Research Article

Design, Synthesis and Characterization of Novel Isoniazid Derivatives and Their Biological Evaluation

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ABSTRACT

Owing to its reactive site, which broadens its range of applications, isoniazid is the most significant heterocyclic moiety in chemical and medicinal chemistry. When treating TB, isoniazid is the most significant first-line medication. It is now employed as a framework for the creation of novel anti-TB drugs.

KEYWORDS: - Isoniazid, Schiff's base, Anti- TB, Almar blue assay, NMR, TLC.

INTRODUCTION

Heterocyclic rings have a key role in medicinal chemistry since many drugs are based on them. Heteroatoms are a very prevalent fraction of a wide range of active medicinal substances as well as excipients, regardless of whether they are carbons that have been isosterically or bio isosterically substituted, carbon foundations in actual heterocycles, or aliphatic structures.

Several scaffolds that are heterocyclic categorized as novel structures. The molecules in question possess unique chemical and pharmacological properties due to the presence of atoms other than carbon, typically nitrogen, oxygen, or sulfur. Heterocyclic rings have several important applications in medicinal chemistry. Among organic chemistry's application fields, nitrogen containing chemistry that is heterocyclic is a highly significant and particularly remarkable class.

A significant level of research in this area is directed toward the creation of novel molecules and compositions in general. Over the past few years, these molecules have gained widespread acceptance and increased importance. They are widely used in the chemical sciences and have contributed to the development of several techniques for organic synthesis.

Without a doubt, the chemical structures of a large range of naturally occurring products and compounds with significant biological efficacy are N-heterocycles. Examples of these compounds are imidazoles, benzimidazoles, isoquinolines, quinazolines, quinazolinones, 1,2,4-oxadiazoles, thiazoles, and thiadiazoles; pyrimidines, 1,2,4-triazoles, 1,3,5-triazines, quimidazoles.

Additionally, the pharmaceutical sector, material chemistry, and chemical synthesis all made significant use of N-heterocycle molecules as intermediates. In order to create N heterocycles, it is especially crucial that the creation of C–N bonds be an attractive method of introducing nitrogen into the organic frameworks. Nature has a large number of widely dispersed N-heterocyclic molecules with physiological and pharmacological properties. Additionally, they serve as the building blocks for a wide range of biologically important compounds, including vitamins, antibiotics, nucleic acids, medicines, dyes, and agrochemicals. Additionally, they are a crucial a part of many compounds that are pharmacologically active. N-heterocyclic substances such as purines, pyrimidines, etc. are also constituents of the guanine, cytosine, adenine, and thymine base pairs of DNA and RNA.

With their distinct qualities and applications, these nitrogen-containing heterocyclic compounds are gaining popularity in the rapidly expanding fields of medical and organic chemistry as well as the pharmaceutical industry. In addition, the electron-rich nitrogen heterocycle is readily able to accept or give up a proton and generate a wide range of weak interactions. The compounds' growing importance in medical chemistry can be attributed to a number of intermolecular forces, such as hydrogen bonding formation, dipole-dipole interactions, hydrophobic effects, van der Waals forces, and π stacking interactions of nitrogen compounds.

These forces also enable the compounds to bind with improved solubility to a wide range of enzymes and receptors found in living things. Because their derivatives have a wide range of bioactivities, their structural characteristics are advantageous. Heterocyclic rings have an attribute called aromaticity that is associated with enhanced stability and unique electrical properties.

Aromatic heterocycles, such as furan and pyridine, are commonly found in pharmaceuticals because of their advantageous pharmacokinetic characteristics and capacity to communicate with biological targets. A drug's properties, including its affinity for binding to receptors, lipophilicity, and solubility, can the size of a heterocyclic ring can have a significant impact.

Smaller rings are frequently greater lipophilicity and flexibility, which facilitates their passage across membranes containing cells. Bigger rings might have a more specific target binding and be stiffer. Since heterocyclic rings can be put to use in many different ways, substituents can be introduced to alter the drug's characteristics. Target, metabolism, and solubility of the drug. The impact of substitutes on element can influence interactions.

One of the most popular areas in medicinal chemistry lately has been hybridization of bioactive compounds, which is based on the combining of two or more distinct combine different bioactive compounds' pharmacophore moieties to create a new molecule. The final molecule's pharmacophores can exhibit numerous pharmacological activities by acting on distinct targets, or one portion of the molecule can balance out the adverse effects induced by another part. It is common practice to combine INH with another active component to overcome resistance. A lot of work has gone into creating INH blends as innovative anti-TB drugs. LL-3858, the most promising INH-pyrrole hybrid, is currently undergoing phase II clinical trials and could soon be licensed for the treatment of tuberculosis. When isoniazid is taken long-term, the rapid acetylators significantly reduce the drug's bioavailability, which causes isoniazid resistance to develop. Given that the main metabolic pathway for INH in humans is the enzymatic acetylation of isoniazid by NAT, chemically altering the Hydrazine container with the appropriate functional group to inhibit acetylation may enhance clinically beneficial effects. Furthermore, adding lipophilic moieties to the isoniazid's structure can improve the drug's ability to enter bacterial cells. More lipophilicity in isoniazid derivatives is therefore become recognized as one of the most

promising anti-TB medicines. An INH derivative hydrazide N′-substituted with a C10 acyl chain was able to counterbalance its decreased reactivity by increasing membrane permeability. This resulted in improved performance against the most prevalent Mycobacterium tuberculosis resistant strain (S315T) as compared to INH. In this work, we have synthesized two new series of isoniazid compounds, alkyl hydrazides and hydrazones, which exhibit promising in silico properties including membrane permeabilities and spontaneous formation of INH radicals. Assessments of the kinetics, cytotoxicity, and biological activity validated the in-silico hypotheses on the exceptionally high reactivity of alkyl hydrazides. However, in contrast to INH, the hydrazones exhibited behavior that was strikingly comparable, especially in biological tests that required more time to finish, suggesting that these compounds are hydrolyzing back to INH. It is crucial to keep in mind that the precise pharmacological effects

of drugs based on benzimidazoles may vary based on the substituents attached to the molecule's core and its overall chemical structure.

Medicinal chemists are still investigating and refining the properties of benzimidazole derivatives to develop new drugs with improved safety, effectiveness, and selectivity profiles. The only reason why isoniazid (INH) derivatives need to be synthesized is because the germs known as Mycobacterium tuberculosis (Mtb) are increasingly resistant to isoniazid and a number of other first-line medications, including pyrazinamide (PZA), ethambutol (EMB), and rifampicin (RIF).

❖ **Schiff Bases:**

A chemical having the overall formula $R_1R_2C=NR_3$ (R_3 = aliphatic or aromatic, but absence of hydrogen) is referred to as a Schiff base in organic chemistry. Depending on their structure, they can be classified as either secondary ketoimine's or secondary aldimines, making them a subclass of imines. A common subset of Schiff bases, known as imines generated from anilines, is referred to as anilines. The word and azomethine, which particularly relates to secondary aldimines, are interchangeable.

Schiff bases are classified as imines, which are compounds containing a heterocyclic carbon double bond that can based on their framework, possibly secondary ketoimines or secondary aldehydes. Schiff bases have the overall framework $R_2C=NR$. The term "imine" frequently used interchangeably with "azomethine," which is a synonym for secondary aldimines. These substances bear Hugo Schiff's name, an Italian chemist. These molecules have many naming schemes.

Schiff bases are substances with an imine (-C=N) or azomethine functional group. These compounds important pharmacophores for the preparation of physiologically in motion chemicals, which may achieve through the reactant and product are condensed to carbonyl compounds with principal amines. Schiff's bases are carbonyl functional group containing compounds that have had the substitution of carbonyl group with azomethine and imine group. These is frequently employed in industrial applications and have a range of biological properties. These are the most frequently utilized organic compounds in various applications such as intermediates in organic synthesis, pigments & dyes, catalysts, polymer stabilizers, etc.

Any amine may react under certain circumstances with a carbonyl compound to create Schiff's bases. Put another any case, it's nitrogen equivalent of a carbonyl compound, where the

carbonyl group has been replaced with an azomethine or imine group. Schiff was the first person to synthesize imines in the 19th century. In order to create Schiff Bases, Schiff employed a traditional process that included using amine to help condense a carbonyl compound, followed by azeotropic distillation and using molecular sieves to remove any water that might have formed in the system. Later, numerous methods for synthesizing Schiff bases are developed.

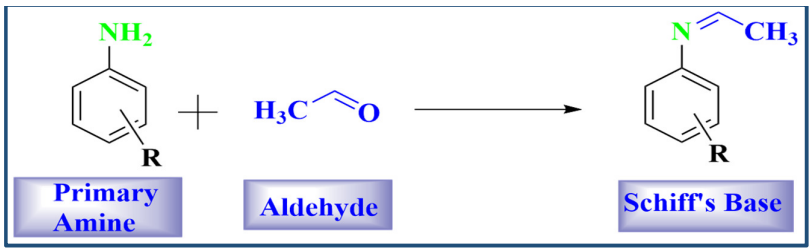

Figure 1. Synthesis of Schiff Base

❖ **History of Isoniazid:**

As a heterocyclic moiety, the pyridine ring has been shown to serve a variety of purposes. Drugs of all kinds, food, plants, adhesives, flavorings, rubber goods, pesticides, and herbicides all include them. Isoniazid (INH) is one such pyridine-containing substance. INH is known by its chemical name is hydrazide of isonicotinic acid. One synthetic medication that was first

identified in 1912 by Meller and Malley. Apart from its capacity to treat tuberculosis, it also has other lesser-known characteristics, including antimycobacterial, antibacterial, anti-virus, antimicrobial, antimalarial, antifungal, anticancer, anti-analgesic, anti-convulsant, anticorrosive, and anti-inflammatory properties. With isoniazid serving as the mainstay of antituberculosis treatment for over 50 years, a great deal has been discovered regarding the complex biochemistry and several mechanisms of action of this first-line medication. In fact, INH has emerged as the most extensively studied antitubercular medication due to the significant worldwide incidence of tuberculosis and the concerning increase in the number of clinical isolates exhibiting treatment resistance or elevated virulence.

Out of the 7.5 billion people on the planet, one-third have tuberculosis (TB), which claims 5,000 lives every day. The disheartening aspect of this information is that although tuberculosis (TB) is treatable and preventable, no new discoveries or breakthroughs has been approved by the WHO to stop this illness from occurring in a shorter amount of time with medicine. As a matter of fact, this illness poses a serious risk to the lives of those who are HIV positive. For more than 60 years, INH has been the first-line treatment for tuberculosis (TB) infection when taken with pyrazinamide (PZA), ethambutol (EMB), and rifampicin (RIF). It is still one of the most potent anti-TB medications.

Numerous impacts on proteins, nucleic acid, biomembranes, lipids, and glycolysis production are all part of its mechanism of action. Sadly, the long-term widespread use—and sometimes abuse— of INH is leading to the emergence of bacterial strains resistant to the treatment,

making the development of novel INH derivatives imperative in order to combat drug resistance. Isoniazid is also used in clinical medicine to treat chronic tuberculosis because it is bacteriostatic against dormant types of mycobacteria.

❖ **Chemistry of Isoniazid:**

Isoniazid chemistry includes the investigation of the structural alterations, chemical characteristics, and reactions of the isoniazid structure system. An additional crucial component is the examination of the structural activity relationship [SAR] of isoniazid. Isoniazid has unique chemical properties and is stable due to the aromaticity of its pyridine ring. Aromaticity, which imparts on the molecule characteristics like planarity, resonance stability, and the ability to participate in several processes, is the outcome of the delocalization of electrons in that pyridine ring of isoniazid. Isoniazid possesses mild basicity because the pyridine ring has a nitrogen atom and para position contain hydrazide group. The chemical compound isoniazid is a synthetic one. It is either a hydrazide of isonicotinic acid or a pyridine derivative of nicotinamide. Isoniazid is also known by the synonyms isonicotinohydrazide and isonicotinic acid hydrozide. In contrast, the IUPAC designation for isoniazid is pyridine-4-carbohydrazide.

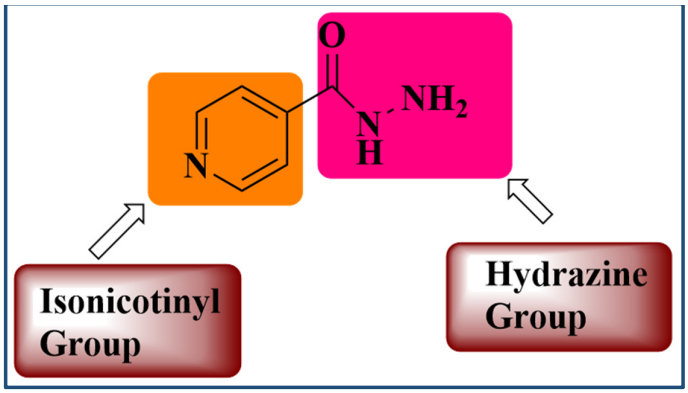

Figure 2. Structural determination of Isonicotinyl & Hydrazine Group

Structural activity relationship of isoniazid is as follows:

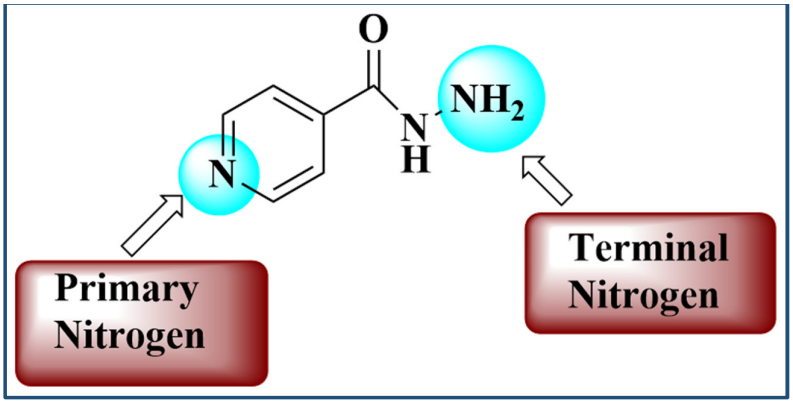

Figure 3. Structural determination of Primary Nitrogen & Terminal Nitrogen Group

- If piperdine were to replace the pyridine ring, the biological activity would be gone.
- A number of active chemicals are created when hydrazide linkage is transformed into hydrazine derivative.
- When the hydrazide is moved from position 4 to positions 2 or 3, the compounds' biological activity decreases.
- For biological activity, carbonyl hydrazide at position no. 4 is necessary.
- INH has two nitrogen atoms, so when an alkyl group is added,
  - ✓ 1. Should N1=compounds stop functioning
  - ✓ 2. If N1 is equal to the original but less active chemicals
- Activity is lost when the hydrazide group is swapped out for an amide or ester group.
- Activity is lost when pyridine nitrogen is oxidized.
- Anti-tubercular action requires terminal nitrogens.
- Anti-tubercular action requires N2-alkyl derivative.
- High activity is shown by dialkyl derivatives. When terminal nitrogens have an isopropyl group added, the molecules behave similarly to INH.

❖ **Biological Importance of isoniazid:**

Apart from its capacity to avert tuberculosis, isoniazid possesses several lesser-known additional characteristics.
as well, including anti-inflammatory, anti-mycobacterial, anti-virus, antimicrobial, antimalarial, anti-fungal, anticancer, and anti-analgesic effects.

- **inflammatory:** Isoniazid has less anti-inflammatory activity than Isoniazid (INH) anti-inflammatory efficacy was assessed using zebrafish inflammation models, and the underlying mechanism was tentatively investigated.
- **Anti-mycobacterial:** An antibiotic called isoniazid is used to treat mycobacterial infections. It is most frequently used in conjunction with other antimycobacterial medications to treat either latent or active tuberculosis.
- **Tuberculous Meningitis:** Tuberculous meningitis is fatal if left untreated. As first-line anti-tuberculosis medications for patients with tuberculous meningitis, the World Health Organization (WHO) recommends isoniazid, rifampicin, and pyrazinamide, typically in combination with a fourth medication such ethambutol or streptomycin.
- **Other:** Isoniazid is indirectly used in acute leukaemia.

MATERIALS AND METHODS

The thermal melting point equipment was used to measure melting points, the Bruker 400 MHz spectrometer was used to record Fourier transform infrared (FTIR) spectra, and the Bruker pectrophotometer was used to record nuclear magnetic resonance (NMR) spectra.

Materials

Isoniazid, *p*-Anisaldehyde, [3,5-Dimethoxybenzaldehyde], 4-Nitrobenzaldehyde, 4-Chlorobenzaldehyde. that Sigma Aldrich was the source of.

Synthesis and characterization

Synthesis of 2-(pyridine-4-carbonyl) hydrazine-1- carbothioamide:

After dissolving isoniazid (0.01 mol) in the smallest volume of 1N HCl, ammonium thiocyanate (0.02 mol) was added. For eight to ten hours, the reaction mixture was heated under reflux. The result was cooled, filtered, and then recrystallized from 100% alcohol after being cleaned with water. 67% yield, m.p. 207°C.

Synthesis of D1, D2, D3, D4

Place 5.88gm (0.30 mol) of 2-(pyridine-4-carbonyl) hydrazide-1-carbothioamide in a RBF of 250 ml and add 0.30 mol of variety of benzaldehyde and stirred for 20min on magnetic stirrer. During stirring glacial acetic acid is added dropwise eventually. Heat the mixture on a water bath at 100 °C with the attachment of reflux for 5 h., with constant and eventually shaking of the flask, until the mixture is just yellowish in colour. After that TLC monitoring is done with the help of chloroform: ethanol as a mobile phase. Reaction mixture is filtered once the reaction is finished and collect crude product. After filter off the synthesized crude product by using the pump, wash with filtrate, drain well and wash again with filtrate.

Dissolve the synthesized product in 100 ml of absolute ethanol, Filter rapidly through a preheated Buchner funnel and a flask at the pump. Allow to evaporate the filtrate to the sunlight, collect the purified synthesized derivative. The yield of pure derivative is about 23%, and M.P. is 78°C.

Derivative D1,m.p. of 78°C–80°C. IR (KBr) ν, cm^{-1}: 3455 cm^{-1} (N-H),2931 cm^{-1} (C-H),1744cm^{-1}(C=O),1567 cm^{-1}(C=N),718cm^{-1}(C=S) Mass (Varian 1200 L,ESI,MS) 314.2772 C^{13} NMR (400 MHz,$CDCl_3$)d, ppm: 160.52 (N-C=O),143.17(C_6H_5-H),42.36 (C-O),118.92 [CH (pyridine)],115.92 [CH(1-benzene)],13.23 (CH_3)

Derivative D2,m.p. of 79°C–82°C. IR (KBr) ν, cm^{-1}:Near to 3500 cm^{-1}(N-H),2925 cm^{-1}(C-H),1744.78 cm^{-1}(C=O),1592cm^{-1}(C=N) Mass (Varian 1200 L,ESI,MS) 344.5841 C^{13} NMR

(400 MHz,$CDCl_3$)d, ppm:161.42 (N-C=O),153.37 (C_6H_5-H),126.01 (C-O),118.92 [CH (pyridine)],110.61 [CH(1-benzene)],18.67 (CH_3)

Derivative D3, m.p. of 81°C–82°C. IR (KBr) ν, cm^{-1}: 3423.85 cm^{-1}(N-H),2925.25 cm^{-1}(C-H),1747.97 cm^{-1}(C=O),1574.51 cm^{-1}(C=N) Mass (Varian 1200 L, ESI, MS) 329.3905 C^{13} NMR (400 MHz, $CDCl_3$) d, ppm: 160.58 (N-C=O),152.64 (C_6H_5-H),128.34 (C-O),110.23 [CH(1-benzene)],20.32 CH_3)

Derivative D4,m.p. of 85°C–86°C. IR (KBr) ν, cm^{-1}:3423.85 cm^{-1}(N-H),2925.25 cm^{-1}(C-H),1747.97cm^{-1}(C=O),1574.51 cm^{-1}(C=N) Mass (Varian 1200 L,ESI,MS) 318.0347 C^{13} NMR (400 MHz,$CDCl_3$)d, ppm: 161.48 (N-C=O),153.32 (C_6H_5-H),126.13 [CH (pyridine)],110.63[CH(1-benzene)],18.66 (CH_3)

Activity study against P388 leukemia murine cells

An activity in-vitro anti-TB was analyzed by almar blue assay, on the basis of colour observation and percentage inhibition with the help of rifampicin as a positive control (standard).

RESULTS AND DISCUSSION

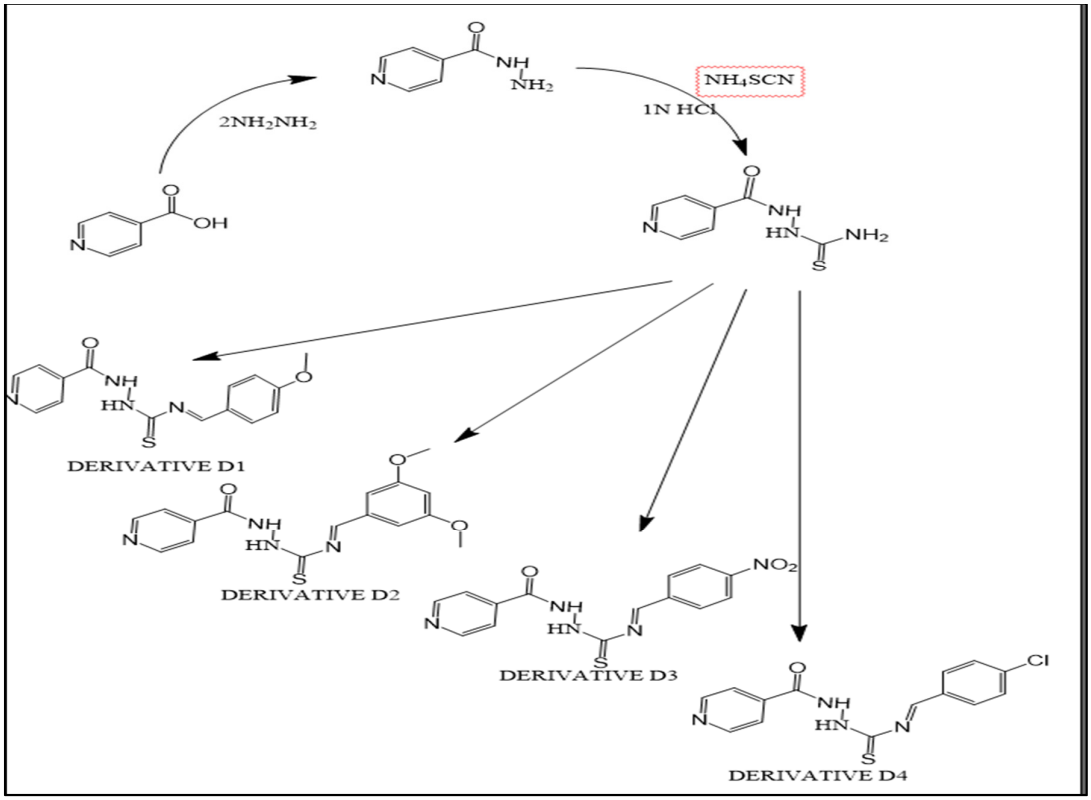

Figure 4. Synthesis pathway

In the Schiff base stage Aldehyde or ketone can be converted into Schiff base in reversible reaction that often proceeds when an A base or an acid catalysed the reaction or when the substance is heated. The carbonyl group's addition of a nucleophile is mechanism of Schiff base. In this case nucleophile is a amine. In the first phase of the process, amine combines with an aldehyde or ketone to form an unstable molecule known as carbinolamine. Through processes mediated by bases or acids, the carbinolamine loses water. As carbinolamine is an alcohol, it is dehydrated by an acid. The completion of reaction takes place after the removal of water molecule. The Schiff base synthesis is a series of two different types of reactions, namely addition and elimination. When Schiff base product treated with different aromatic amine to form different amide derivatives of Schiff base with elimination of water molecule.

Compounds D1, D2, D3, and D4 were successfully converted as evidenced by the presence of an N-H IR absorption band at around 3420-3500 cm−1. All compounds showed an absorption band of acetyl C=O at around 1744–1747 cm−1, and C=N at approximately 1567–1592 cm−1, in their infrared spectra.

Compound D1, D2, D3, and D4 have all had their structures verified by 13CNMR and mass spectrometry. The two spectrums are displayed in Figures 2 and 3.

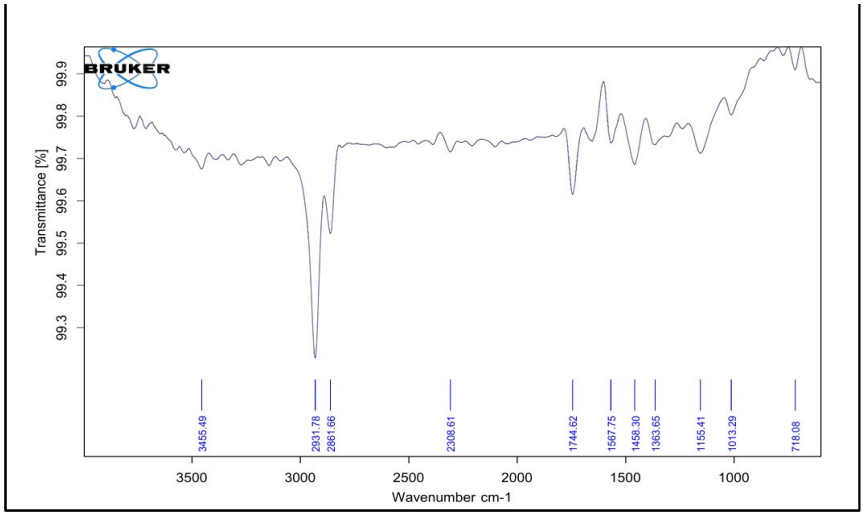

Fig. 1. IR Spectra of D1

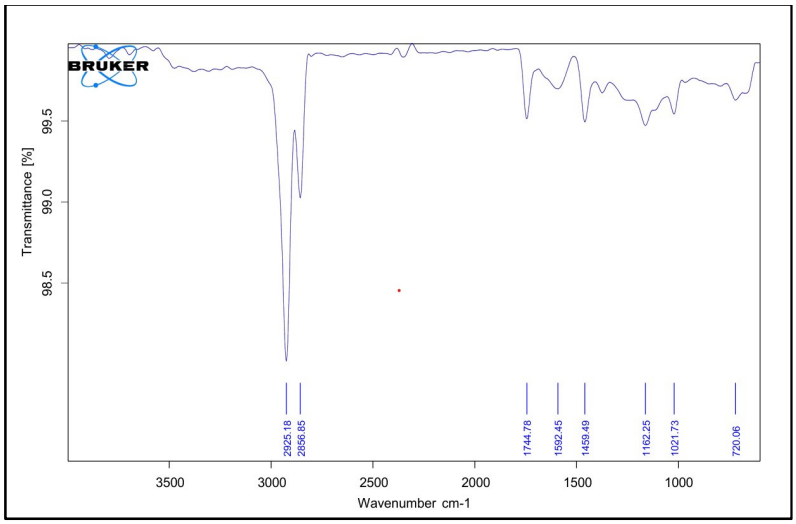

Fig. 2. IR Spectra of D2

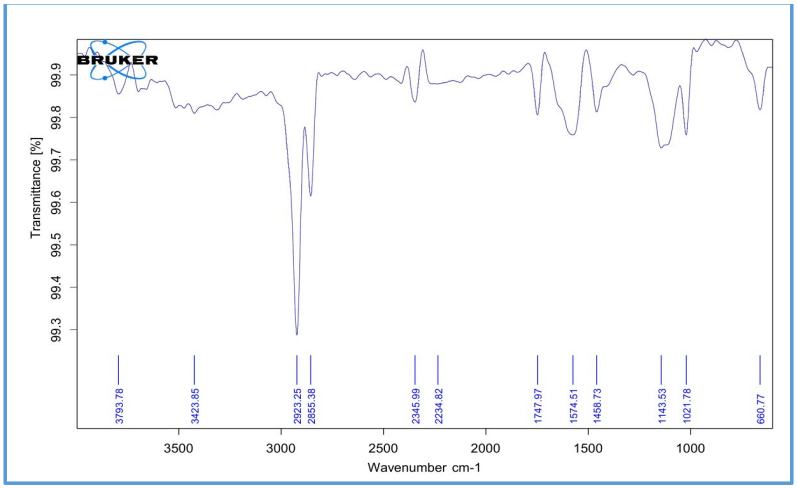

Fig. 3. IR Spectra of D3

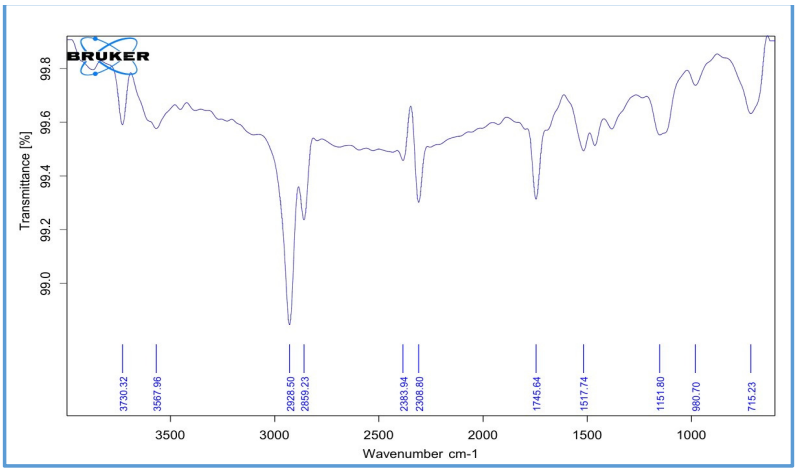

Fig. 4. IR Spectra of D4

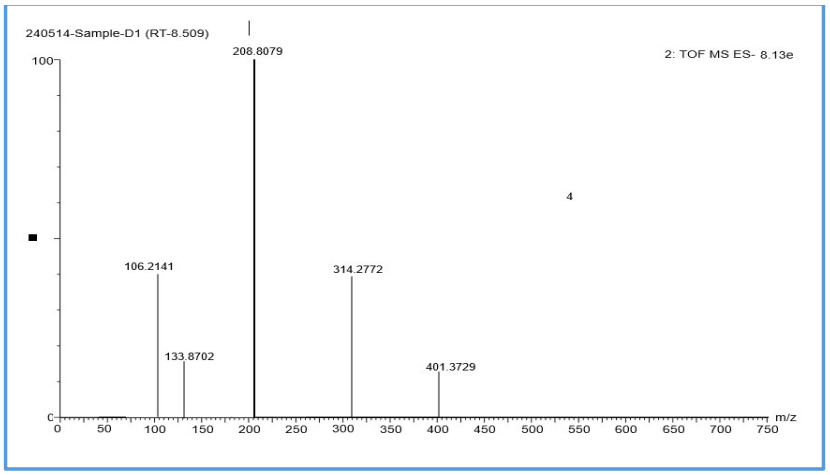

Fig. 5. Mass Spectra of D1

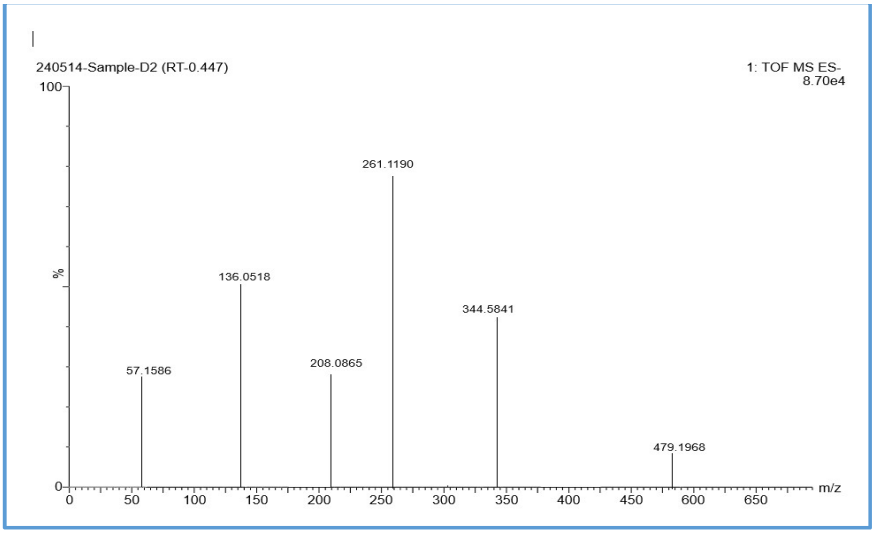

Fig. 6. Mass Spectra of D2

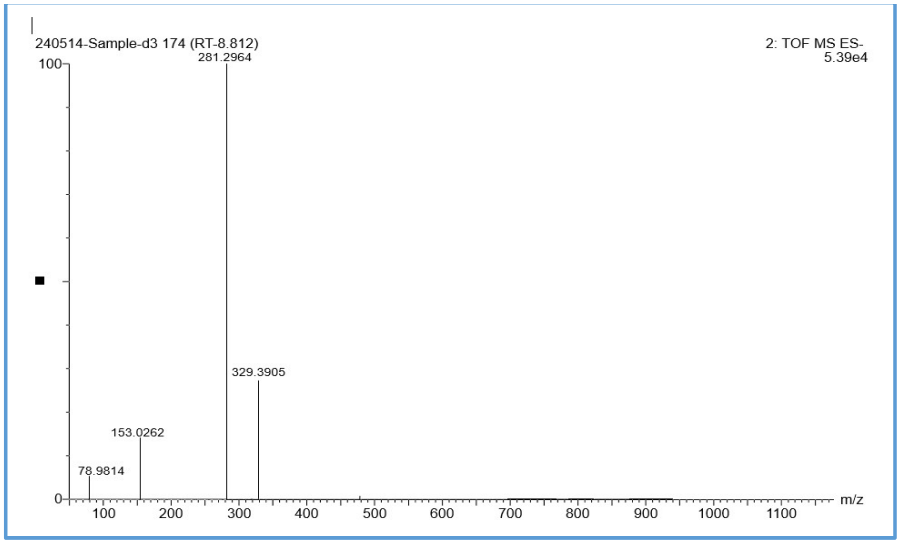

Fig. 7. Mass Spectra of D3

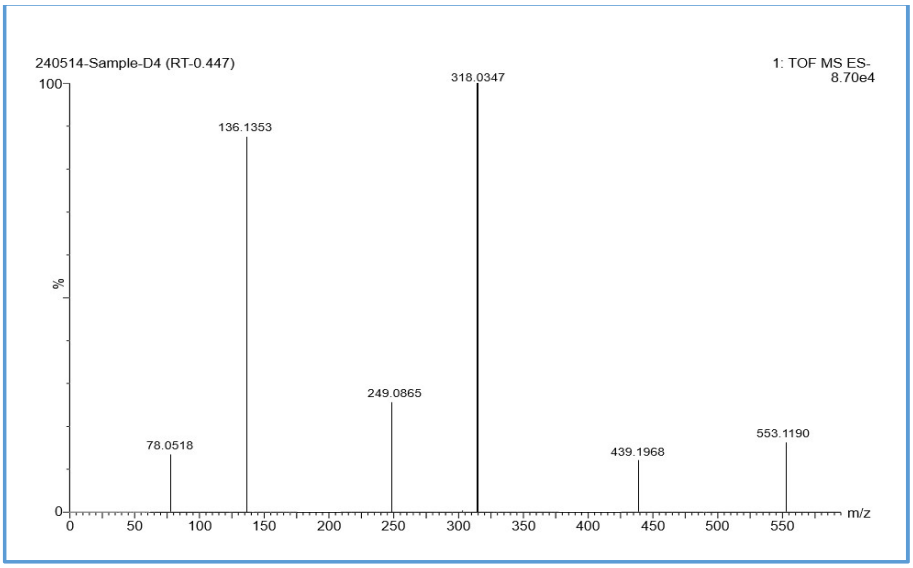

Fig. 8. Mass Spectra of D4

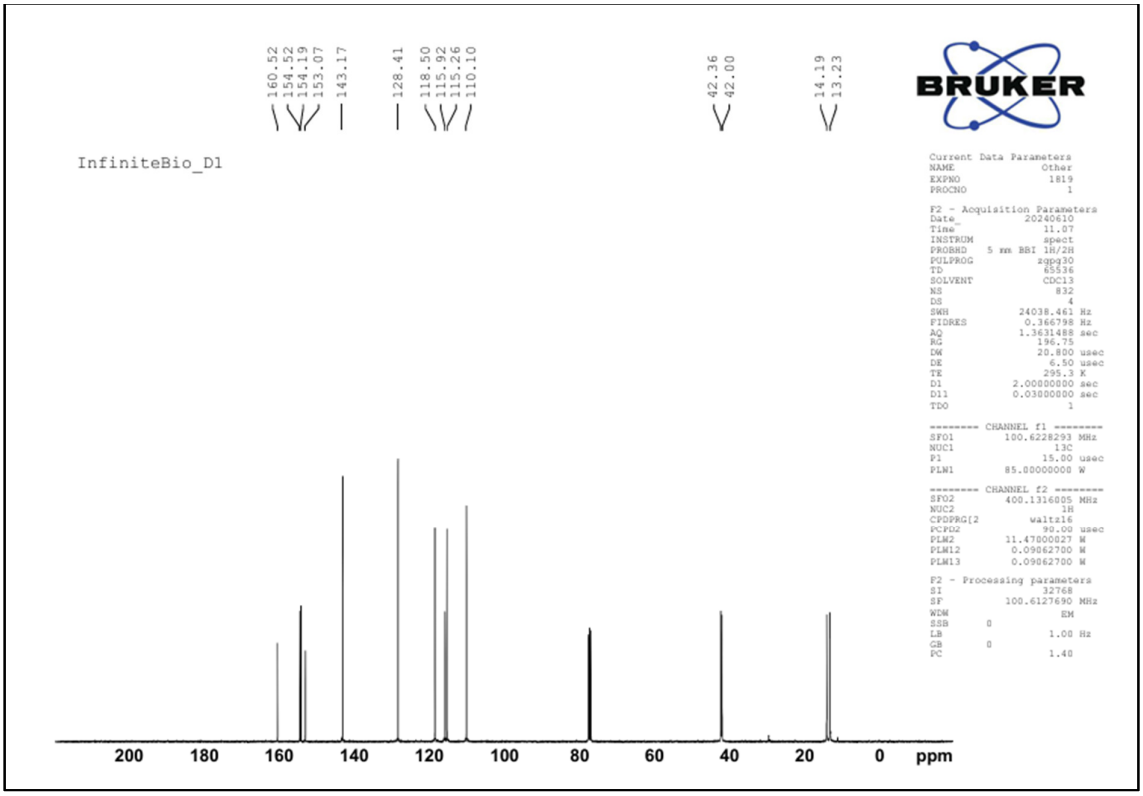

Fig. 9. ^{13}CNMR Spectra of D1

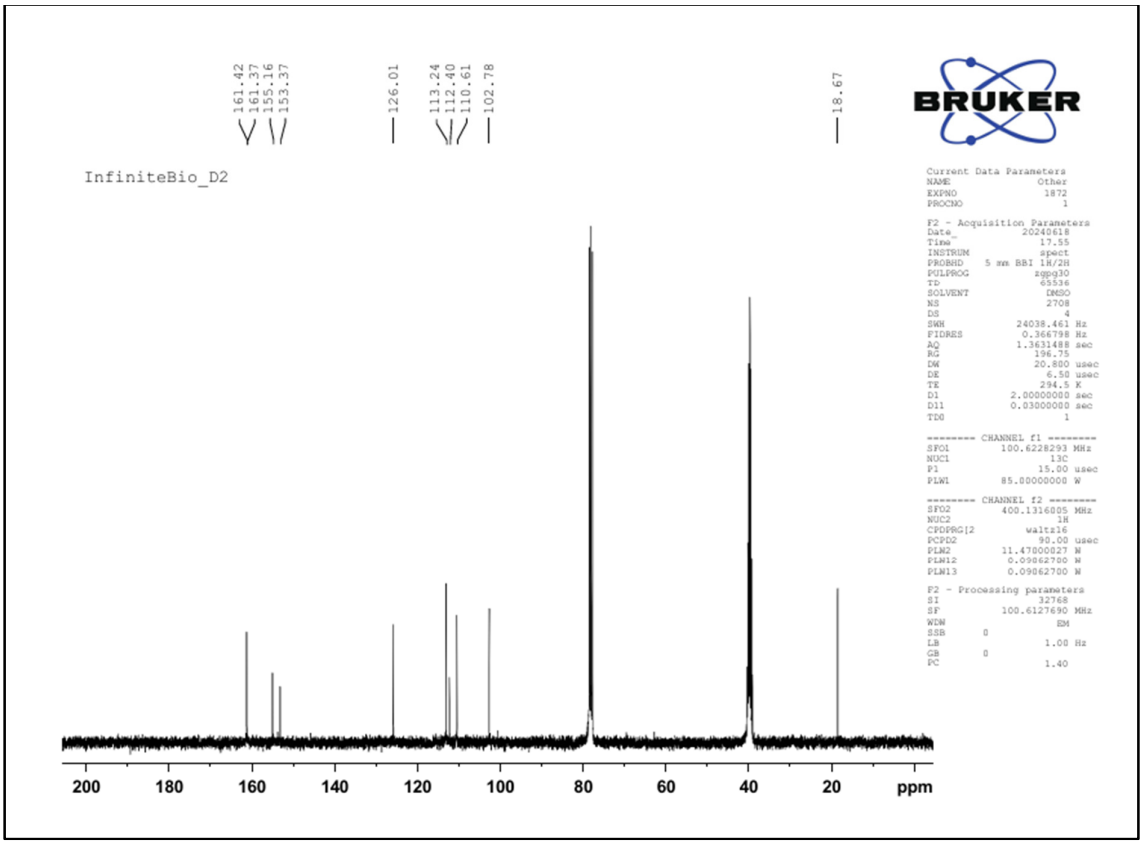

Fig. 10. ^{13}C NMR Spectra of D2

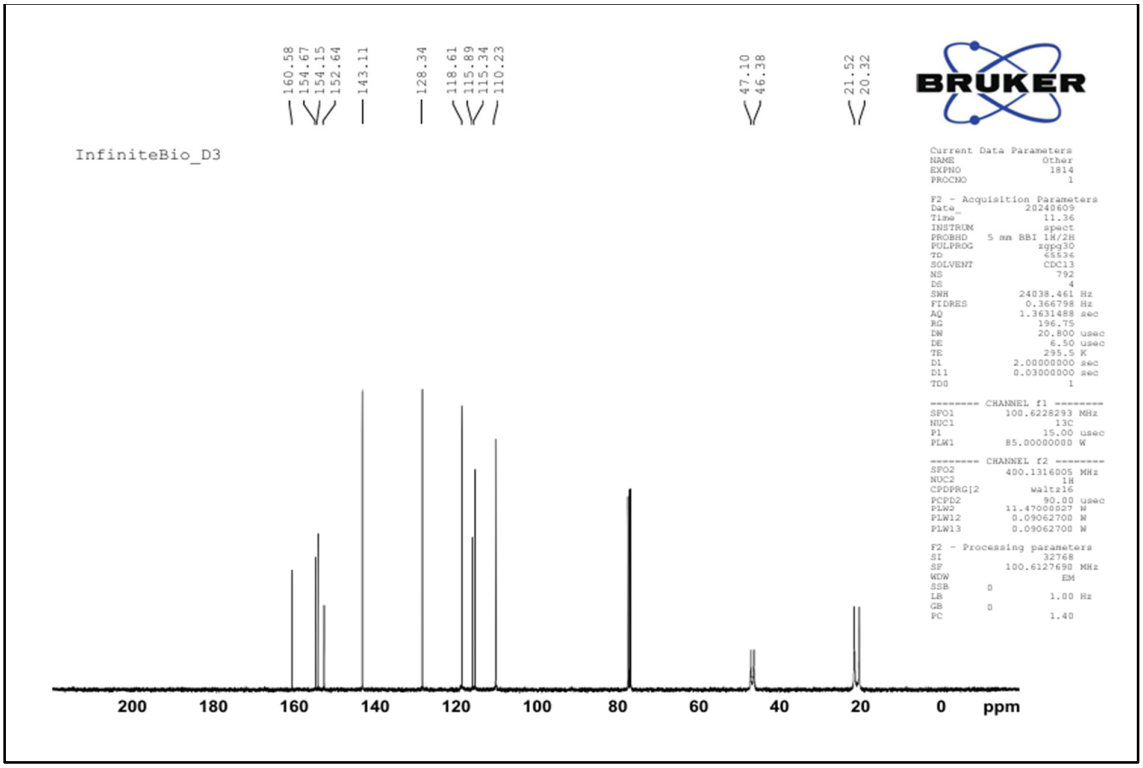

Fig. 11. ^{13}C NMR Spectra of D3

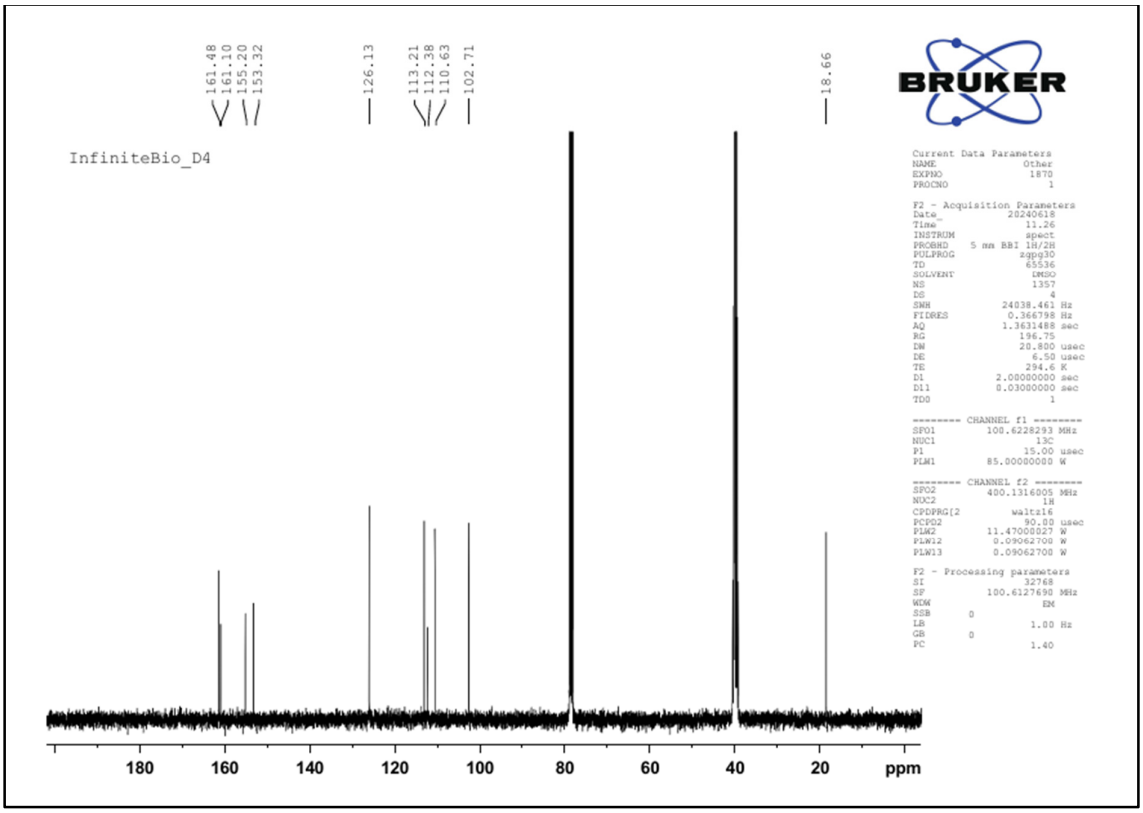

Fig. 12. ^{13}C NMR Spectra of D4

Table 1. The in-vitro anti-TB activity values of synthesized compounds

Sr. No.	Sample code	Concentra-tion(µg/ml)	Absorbance at 600 nm			Mean	%Of Growth Inhibition
			Test 1	Test 2	Test 3		
1	Growth c		1.603	1.603	1.603	**1.603**	
2	Standard	7.8	1.593	1.594	1.594	**1.594**	**NE**
	(Rifampicin)	15.6	1.538	1.538	1.538	**1.538**	**NE**
		31.2	1.421	1.421	1.421	**1.421**	**11.35%**
		62.5	1.397	1.398	1.397	**1.397**	**12.85%**
		125	0.834	0.834	0.834	**0.834**	**47.97%**
		250	0.611	0.611	0.611	**0.611**	**61.88%**
		500	0.531	0.531	0.531	**0.531**	**66.87%**
		1000	0.422	0.422	0.422	**0.422**	**73.67%**

3	D1	7.8	-	-	-	**-**	**-**
		15.6	-	-	-	**-**	**-**
		31.2	-	-	-	**-**	**-**
		62.5	-	-	-	**-**	**-**
		125	1.483	1.483	1.482	**1.482**	**7.54%**
		250	1.215	1.216	1.215	**1.215**	**24.20%**
		500	1.035	1.035	1.036	**1.035**	**35.43%**
		1000	0.977	0.976	0.977	**0.976**	**39.11%**
4	D2	7.8	-	-	-	**-**	**-**
		15.6	-	-	-	-	**-**
		31.2	-	-	-	**-**	**-**
		62.5	1.541	1.542	1.542	**1.541**	**3.86%**
		125	1.289	1.262	1.257	**1.269**	**20.83%**
		250	1.032	1.025	1.025	**1.027**	**35.93%**
		500	0.956	0.956	0.956	**0.956**	**40.36%**
		1000	0.732	0.732	0.732	**0.732**	**54.33%**
5	D3	7.8	1.474	1.474	1.474	**1.474**	**8.04%**
		15.6	1.460	1.460	1.460	**1.460**	**8.92%**
		31.2	1.420	1.420	1.420	**1.420**	**11.41%**
		62.5	1.235	1.235	1.235	**1.235**	**22.95%**
		125	1.574	1.574	1.574	**1.574**	**1.80%**
		250	0.943	0.943	0.943	**0.943**	**41.17%**
		500	0.730	0.730	0.730	**0.730**	**54.46%**
		1000	0.648	0.648	0.648	**0.648**	**59.57%**
6	D4	7.8	-	-	-	**-**	**-**
		15.6	-	-	-	**-**	**-**
		31.2	1.330	1.330	1.330	**1.330**	**17.03%**
		62.5	1.112	1.112	1.112	**1.112**	**30.63%**

		125	1.576	1.576	1.576	**1.576**	**1.68%**
		250	0.960	0.960	0.960	**0.960**	**40.11%**
		500	0.737	0.737	0.737	**0.737**	**54.02%**
		1000	0.650	0.650	0.650	**0.650**	**59.45%**

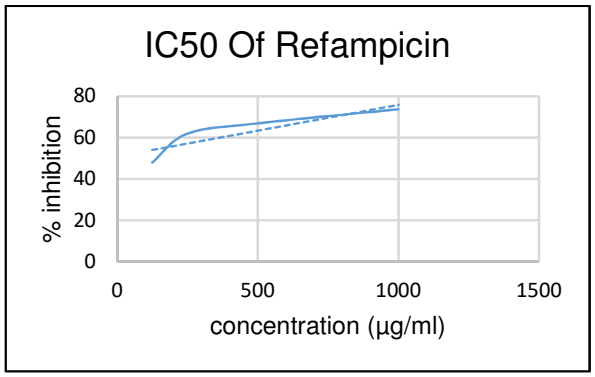

Fig. 13 IC50 value of rifampicin

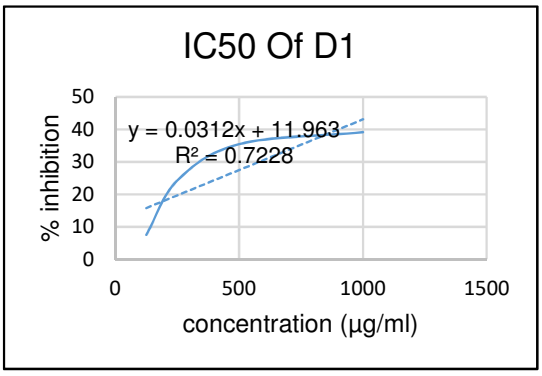

Fig. 14 IC50 value of Derivative D1

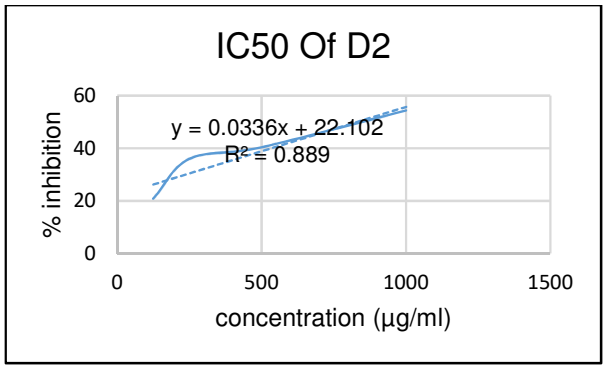

Fig. 15. IC50 value of Derivative D2

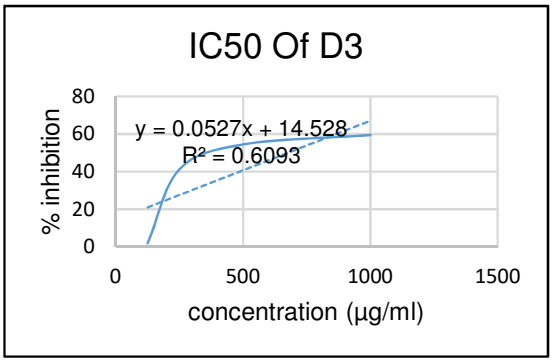

Fig. 16. IC50 value of Derivative D3

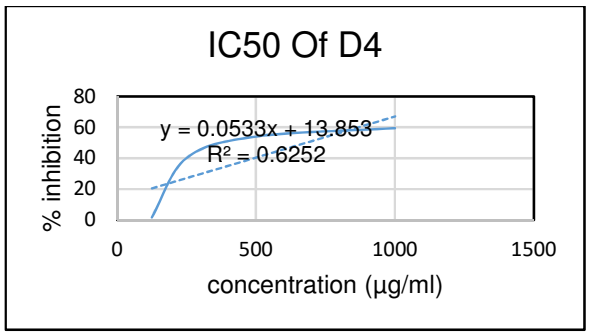

Fig. 17. IC50 value of Derivative D4

CONCLUSION

To create a novel heterocyclic moiety, the suggested synthesis approach is investigated. This is among the greatest examples of developing drugs, molecules, or heterocyclic synthesis. The isoniazid moiety is synthesized in a new, simple way in the current work using Schiff's base synthesis method. With an average yield ranging from 30% to 70%, isoniazid combines with ammonium thiocyanate in 1N HCl to form isonicotinoylthiosemicarbazide. This chemical then passes through Schiff's base reaction using both primary and secondary carbonyl compounds, such as aldehydes and ketones. Thin Layar Chromatography helps to monitor the reaction. Several chromatographic and spectroscopic techniques were used to forecast each produced molecule. In order to anticipate the synthesized derivatives, IR, NMR, and MASS spectroscopy were employed.

The Auto Dock Vina software was used to inspect and dock the synthesized derivatives. Since the protein 4F4Q Code is A M. smegmatis DprE1 crystal structure in conjunction covalently linked BTZ043 and FAD, it was selected from the protein data library due to its antitubercular characteristics. A table displaying the dock scores for each compound is found, and it is found that compound coding D1 has a dock value of -7.2.

The property of new synthesized derivatives against mycobacterium tuberculosis was examined. The addition of 4-nitrobenzaldehyde in proposed scheme it will gives derivative D3, resulting derivative having inhibitory concentration i.e. IC50 value is 11.41 which is nearly similar to the reference drug (Rifampicin) having IC50 value is 11.35. On the other side, with the replacing 4-nitrobenzaldehyde with other aldehyde which leads to differ in IC50 value.

The anti-tubercular profile of derivatives D1-D4 was analysed by almar blue assay; on the basis of colour change observation and % inhibitory concentration of derivative D3 is good anti-tubercular activity as compared with standard rifampicin drug.

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