result analysis.

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Synthesis, Characterization and Antitubercular Evaluation of Novel Isatin Derivatives

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Abstract – Tuberculosis is caused by a bacterium called Mycobacterium tuberculosis, attacks the lungs but may also attack other parts of the body such as the kidney, spine, and brain. Tuberculosis may also be linked to certain risk factors, including alcoholism, IV drug abuse, and homelessness. Infection with Tubercle bacillus (most often M. tuberculosis) is characterized by the formation of tubercles.

Since the main antitubercular drugs have developed resistant to the large proportion of TB patients. Bacteria's are showing tolerances for more of antibiotics. The long treatment due to resistance causes heptotoxicity, nephrotoxicity etc. So there the Isatin was selected as template for modification and development of a compound library because anti-tubercular potential of novel Isatin derivatives like the first antitubercular potential of Isatin was confirmed by Erdman and Laurent in 1841. Isatin has medicinal potential to be used as drug template for identifying the novel drug candidates which can eliminate these treatments associated problems and can work potentially over first line drug resistant. The synthesized derivatives were potentially characterized for their purity through the chromatography and spectroscopic techniques.

The confirmed derivatives were tested, the results showed like the few compounds from Bromoisatin derivatives found to show 50% MTB inhibition at concentration between 20µM to 30µM. The compounds with hydrocarbon attached substituent could show little good activity that may be due the little enhanced liphophilicity of the compounds which made the entry of the compounds easy into the MTB.

The Pyrimidine derivatives of Bromoisatin were showed good results than the Bromoisatin derivatives due the significant medicinal effect of attached Pyrimidine ring which may have enhance the liphophilicity and metabolic resistance as well. Due that two compounds 32 and 33 could kill the 50% of MTB even at concentration lower than 20µM. The study led to the knowledge that the isatin has antitubercular value. The Isatin scaffold may be utilized as template for further modification and development of drug like candidate.

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INTRODUCTION

Tuberculosis is caused by a bacterium called *Mycobacterium tuberculosis*. This bacterium typically attacks the lungs but may also attack other parts of the body such as the kidney, spine, and brain. Tuberculosis may also be linked to certain risk factors, including alcoholism, IV drug abuse, and homelessness. Infection with *Tubercle bacillus* (most often *M. tuberculosis*) is characterized by the formation of tubercles, hard nodules in the lungs that are the result of interaction between the bacteria and the host's immune system. The infected macrophages result in an inflammatory response (heat, swelling, dilated capillaries) which attracts more macrophages until the site of infection is completely surrounded by many of these compressed phagocytic cells. Inflammation triggers other cells within the host to essentially quarantine the area by depositing collagen fibers around the packed macrophages, forming an enclosed infection within the lung called a tubercle. The cells at the center of the tubercle may eventually die, producing either an area of necrosis or an actual cavity. Tuberculosis usually attacks the lungs (pulmonary tuberculosis) but it also affects the central nervous system, the lymphatic system, the circulatory system, the genitourinary system, the gastrointestinal system, bones, joints, and even the skin **(Wehenkel, *et al.* 2008).**

Resistance is growing for standard anti-tubercular drugs have been used frequently. Disease strains

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that are resistant to a single anti-TB drug have been documented in every country surveyed. Multidrug-resistant tuberculosis (MDR-TB) is a form of TB caused by bacteria that do not respond to, at least, isoniazid, ethambutol and rifampicin, the two most powerful, first-line (or standard) anti-TB drugs. About 450 000 people developed MDR-TB in the world in 2012. More than half of these cases were in India, China and the Russian Federation. It is estimated that about 9.6% of MDR-TB cases had XDR-TB. **(WHO Fact sheet 2019,** http://www.who.int/mediacentre/factsheets/fs104/en/) .

Disease caused by resistant bacteria fails to respond to conventional, first-line treatment. MDR-TB is being treated by using second-line drugs but there is no satisfactory results have been found. However second-line treatment options are limited and recommended medicines are not always available. The extensive chemotherapy required (up to two years of treatment) is more costly and can produce severe adverse drug reactions in patients. Isoniazid, rifampicin and ethambutol main first line antitubercular drugs have been found not effective for resistant mycobacterium tuberculosis individually or not in combination therapy. Isoniazid has been experimentally identified resistant towards tuberculosis. Isoniazid is also responsible for heptotoxicity at the cost of long term treatment. Rifampicin is also a first line drug that has been found not effective against mutated mycobacterium tuberculosis (MTB) in combination drug therapy. Rifampicin has become resistant to kill MTB pathogen.

Because INH is the most commonly used antitubercular drug, resistance to INH occurs more frequently among clinical isolates than resistance to any other agent **(Karakousis, 2009).** Mutations have been commonly detected in the *katG* gene in INH-resistant clinical isolates occurring in 50–80% of cases. Mutations in *katG* gene reduce or affect the ability of the catalase-peroxidase to activate the INH pro-drug. Commonly point mutations in *katG* are observed and a single point mutation which is responsible for substitution of threonine for serine at residue 315 (S315T) accounts for the majority of INH resistance among clinical isolates **(Marttila, *et al.* 1998; Abate, *et al.* 2001).** INH resistance has also been found to arise from mutations in *inhA* that can also result in reduced affinity of the enzyme for NADH without affecting enoyl reductase activity of NADH **(Basso, *et al.* 1998).** Mutations in *inhA* also have been found to cause resistance to the structurally related second-line drug ethionamide.

The rifamycins were first isolated in 1957 from *Amycolatopsis mediterranei* as part of an Italian antibiotic screening program **(Sensi, 1983).** While INH resistance alone is more common in *M. tuberculosis* than resistance to rifampin alone and more than 90% of rifampin-resistant isolates has been found also resistant to INH. Therefore, rifampin resistance isolates has been used as a substitute marker for MDR-TB. Resistance to rifampin in *M. tuberculosis* is caused most commonly as single point mutations in the *rpoB* gene, which encodes the RNA polymerase **(Telenti, *et al.* 1993).** Point mutations cluster in an 81-base pair “hot-spot” region between codons 507 and 533 of the *rpoB* gene, with mutations in codons 531 encodes Serine and codons 526 encodes Histidine predominatly in More than 90% of rifampin-resistant clinical isolates **(Ramaswamy and Musser 1998).**

Duration of treatment vital to achieve acceptable relapse rates has been reduced to six months from 9–12 months since the discovery of pyrazinamide (PZA) **(Steele and Des Prez 1988).** PZA resistance has been recognized primarily due to mutations in the *pncA* gene which encods PZase **(Scorpio and Zhang 1996).** Most of mutations found are due to point mutations, deletions, and insertions which have been reported in a 561-bp region of the open reading frame or in an 82-bp region of its putative promoter **(Scorpio, *et al.* 1997; Jureen, *et al.* 2008).** A small percentage of isolates with high-level PZA resistance contain no mutations in *pncA* or its promoter that suggests about alternative mechanisms of resistance such as deficient uptake and enhanced efflux and altered *pncA* regulation **(Raynaud, *et al.* 1999).**

Resistance to ethambutol in *M. tuberculosis* is commonly found to be caused due to point mutations in the *embCAB* operon **(Belanger, *et al.* 1996).** The *EmbA* and *EmbB* proteins are found to involve in the formation of the proper terminal hexaarabinofuranoside motif during arabinogalactan synthesis **(Escuyer, *et al.* 2001)** where *EmbC* is found to involve in lipoarabinomannan synthesis **(Zhang, *et al.* 2003).** *EmbB* is considered to be the main target of ethambutol because more percentage of EMB-resistant clinical isolates found to have mutations in the *embB* gene **(Sreevatsan, *et al.* 1997; Telenti, *et al.* 1997; Ramaswamy, *et al.* 2000).**

Antibiotic tolerance is the capability of non-replicating bacteria to get resistant againt particular antibiotic i.e the bacteria resist killing by cell wall-active antibiotics **(Tomasz, *et al.*1970).** This occurrence of tolerance is distinct from drug resistance as that can be intrinsic or acquired tolerance since it is not attributable to genetic mutations, and the organisms regain susceptibility to these antibiotics once the stress conditions have been removed and bacterial growth resumes. The prolonged treatment with antibiotics required to eradicate TB is supposed to alter the physiological state of persistent bacilli which have developed tolerance to standard anti-tuberculosis drugs, particularly to isoniazid, which inhibits mycolic acid synthesis **(Karakousis, *et al.* 2008; Adhikari, 2010).**

METHODOLOGY

Synthesis of Isatin derivatives

The chalcone and pyrimidine derivatives of Isatin as Series-I and Series-II were synthesized and characterized by recording IR, ^{1}HNMR, ^{13}C NMR and MASS spectra as published in our previous research paper **(Ramesh Kumar and Mahesh Kumar 2019; Ramesh Kumar and Mahesh Kumar 2018)** .

Anti-tubercular activity

All the synthesized were tested *in-vitro* for anti-tubercular activity. Alamar blue susceptibility test (MABA): Antimicrobial susceptibility testing will be performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, Conn.) in order to minimize background fluorescence. Outer perimeter wells will be filled with sterile water to prevent dehydration in experimental wells. **(Collins and Franzblau 1997).** The M. tuberculosis (RCMB 010126) strain was used for anti-tubercular activity and the reference drugs Isoniazide and pyrazinamide were used. There the anti MTB activity was of the derivatives was done based on the Alamar blue assay (MABA). Assay was done in black, clear-bottomed, 96 well microplates. The serial dilutions of the each derivative were made for the testing. The plates containing MTB and test compounds were incubated at 37 °C. The compounds were tested in triplicates. The IC_{50} of the derivatives was calculated.

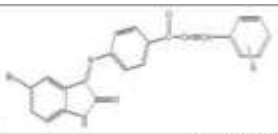
RESULTS

Compound library enumeration and Druglikeness prediction

The compound library developed was screened for their druglikeness using the online available tool DataWarriar. The compounds were filtered through Lipinski rule of five which decides the physicochemical properties of the compounds needed for being a druglike compound. The compounds were found to possess druglike properties except comp. 3j P-Dimethylamino substituted analog which showed high risk of carcinogenicity and mutagenicity. The comp. 3c, 4-OCH_3 substituted analog and 3j also showed little probability of reproductive effects as shown in Table (1 & 2).

Chalcone derivatives of 5-Bromoisatin

Table.1: The substituent (R) groups for the compound designing (Series-I)



Substituent's (R)			
3a	H	**3i**	2,4-Cl
3b	2-OCH_3	**3j**	P-Dimethylamino
3c	4-OCH_3	**3k**	2-Cl
3d	4-OH	**3l**	2-Br
3e	4-Cl	**3m**	3-NO_2
3f	4-Br	**3n**	3,4-OCH_3
3g	2-NO_2	**3o**	4-OH, 3OCH_3
3h	4-NO_2		

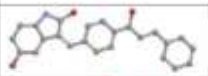
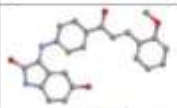
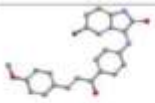
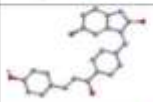
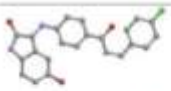
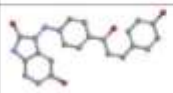
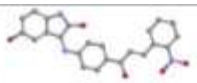
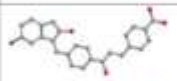
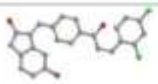
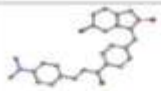
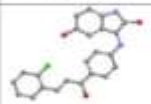
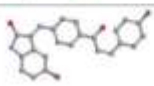
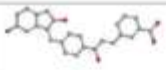
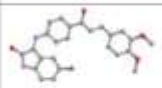
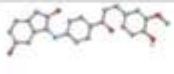
Table.2: Physicochemical parameters of compounds as per Lipisnki rule of five.

Comp.Id	HB A	HBD	cLogP	MW	PSA	LRV	M	C	RE
3a	4	1	4.028	431.288	58.53	0	False	False	False
3b	5	1	3.958	461.314	67.76	0	False	False	False
3c	5	1	3.958	461.314	67.76	0	False	False	**low**
3d	5	2	3.6823	447.287	78.76	0	False	False	False
3e	4	1	4.634	465.733	58.53	0	False	False	False
3f	4	1	4.7532	510.184	58.53	**1**	False	False	False
3g	7	1	3.1064	476.285	104.35	0	False	False	False
3h	7	1	3.1064	476.285	104.35	0	False	False	False
3i	4	1	5.24	500.178	58.53	**2**	False	False	False
3j	**5**	**1**	**3.9244**	**474.357**	**61.77**	**0**	**High**	**High**	**low**
3k	4	1	4.634	465.733	58.53	0	False	False	False
3l	4	1	4.7532	510.184	58.53	**1**	False	False	False
3m	7	1	3.1064	476.285	104.35	0	False	False	False
3n	6	1	3.888	491.340	76.99	0	False	False	False
3o	6	2	3.61235	477.313	87.99	0	False	False	False

HBA: Hydrogen bond acceptor; **HBD:** Hydrogen bond donor; **MW:** Molecular weight; **PSA:** Polar surface area; **LRV:** Lipinski rule violations; **M:** Mutagenic; **C:** Carcinogenic; **RE:** Reproductive effects.

A series Isatin derivative was synthesized. A significant number of derivatives showed potential anti-TB activities with IC_{50} in a range of 20.2 to 58.0 µM concentration. The IC50 was found very high than the references drugs Pyrazinamide and INH (IC50: 0.083µg/mL and 0.105µg/mL). The derivatives containing methoxy substituent showed the better inhibition (50% inhibition) of MTB growth below 30 µM.

Table.4: 50% inhibition of MTB parasite at incubation with the synthesized compounds.

S.No.	Comp. Id	Structure	IUPAC Name	IC_{50} (µM)
1.	3a		(3Z)-5-bromo-3-{[4-[(2E)-3-phenylprop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	32.1
2.	3b		(3Z)-5-bromo-3-{[4-[(2E)-3-(2-methoxyphenyl)prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	20.2
3.	3c		(3Z)-5-bromo-3-{[4-[(2E)-3-(4-methoxyphenyl)prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	22.1
4.	3d		(3Z)-5-bromo-3-{[4-[(2E)-3-(4-hydroxyphenyl)prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	35.3
5.	3e		(3Z)-5-bromo-3-{[4-[(2E)-3-(4-chlorophenyl)prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	46.2
6.	3f		(3Z)-5-bromo-3-{[4-[(2E)-3-(4-bromophenyl)prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	58.0
7.	3g		(3Z)-5-bromo-3-{[4-[(2E)-3-(2-nitrophenyl)prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	28.7
8.	3h		(3Z)-5-bromo-3-{[4-[(2E)-3-(4-nitrophenyl)prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	30.2
9.	3i		(3Z)-5-bromo-3-{[4-[(2E)-3-(2,4-dichlorophenyl]prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	49.7
10.	3j		CN(C)c1ccc(cc1)/C=C/C(=O)c1ccc(cc1)/N=C1/c2cc(Br)ccc2NC1=O	34.1
11.	3k		(3Z)-5-bromo-3-{[4-[(2E)-3-(2-chlorophenyl]prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	51.2
12.	3l		(3Z)-5-bromo-3-{[4-[(2E)-3-(4-bromophenyl]prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	28.6
13.	3m		(3Z)-5-bromo-3-{[4-[(2E)-3-(3-nitrophenyl]prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	36.7
14.	3n		COc1ccc(cc1OC)/C=C/C(=O)c1ccc(cc1)/N=C1/c2cc(Br)ccc2NC1=O	32.6
15.	3o		(3Z)-5-bromo-3-{[4-[(2E)-3-(4-hydroxy-3-methoxyphenyl]prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	25.3
16.		Isoniazide	Reference drug	0.105 µg/mL
17.		Pyrazinamide	Reference drug	0.83 µg/mL

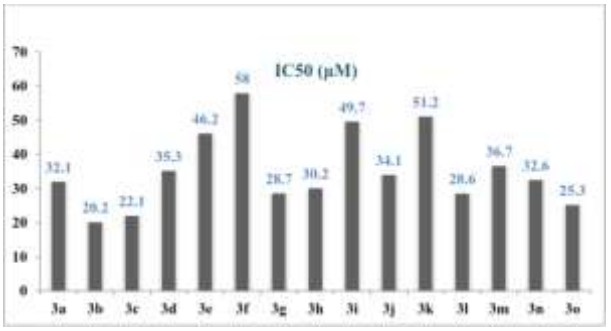
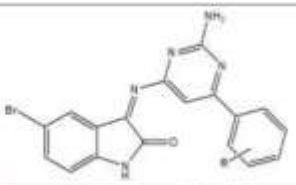


Figure.1: IC_{50} of chalcone derivatives of 5-Bromoisatin.

Activity of Pyrimidine derivative of 5-Bromoisatin

The Pyrimidine derivatives showed little better antitubercular activity. There derivatives 32 and 33 were found to show the 50% inhibition of MTB bacteria at the concentration below the 20 micromolar of concentration. The other twelve compound derivatives also showed the IC_{50} below the 50µM except 36th compound. The activity of the derivatives was found very far higher than the reference drugs.

Table.5: The substituent (R) groups for the compound designing



Substituent (R)

Compounds	-R	Compounds	-R
31	-H	39	2,4-Cl
32	2-OCH_3	40	*p*-Dimethylamino
33	4-OCH_3	41	2-Cl
34	4-OH	42	2-Br
35	4-Cl	43	3-NO_2
36	4-Br	44	3,4-OCH_3
37	2-NO_2	45	4-OH,3-OCH_3
38	4-NO_2		

Table.6: Physicochemical parameters of compounds as per Lipisnki rule of five.

Comp.Id	HB A	HBD	cLogP	MW	PSA	LRV	M	C	RE
3a	4	1	4.028	431.288	58.53	0	False	False	False
3b	5	1	3.958	461.314	67.76	0	False	False	False
3c	5	1	3.958	461.314	67.76	0	False	False	**low**
3d	5	2	3.6823	447.287	78.76	0	False	False	False
3e	4	1	4.634	465.733	58.53	0	False	False	False
3f	4	1	4.7532	510.184	58.53	**1**	False	False	False
3g	7	1	3.1064	476.285	104.35	0	False	False	False
3h	7	1	3.1064	476.285	104.35	0	False	False	False
3i	4	1	5.24	500.178	58.53	**2**	False	False	False
3j	**5**	**1**	**3.9244**	**474.357**	**61.77**	**0**	**High**	**High**	**low**
3k	4	1	4.634	465.733	58.53	0	False	False	False
3l	4	1	4.7532	510.184	58.53	**1**	False	False	False
3m	7	1	3.1064	476.285	104.35	0	False	False	False
3n	6	1	3.888	491.340	76.99	0	False	False	False
3o	6	2	3.61235	477.313	87.99	0	False	False	False

HBA: Hydrogen bond acceptor; **HBD:** Hydrogen bond donor; **MW:** Molecular weight; **PSA:** Polar surface area; **LRV:** Lipinski rule violations; **M:**

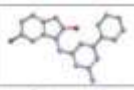
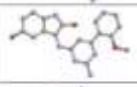
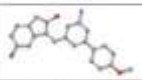
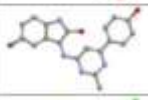
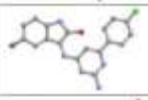
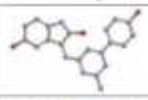
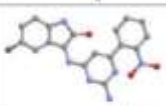
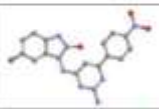
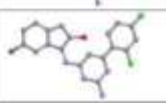
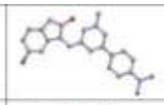
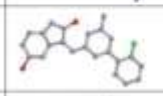
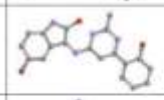
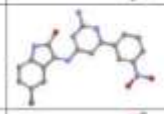
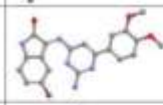
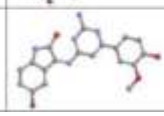
Mutagenic; **C:** Carcinogenic; **RE:** Reproductive effects.

Table.7: Physicochemical properties of synthesized compounds (31-45).

Compounds	Molecular Formula	Mol. Weight	Melting Point (°c)	Rf value	% Yield
31	$C_{10}H_{12}BrN_5O$	394.22	190-193	0.68	70.0
32	$C_{19}H_{14}BrN_5O_2$	424.25	212-214	0.71	68.0
33	$C_{19}H_{14}BrN_5O_2$	424.25	206-209	0.76	65.0
34	$C_{18}H_{12}BrN_5O_2$	410.22	196-199	0.65	71.0
35	$C_{18}H_{11}BrClN_5O$	428.67	230-233	0.69	76.0
36	$C_{10}H_{11}Br_2N_5O$	473.12	224-226	0.73	71.0
37	$C_{18}H_{11}BrN_6O_3$	439.22	228-230	0.77	64.0
38	$C_{18}H_{11}BrN_6O_3$	439.22	226-228	0.75	69.0
39	$C_{18}H_{10}BrCl_2N_5O$	463.11	218-221	0.65	61.0
40	$C_{20}H_{17}BrN_6O$	437.27	202-205	0.78	74.0
41	$C_{18}H_{11}BrClN_5O$	428.67	226-229	0.67	70.0
42	$C_{18}H_{11}Br2N_5O$	473.12	228-230	0.72	75.0
43	$C_{18}H_{11}BrN_6O_4$	455.22	222-224	0.74	68.0
44	$C_{20}H_{16}BrN_5O_3$	454.28	237-239	0.60	78.0
45	$C_{19}H_{14}BrN_5O_3$	440.25	234-236	0.64	80.0

(Solvent front: chloroform: benzene: acetic acid)

Table.8: 50% inhibition of MTB parasite at incubation with the synthesized compounds.

S.No.	Structure	IUPAC Name	IC_{50} (µM)
31		(3Z)-3-{[2-amino-6-phenylpyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	22.3
32		(3Z)-3-{[2-amino-6-(2-methoxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	12.4
33		(3Z)-3-{[2-amino-6-(4-methoxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	16.7
34		(3Z)-3-{[2-amino-6-(4-hydroxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	31.5
35		(3Z)-3-{[2-amino-6-(4-chlorophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	41.1
36		(3Z)-3-{[2-amino-6-(4-bromophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	51.6
37		(3Z)-3-{[2-amino-6-(2-nitrophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	20.9
38		(3Z)-3-{[2-amino-6-(4-nitrophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	20.9
39		(3Z)-3-{[2-amino-6-(2,4-dichlorophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	41.2
40		(3Z)-3-{[2-amino-6-[4-(dimethylamino)phenyl]pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	32.4
41		(3Z)-3-{[2-amino-6-(2-chlorophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	46.1
42		(3Z)-3-{[2-amino-6-(2-bromophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	22.5
43		(3Z)-3-{[2-amino-6-(3-nitrophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	33.6
44		(3Z)-3-{[2-amino-6-(3,4-dimethoxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	24.7
45		(3Z)-3-{[2-amino-6-(4-hydroxy-3-methoxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	22.8

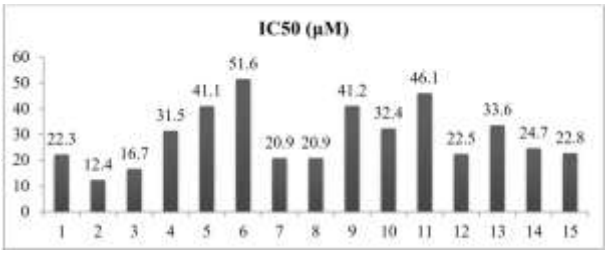


Figure.2: IC_{50} of Pyrimidine derivative of 5-Bromoisatin (Series-II).

CONCLUSION

The Isatin was selected as template for modification and development of a compound library because anti-tubercular potential of novel Isatin derivatives like the first antitubercular potential of Isatin was confirmed by Erdman and Laurent in 1841. Isatin has medicinal potential to be used as drug template for identifying the novel drug candidates which can eliminate these treatments associated problems and can work potentially over first line drug resistant. The synthesized derivatives were potentially characterized for their purity through the chromatography and spectroscopic techniques.

The confirmed derivatives were tested, the results showed like the few compounds from Bromoisatin derivatives found to show 50% MTB inhibition at concentration between 20µM to 30µM. The compounds with hydrocarbon attached substituents could show little good activity that may be due the little enhanced liphophilicity of the compounds which made the entry of the compounds easy into the MTB.

The Pyrimidine derivatives of Bromoisatin were showed good results than the Bromoisatin derivatives due the significant medicinal effect of attached Pyrimidine ring which may have enhance the liphophilicity and metabolic resistance as well.

Due that two compounds 32 and 33 could kill the 50% of MTB even at concentration lower than 20µM. The study led to the knowledge that the isatin has antitubercular value. The Isatin scaffold may be utilized as template for further modification and development of drug like candidate.

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