Government of Maharashtra's
Ismail Yusuf College of Arts, Science and Commerce
Jogeshwari (East), Mumbai 400 060

Tel No. (Office) 022-28352881 **Tele-Fax (Principal) 022-28202**

No.IYC/2019-2020/ Date: 30-09-2019

To,
The Registrar,
University Of Mumbai
Fort, Mumbai -400032.

Subject: Submission of MINOR RESEARCH PROJECT.

Respected Sir,

This is with reference to MINOR RESEARCH PROJECT submitted to University of Mumbai online by teachers of our College. Kindly received the hard copy of the same.

Thanking You.

Yours Sincerely

PRINCIPAL
PRINCIPAL
Government of Maharashtra's
Ismail Yusuf College of
Arts, Science & Commerce.
Jogeshwari (East), Mumbai -400 060.

मुंबई विद्यापीठ
सामान्य पोच
आयएस/आयसीडी/ईसीडी/
7/10/19
आवक विभाग

ISMAIL YUSUF COLLEGE ★ MUMBAI ★

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ISMAIL YUSUF COLLEGE ★ MUMBAI ★

	project NO	Amt
Dr. Amit Yadaorao Saraf	713	28,000/-
Dr. Thorat Bapu Rangonath	631	28,000/-
Nitin Devram shelake	716	24500/-
Rayshree N. Vyas	220	18900/-
Dr. Eknath. Shripali phutane	26	17500/-
		1,16,900

Dr. Thorat Bapu Rangaiah.

University of Mumbai

Academic Planning and Development Section
No. APD/ICD/2019-20/762
17th March, 2020

Sub : Minor Research Grant Project 2019-20

Sir/Madam,

I am directed to inform you that the said proposal has been considered by the University and the research grant as quoted above is sanctioned to the researcher.

The sanctioned amount will be disbursed in two installments. The first installment of 40% of the sanctioned amount will be disbursed within the month of March. The remaining 60% amount will be disbursed up to 31st December, 2020.

The researcher is expected to spend 60% amount initially from his/her own resources to carry out the work.

Further, I am to inform you that the researcher will have to utilize the 40% sanctioned amount on or before 31st March, 2020 and submit original bills/vouchers of the expenditure along with Utilization Certificate duly certified by the Principal/Director/Head/Institute/University Department/College to the Accounts Section of the University.

Please note that 60% balance amount, out of sanctioned grant will be released after Poster Presentation & final approval of the committee. Therefore you need to submit of utilization certificate after presentation of your research including bills/vouchers/receipts in original through University Account Section.

The report of the research work carried out by the concerned researcher will have to be submitted to the University on or before 31st December, 2020.

The Principal/Head of the Institute are requested to inform the researcher accordingly and arrange to forward his/her undertaking immediately to enable this office to release first installment of the research grant.

Yours faithfully,

Deepak V. More
Assistant Registrar
(APD Section)

ISMAIL YUSUF COLLEGE ★ MUMBAI ★

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616	Dr. Sharad Pandit Panchgalle	Khalapur Taluka Shikshan Prasarak Mandal's Khopoli Municipal Council College	50000
617	Dr. Sachin Vasant Bangale.	Gopinath Mahadeo Vedak Pratishthan GVM College of Science	40000
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619	Dr.SURABHI	\"Mahatma Education Societyâ€™s Pillai College of Engineering \r\n Dr. K. M. Vasudevan Pillai Campus Sector 16 New Panvel Navi Mumbai- 410206 .\"	40000
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640	Kartiki .Anilkumar.Bhave	The Bombay Salesian Society's Don Bosco Institute of Technology	55000

ISMAIL YUSUF COLLEGE MUMBAI

FORMAT FOR SUBMISSION OF REPORT
MINOR RESEARCH PROJECT

1. Title of project	: **Synthesis and Anti-TB properties study of substituted Acetohydrazones**
2. Area of Research	: Applied Chemistry
3. Name of the faculty	: Faculty of Science
4. Name of Subject	: Chemistry (Organic Chemistry)
5. Principal Investigator	
i. Name	: **Dr. Thorat Bapu Ranganath**
ii. Sex (M/F)	: Male
iii. Date of Birth	: 06.09.1978
iv. Qualification	: M. Sc. SET, NET, Ph. D.
v. Designation	: Assistant Professor
vi. Address	
a. Office	: Department of Chemistry, Government of Maharashtra, Ismail Yusuf Arts, Science and Commerce College, Jogeshwari (East), Mumbai 400060.
b. Residence	: A-18/3, Government Colony, Kherwadi, Bandra (East), Mumbai 400051.
Email/Phone	: iycbrthorat@gmail.com
6. Name of the College/Institute/Department where the proposal will be executed	: Government of Maharashtra, Ismail Yusuf Arts, Science and Commerce College
7. Full Address of the College/Institute/Department	: Jogeshwari (East), Mumbai 400060.

Project Title: Synthesis and Anti-TB properties study of substituted Acetohydrazones

Introduction:

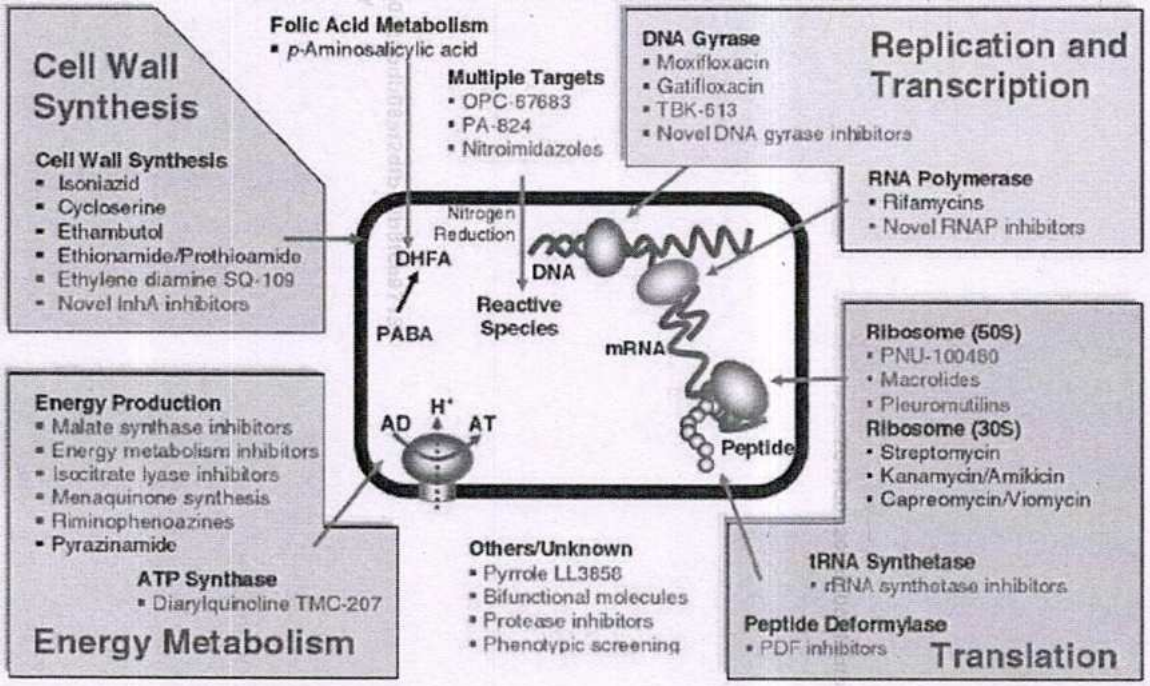
Tuberculosis (TB) is a lung infection caused mainly by Mycobacterium tuberculosis (M. tuberculosis [MTB]). It is considered to be one of the most contagious and deadly diseases and is a major threat for public health. The overviews of the current status of TB drug development with a focus on recent strategies are not directed toward "genetic" based efforts. Such genetic strategies have recently dominated the field of TB and antibiotic drug discovery with little effect, arguably, and now the identification of new chemical entities is exploiting other strategies. These programs include phenotypic screening, repurposing of existing antimicrobials and drugs for noninfection indications, and the coadministration of positive regulators of prodrug activation. The Miniperspective will complement other detailed review articles published in recent years that discuss the mechanisms of action of existing drug classes [01,02] and strategies [03,04] to elucidate the targets of newly discovered chemical entities which is required for their downstream development and regulatory approval.

Antibiotics are most effective against actively growing M. tuberculosis, [05] as these persistent organisms exhibit a phenotypic drug resistance; i.e., their resistance is not associated with genetic changes but with their extant metabolic state. The structures of the developing tuberculosis lesions may effectively define the metabolic status of their bacterial inhabitants, and it has been speculated that at least four significant subpopulations of bacteria exist for which different drugs could be efficacious. These might include active growers that may be killed by isoniazid (INH), those with sporadic metabolic bursts that could be killed by rifampicin (RIF), a population with low metabolic activity that is considered likely to experience acidic surroundings and hypoxia that may be susceptible to pyrazinamide (PZA), and finally dormant bacilli that are not killed by any current agents.[06,07] These complex phenomena are poorly understood and add a further barrier to the already formidable challenges associated with drug development and treatment of the disease.

Despite its superlative early bactericidal activity (EBA), INH is no more effective than other drugs after this period and RIF becomes the most significant bactericidal drug. Its activity against sporadically active M. tuberculosis is crucial for preventing relapses, and INH then serves to limit the emergence of RIF resistance. [08] Because of its apparent ability to kill a subset of bacteria not killed by the other drugs, supposed sporadically active organisms subject to an hypoxic and possibly acidic environment, [06,09,10] PZA represents an important component of combination therapy.

Drug	Chemical class	Cellular target
Isoniazid (INH)	Isonicotinic acid	Enoyl-ACP reductase, mycolic acid elongation
Rifampicin (RIF)	Rifamycin	DNA primed RNA polymerase
Pyrazinamide (PZA)	Pyrazine	Fatty acid biosynthesis/membrane depolarization/ ribosomal protein S1 (RpsA), protein translation and the ribosome sparing process of trans-translation
Ethambutol (EMB)	Ethylenediamine	Cell wall arabinan deposition

Fig 01: Mechanism of action of various drugs on bacteria of TB:



Current drugs: Black **Compounds in clinical: Blue** **Compounds in preclinical: Green** **Discovery projects: Red**

SOURCE: Ginsberg, 2008.

The current drug classes, both first and second line drugs were discovered between the 1940s and the 1970s. From then until last few years ago, there was little work on TB drug development. To find effective treatments for TB, MDR and XRD TB, it is important to established treatment regimens that are better tolerated, more efficacious, and more affordable. The root of the drug resistant problem is the complexity and length of drug sensitive regimens. To meets this need, it will be necessary to develop new drugs that will shorten and simplify treatment. They must be effective against those mycobacteria that persist now in the face of drugs to which they are genetically susceptible. Now it is need of today to develop the drugs with novel mechanisms of action that are equally effective against MDR and XDR and drug sensitive strains of TB. They must also be effective and have minimal drug-drug interactions for both HIV-positive and HIV-negative patients. Additionally, they should be able to deliver orally once a day or less frequently if possible, and obviously be low cost.

The organic chemist shows more interest towards the acid hydrazides and their derivatives because of their properties. These derivatives having wide applications as chemical preservers for plants, drugs, for manufacturing polymers, glues, etc., in industry, and for many other purposes [11]. These acid hydrazides and their derivatives were found to be useful synthons for various heterocyclic five, six or seven membered rings with one or more heteroatoms that were exhibited great biological, pharmacological and industrial applications such as antibacterial agents [12], pharmaceuticals [13], herbicides [14], antimalarial [15], antimycobacterial [16], anticonvulsant [17], antiinflammatory [18], antidepressant [19], anticancer [20], antimicrobial [21] activities and dyes [22]. The hydrazides and their derivatives were converted to heterocyclic compounds either by cyclisation or cyclo-addition with numerous reagents.

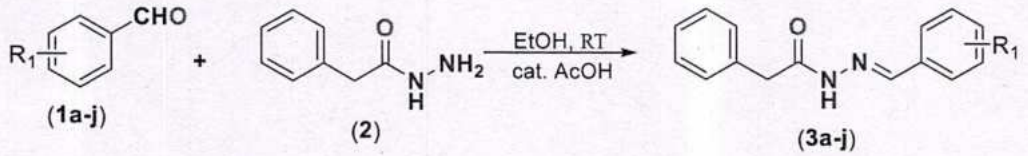
Working Scheme:

Materials and Methods:

All chemicals and solvents were purchase from commercial sources (LOBA chemicals) and purified if necessary before used. The Thin layered chromatography (TLC 0.25 mm E-Merck silica gel 60 F254 pre-coated plates) was used to monitor the reactions, which were visualized with UV light. Melting points were measured on standard melting point apparatus from Sunder industrial product, Mumbai and are uncorrected. ^{1}H NMR spectra were recorded on a 300 MHz instrument of Agilent Technology. The FT-IR spectral analysis was performed by using Perkin Elmer Tensor-II model. The absorption spectra of the compounds were recorded on a Perkin Elmer Lambda-25 double beam spectrophotometer.

Synthesis of hydrazones (3a-j): Equimolar quantity of phenyl acetohydrazine (**2**) and aldehyde (**1a-j**) in ethanol containing catalytic amount of acetic acid (pH should be 6 – 6.5) is stirred at room temperature in water bath till reaction get completed (monitored by TLC). Filtered the solid, wash with cold aqueous alcohol. Record m.p. and characterized by spectral analysis. The yield, reaction time and other physical properties of the product was recorded in following observation table.

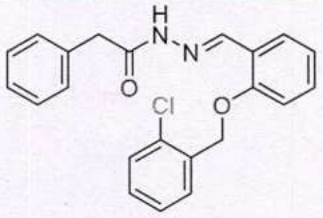
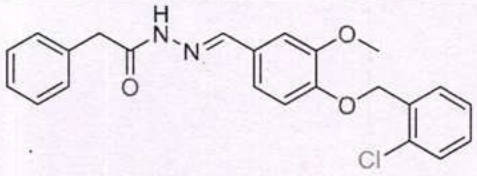
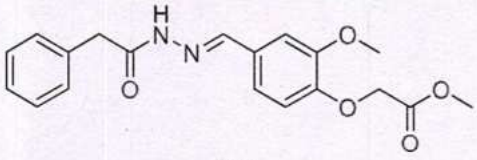
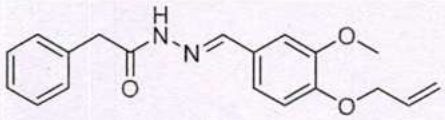
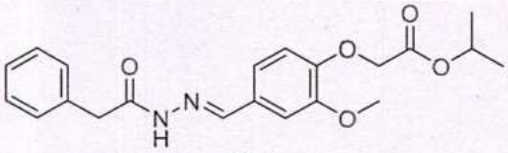
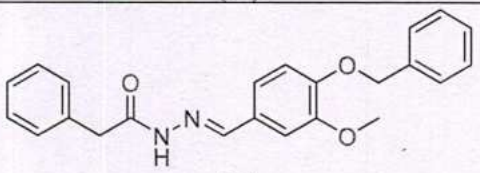
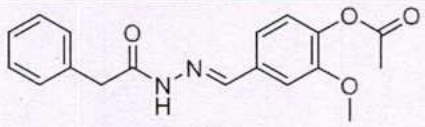
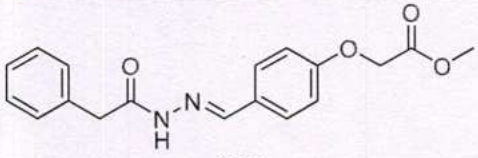
Reaction Scheme:

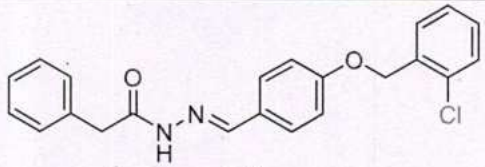
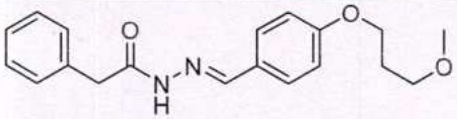


(1a-j) (2) (3a-j)

Table 01: Synthesis of different hydrazones of phenyl acetohydrazide (**2**):

Product (Hydrazone)	Reaction time (T in min)	Colour	m.p. (^{0}C)

 **(3a)**	90	**White solid**	**170**
 **(3b)**	80	**White solid**	**165**
 **(3c)**	75	**White solid**	**141**
 **(3d)**	60	**White solid**	**141**
 **(3e)**	65	**White solid**	**138**
 **(3f)**	45	**White solid**	**172**
 **(3g)**	75	**White solid**	**175**
 **(3h)**	65	**White solid**	**124**

 (3i)	40	**White solid**	**172**
 (3j)	90	**White solid**	**146**

UV spectral analysis: All electronic spectral analysis was performed by using Double beam UV spectrophotometer. UV spectral analysis of hydrazones (**3a-j**) were performed in DMSO in order to determine their λ_{max} values at a concentration 2 μM (Table 2). All compounds shows strong electronic excitation at 292.6 – 319.0 nm.

Table 02: Electronic spectral (UV spectra) of **3a-j**:

Test sample	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**	**3j**
λ_{max} (nm)	319	317.3	316.3	317.4	295.3	317.7	317.1	292.6	294.8	296.1

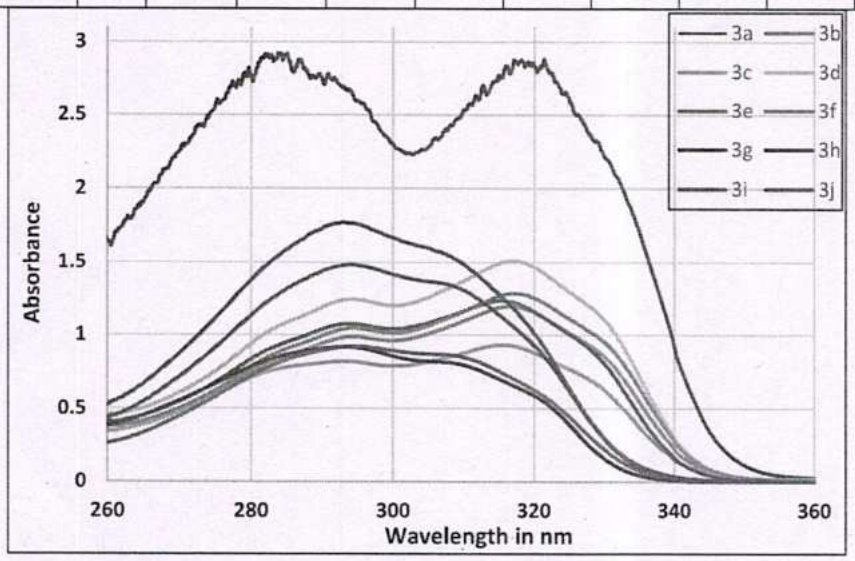


Fig 01: UV spectra of **3a-j**.

Photoluminescence study: Luminescence properties of the hydrazones (**3a-j**) of benzohydrazide (**2**) have been checked by using Spectrofluorophotometer model number RF5301. A Xe laser lamp was used for emission spectra were scanned from the range 250 nm to 600 nm at their excitation wavelength (λ_{max}). For fluorescence study of the hydrazones, dimethylformamide is used as solvent and reference material. The excitation of the molecule is occurred due to the n → π* and π → π* electronic transitions. Slit width:

Emission is 5 mm; Concentration of solution is 2 μM; Solvent used is DMF. Quantum efficiency of hydrazones is low which can be easily determined from intensities of excitation and emission wavelengths. All compounds shows more intense emission in the 348 – 365 nm region except **3i** is may be due to C=N (imine) bond. The emission wavelengths and their intensities were reported in table 3.

Table 03: Photoluminescence spectra (emission spectra) of **3a-j** at their λ_{max} value:

Molecules	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**	**3j**
Excitation wavelength (nm)	326	325	325	326	325	327	316	316	317	318
Emission wavelength (nm) (intensity)	363 (155.0)	355 (183.8)	359 (190.4)	365 (88.7)	357 (142.1)	354 (200.3)	352 (149.7)	348 (142.4)	350 (75.7)	351 (227.0)

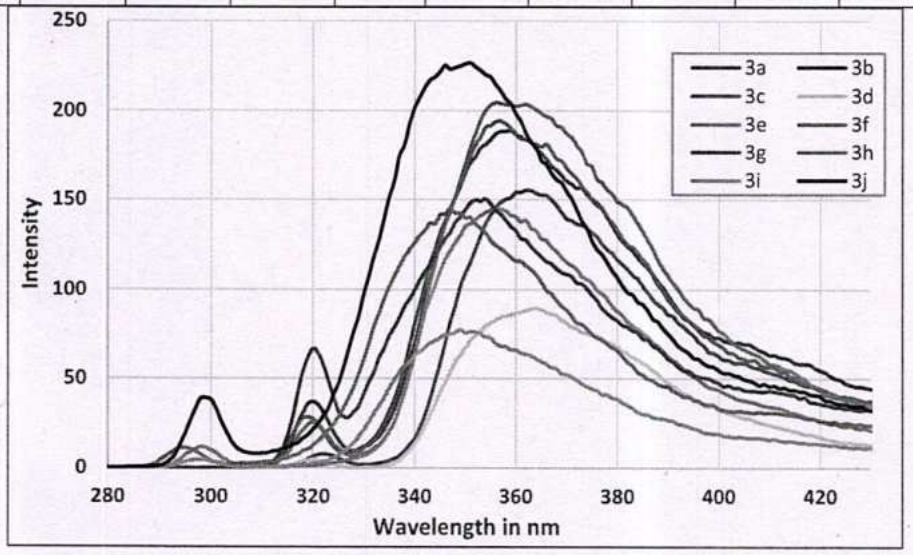


Fig 02: Photoluminescence spectra of **3a-j**.

Study of anti-mycobacterium tuberculosis activity:

Mycobacteria strain used for the analysis is ***Mycobacteria tuberculosis*** (Vaccine strain, *H37RV strain*): ATCC No. 27294. The anti-TB study is performed by using microplate Alamar Blue assay (MABA) which is one of the best method for analysis. Add 200μL of sterile deionized water into all perimeter well of sterile wells plate to minimize evaporation of test medium in the wells during incubation. All wells plate received 100 μL of the Middlebrooks 7H9 broth and serially diluted with test compounds directly on plate. The test compound concentration were varied from 100 to 0.2 μg/mL. All plates were covered and sealed with parafilm and incubated at 37^{0}C for 5 days. After that, 25μL of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added into each plate and incubated further for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. Record MIC (lowest drug

concentration which prevented the color change from blue to pink) value with reference to three anti-TB drugs such as pyrazinamide, ciprofloxacin and streptomycin as standard.

Table 06: MIC values of **3a-j** against H37 RV strain:

Test sample	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j	Pyr.	Cipro.	Strep.
MIC value (µg/mL)	12.5	12.5	25.0	25.0	25.0	12.5	12.5	25.0	25.0	100	3.125	3.125	6.250

Pyr – Pyrazinamide; Cipro – Ciprofloxacin; Strep - Streptomycin

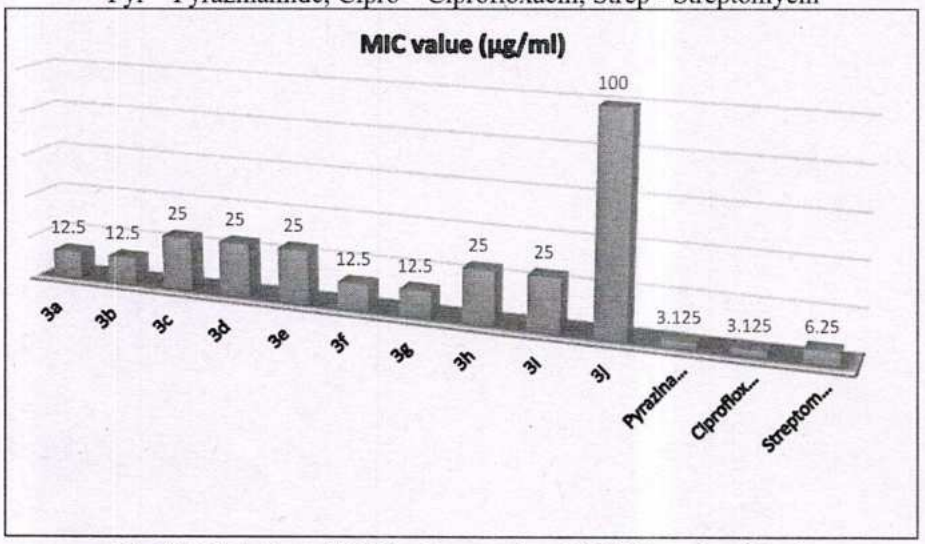


Fig 04: Variation of MIC values against H37 RV strain of **3a-j**:

Result and Discussion:

Phenyl acetohydrazide (**2**) is condensed with substituted aryloxybenzaldehydes (**1a-j**) in ethanol at room temperature in acidic condition forming hydrazones (**3a-j**). All the derivatives are novel and characterized by ^{1}HNMR Spectroscopy, it has been found that the data obtained is appropriate and gives information about all the protons present in compounds. The details of IR stretching frequencies of important groups and chemical shift of protons and interpretation of chemical shifts is shown in table 07. The absorption spectra of hydrazones (**3a-j**) are recorded in DMSO. All compounds are shows strong absorption in the region 292.6 – 319 nm. The photoluminescence spectra is recorded at absorption wavelength. All hydrazones are shows more intense emission in the 348 – 365 nm region except **3i** is may be due to C=N (imine) bond.

Table 07: Spectral Characterization of phenyl acetohydrazones:

	FT-IR data (cm^{-1})										
Hydrazone	**2**	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**	**3j**

-NH/NHNH$_2$	3338, 3301, 3202	3200	3199	3197	3190	3211	3217	3174	3185	3191	3180
>N-C=O (amide)	1643	1659	1653	1654	1643	1657	1665	1663	1646	1651	1661
>C=O (ester)	-	-	-	1753	-	1738	-	1752	1736	-	-
-N=CH	-	1602	1600	1602	1599	1599	1599	1598	1600	1603	1603
	Chemical shift (δ in ppm, $CDCl_3$)										
-NH	-	8.810	9.180	9.849	9.678	9.686	9.739	9.774	9.465	9.331	9.212
-OH	-	8.415	8.293	8.550	8.453	8.484	8.475	8.550	8.470	9.291	8.287
>N=**CH** (amide form)	-	8.221	7.662	7.810	7.703	7.701	7.687	7.725	7.709	7.906	7.685
>N=**CH** (enol form)	-	8.320	7.878	7.876	7.883	7.886	7.855	7.907	7.889	8.324	7.861
-CH_2-CO	-	4.090	4.095	3.951	4.100	4.096	4.090	4.096	4.088	4.090	3.774

Hydrazones compounds shows weak absorption in light atom and polar region (N-H group - 3217-3174 cm^{-1} except **3h**; C-H group – 3070-2955 cm^{-1} lower energy side than parent hydrazide) and absorption in amide carbonyl group (hydrazones) in the region 1665–1643 cm^{-1} confirm the formation of acid hydrazones. The N-H group also shows out of plane bending absorption in the region 850 – 810 cm^{-1}. All molecules shows medium to strong absorption at 1340-1100 cm^{-1} due to bending vibrations and at 1450-1400 cm^{-1} due to stretching vibration of C-N bond. All molecules having >C=N bond showing absorption in the region 1603–1598 cm^{-1}. The molecule 3d containing allylic group shows addition absorption band at 1580 cm^{-1} in it spectra. All molecules shows strong absorption band in the region 1250-1150 cm^{-1} confirm the presence of ether linkage. Some hydrazones such as **3c**, **3e**, **3g** and **3h** showing additional carbonyl stretching vibrations in the region 1753-1736 cm^{-1} confirm the presence of ester group.

The amide proton get enolized to some extent so there are two peaks for amide (NH) and enol (OH) protons (with ratio 4:1, obtained from integration area under the peak). These enolisation also affect the chemical shift of other protons of the molecule basically imine proton shift to deshielded zone marginally. Other protons shows two signals for each chemically equivalent proton/s. The OH proton is more shielded than NH proton. The -CH_2-CO- protons also shows weak singlet on shield zone. The data of chemical shift of imine (N=CH) proton, –NH- and OH protons of **3a-j** is summarized in table 07. The hydrazide CO-NH proton in **3a-j** compounds is highly deshielded and shows broad singlet at 9.849-8.810 ppm in $CDCl_3$. The imine protons in **3a-j** shows singlet at 8.221 – 7.662 ppm. The –CH_2-O protons get deshielded due to electronic effect and anisotropy effect of phenyl ring and carbonyl group and it shows singlet in deshielded zone at 4.096-3.774 ppm. The –CH_2-O protons shows singlet (except **3d** shows doublet and **3j** shows triplet) in deshieded zone (5.298 – 4.124 ppm).

The synthetic route was initiated with the need of efficient anti-TB candidates and biological importance of hydrazones. All hydrazones shows good drug scores and druglikeness scores as compared to compound **2** along with good bioactivity score which suggest that they shows stronger interactions with different receptors, ligands, and enzymes. Cardiac toxicity was predicted using Pred-hERG, binary model predicted positive (blocker) response for **3d**, **3e** and **3j** hydrazones and are showing moderate to strong cardiotoxicity while remaining molecules shows negative response (Non-blocker) in multiclass model.

Toxicity risk of phenyl acetohydrazide and hydrazones was predicted by using Osiris program, phenyl acetohydrazide (**2**) was found to be mutagenic and tumorigenic while **3d**, **3f** and **3g** are found to be irritant. Among all the molecules, Phenyl acetohydrazide (**2**, least active) and hydrazone **3d** and **3f** shows low drug score. Druglikeness score predicted by using Molinspiration technology confirm that among all synthesized molecules, **3a** and **3f** are found to be best candidate for drug development.

Different sensitization and toxicity study of hydrazones was predicted by using STopTox tool. All synthesized are not showing any acute inhalation toxicity and acute dermal toxicity against OECD TG 403 and 436 and OECD TG 402 respectively of rat. These compounds are not showing any Skin Irritation and Corrosion (over all activity is negative). Compounds **2**, **3a**, **3d**, **3i** and **3j** are showing positive Acute oral toxicity test (OECD TG 401, 420, 423 and 425) of rat i.e. acute oral toxicity. All hydrazones **3a-j** and phenyl acetohydrazide showing Eye Irritation and Corrosion toxicity against OECD TG 405 of rabbit. Few hydrazones including **3a**, **3b**, **3d**, **3e** and **3h-j** are showing skin sensitization (assay type – LLNA test OECD TG 429 and 442) of mouse and guinea pig.

All molecules are showing good human intestinal absorption (HIA% is >30%). Human colon adenocarcinoma (Coco-2, monolayer cell culture model) used to design good intestinal model for the determination of absorptive and defensive properties of the intestinal mucosa. All molecules shows high Coca-2 permeability (intestinal absorption) except **3a**, **3e**, **3g** and **3h** which shows moderate poor permeability. All hydraones shows good Human oral bioavailability except **3a** (-0.5571). P-Glycoprotein (P-gp, an efflux transporter) plays a crucial role in drug pharmacokinetic properties (ADME) and is critical for multidrug resistance (MDR) by mediating the active transport of anticancer drugs from the intracellular to the extracellular compartment. All hydrazones shows p-gp inhibition property except **3d**, **3g** and **3h** hydrazones. Hydrazones **3d**, **3f**, and **3h-j** were act as CYP450 3A4 Inhibitor. Hydrazones **3d** and **3f-j** were showing non-inhibition properties of CYP450 2C9 inhibitor. Hydrazones **3e** and **3h** are act as noninhibitor against CYP450 2C19 Inhibitor and CYP450 1A2 Inhibitor. Ames mutagenesis test (bacterial reverse mutation assay) was used to identify revert mutations and mutagenicity of environmental samples. It was also used to detect suitable mutant. Compounds (mutant) **3h** and **3j** were shows positive Ames mutagenesis test. All molecules were shows hepatotoxicity. All molecules were shows class III moderate acute oral toxicity. All Hydrazones does not shows estrogen receptor binding while **3a** and **3g** were shows androgen

receptor binding. All molecules does not shows any binding with thyroid receptor and glucocorticoid receptor. They are also not showing any honey bee toxicity but shows strong Fish aquatic toxicity except **3f** and **3j**. Some hydrazones such as **3d, 3f-h** and **3j** are showing crustacea aquatic toxicity.

Lazar Toxicity Prediction tool was used to predict different toxicity, mutagenicity, BBB, etc. Some hydrazones **3b-g** shows acute toxicity (fathead minnow) in the range 8.18-49.5 mg/kg_bw/day. Also **3f** (102 mg/L) and **3h** (61.9 mg/L) are showing acute toxicity (Daphnia manga). All hydrazones does not shows any mutagenicity and carcinogenic property (rat). Some hydrazones including **3a-b, 3f-g**, and **3i-j** shows penetration of blood brain barrier so not directly used as drug candidate. **3a-b** also showing potent carcinogenic property against mouse. **3b**, **3c** and **3e** are carcinogenic against rodents.

The synthetic route was initiated with the need of efficient anti-TB candidates and biological importance of hydrazones. All tested compounds shows moderate to good anti-TB activity. Compounds **3a**, **3b**, **3f** and **3g** were found to be most active compounds and shows MIC values 12.5 µg/ml. Remaining compounds are also shows moderate activity except **3j** so after some structural modifications they may be become anti-TB drug candidate. The compound **3j** shows higher MIC value (100 µg/ml).

Conclusion:

Derivatives of phenyl acetic acid hydrazones were shows important biological activities. We have synthesized different hydrazones from phenyl acetohydrazine and subjected for the study of anti-TB activity. Compound **3a**, **3b**, **3f** and **3g** does not shows any oral toxicity but most active against *H37RV strain*.

Expenses:

Sr. No.	Items	Bill Number	Amount
1	Consumables & Chemicals	--	28000.00
2	Hiring Services	8000.00	8000.00
3	Field Work and Travel		0.00
4	Books and peripherals		0.00
5	Contingency (including special needs)	--	4000.00
Grant Total			40,000.00

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Fig.: FT-IR spectra of (E)-N'-(2-((2-chlorobenzyl)oxy)benzylidene)-2-phenylacetohydrazide (3a):

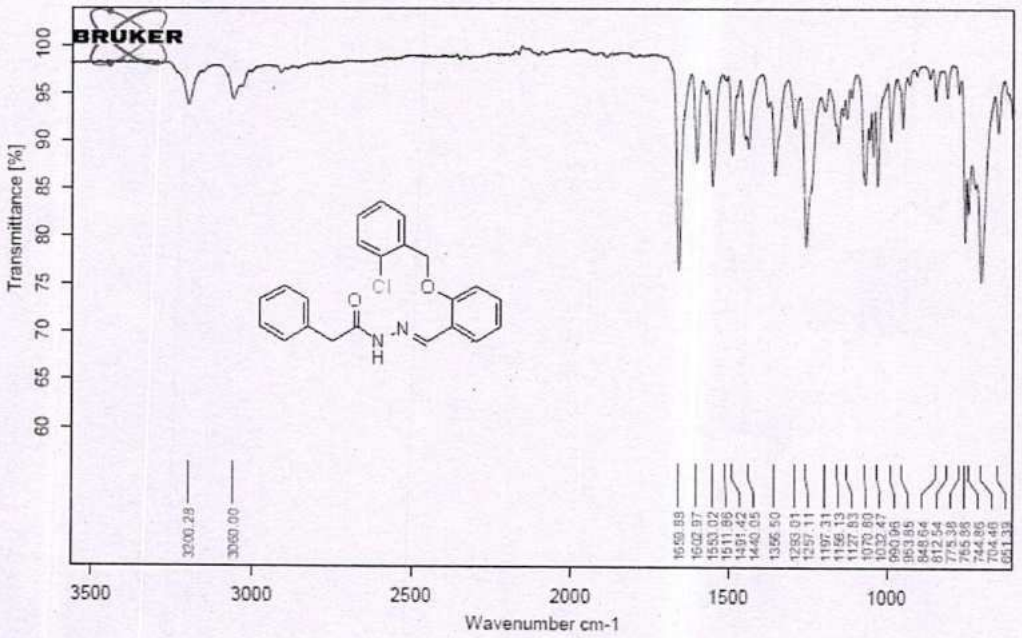

Fig.: ^{1}H-NMR spectra of (E)-N'-(2-((2-chlorobenzyl)oxy)benzylidene)-2-phenylacetohydrazide (3a):

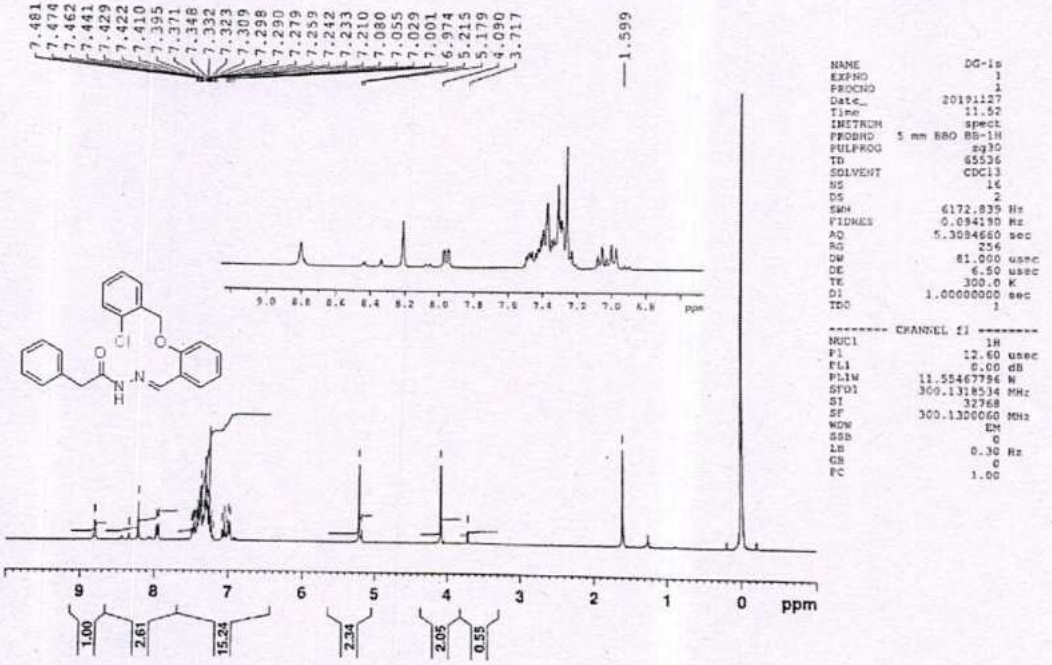

Fig.: FT-IR spectra of (E)-N'-(4-((2-chlorobenzyl)oxy)-3-methoxybenzylidene)-2-phenylacetohydrazide (3b):

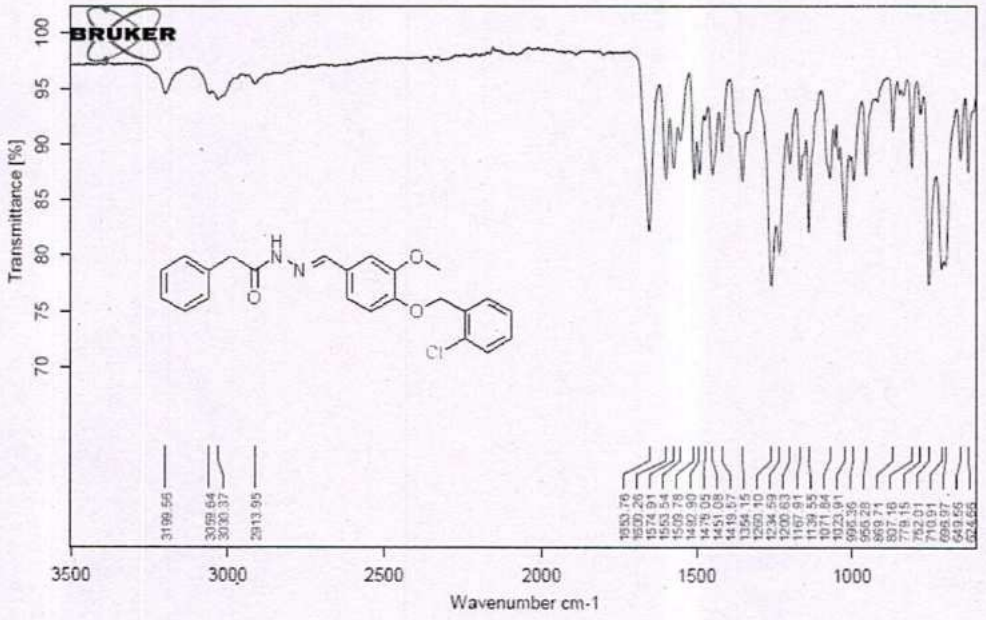

Fig.: ^{1}H-NMR spectra of (E)-N'-(4-((2-chlorobenzyl)oxy)-3-methoxybenzylidene)-2-phenylacetohydrazide (3b):

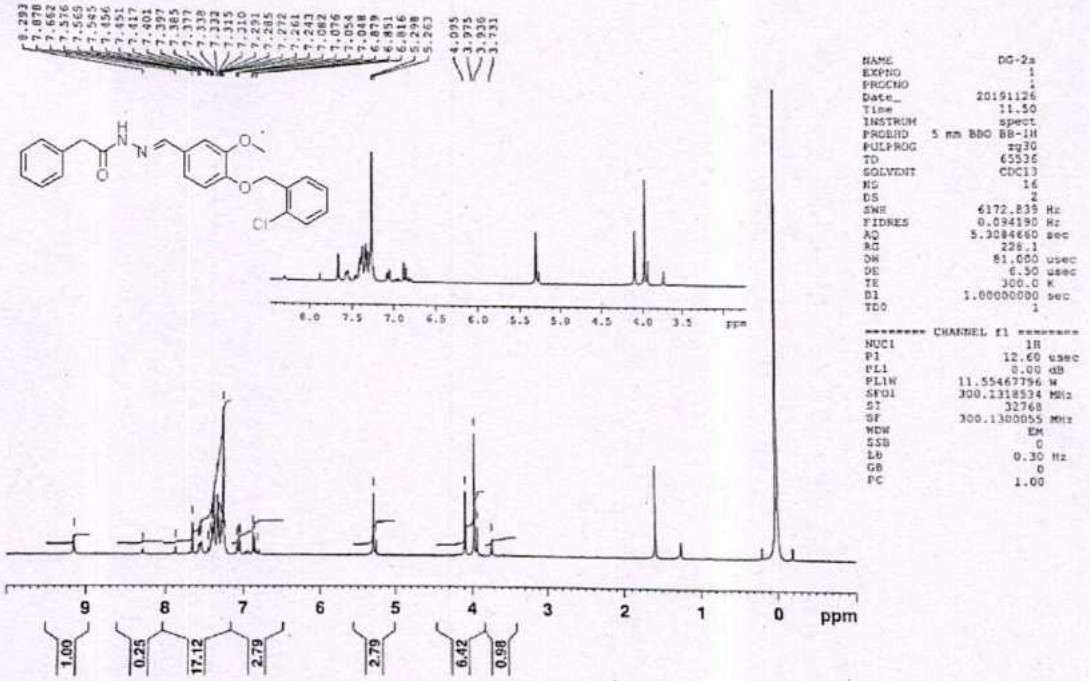

Fig.: FT-IR spectra of methyl (E)-2-(2-methoxy-4-((2-(2-phenylacetyl)hydrazono)methyl)phenoxy)acetate (3c):

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1. **Bapu R. Thorat et al.** Synthesis, Spectroscopic, *In-vitro* and Computational Analysis of Hydrazones as Potential Antituberculosis Agents: (Part-I), *Combinatorial Chemistry & High Throughput Screening,* **2020,** *23. DOI:10.2174/1386207323999200325125858.*
2. **Bapu R. Thorat et al.** Review of the importance of Hydrazide and its Derivatives in 2 Organic synthesis, *Proceedings* **2021**, 68.

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Acknowledge Receipt

Total number of samples:	20
Charges per sample including GST:	400.00
Total amount received:	8000.00

Results:

Sl. No.	Sample	100 µg/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
01	01K	S	S	S	S	S	R	R	R
02	02K	S	S	S	S	S	R	R	R
03	03K	S	S	S	S	S	S	R	R
04	04K	S	S	S	S	S	S	R	R
05	05K	S	S	S	S	S	S	R	R
06	06K	S	S	S	S	S	R	R	R
07	07K	S	S	S	S	S	R	R	R
08	08K	S	S	S	S	S	R	R	R
09	09K	S	S	S	S	S	R	R	R
10	10K	S	S	S	S	S	R	R	R
11	01S	S	S	S	S	R	R	R	R
12	02S	S	S	S	S	R	R	R	R
13	03S	S	S	S	R	R	R	R	R
14	04S	S	S	S	R	R	R	R	R
15	05S	S	S	S	R	R	R	R	R
16	06S	S	S	S	S	R	R	R	R
17	07S	S	S	S	S	R	R	R	R
18	08S	S	S	S	R	R	R	R	R
19	09S	S	S	S	R	R	R	R	R
20	10S	S	R	R	R	R	R	R	R

NOTE: S – Sensitive; R- Resistant

Reference: Evaluation of anti-Tubercular activity of nicotinic and isoniazid analogues. *ARKIVOC* **2007** (xv), 181-191; Maria C. S. Lourenco, Marcus V. N deSouza, Alessandra C Pinheiro, Marcelle de L. Fer reira, Rasnisb B, Goncalves, Thais Cristina M Nogneira, Monica A Peralta.

Date: 04.10.2019
Place: Belgaum

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