SYNTHESIS OF NOVEL PYRIDINE-SCHIFF BASE DERIVATIVES FOR POTENTIAL TREATMENT OF TUBERCULOSIS

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Abstract

Heterocyclic compounds have occupied a prominent place among various classes of organic compounds by virtue of their diverse biological activities and chemistry. Pyridine is important pharmacophores in modern drug discovery. Among pyridine skeleton occupies a significant place in numerous bioactive molecules with wide range of activities like anti microbial, anticancer, analgesic, anti-inflammatory and anticonvulsant the aim is to synthesize a drug with better efficacy, less toxicity and fewer side effects. In view of the important biological activities in the present work an attempt has been made to synthesize some six novel pyridine Schiff base derivatives (AZ-1-AZ6) and evaluated the biological profile of these compounds.

The title compounds were evaluated for anti-TB activity using Microplate Alamar Blue Assay method, from the results revealed that these derivatives shown potent anti-TB activity when compare to standards pyrazinamide, streptomycin and isoniazid respectively

Keywords: Pyridine, Schiff base, diabetis mellitus, anti-diabetic agents.

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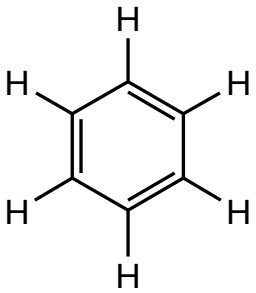
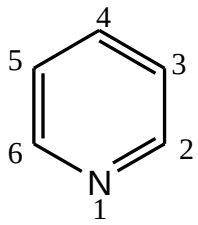
1. INTRODUCTION

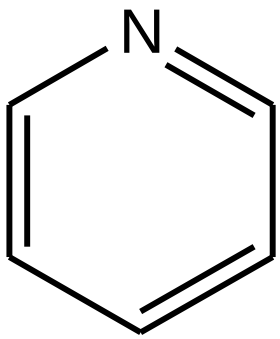
PYRIDINE:

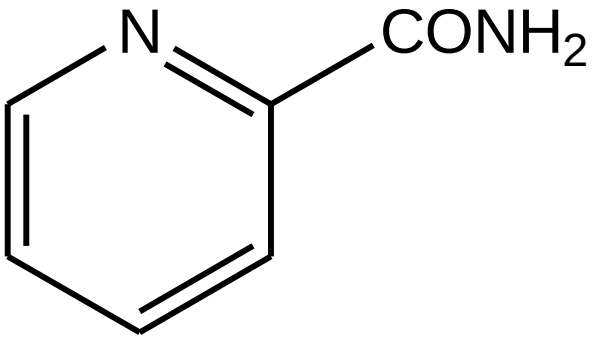
Pyridine is a basic heterocyclic organic compound of the chemical formula C5H5N. It is structurally related to benzene with one methine group (=CH-) replaced by a nitrogen atom. The pyridine ring occurs in many important compounds, including azines and the vitamins niacin and pyridoxal[1-2].





Pyridine is a six membered heterocyclic compound and after the discovery it gained attraction by researchers due to their diversified biological and pharmacological activities showing by them. . Pyridine moiety is observed in Vitamin B-complex, Antidiabetic drugs (Pioglitazone, Rosiglitazone), Neonicotinoids (eg. Imidacloprid, Acetamiprid). Nicotinic acid and its amide (Niacin) are part of the vitamin B complex. Furthermore, different congeners of azetidinone, thiadiazole, thiazolidinone have also reported to exhibit potent antifungal activities. Incorporating thiadiazole, thiazolidinone and azetidinone moieties at 2-position of pyridine nucleus with a hope to develop better antifungal agents. NAD and its derivative NADP are ubiquitous in living cells. They mediate hundreds of redox reactions and thus impact on virtually every metabolic pathway in the cell. Pyridine nucleotides are also key players in signalling through reactive oxygen species (ROS) since they are crucial in the regulation of both ROS-producing and ROS-consuming systems in plants. ChloroplastROS production is influenced by NADP+:NADPH ratios.Mitochondrial NAD(P) status is important in determiningROS formation by the respiratory electron transport chain,and NAD(P)H oxidases are key players in the generation of ROS at the plasmalemma. Similarly, ROS processing partly depends on ascorbate and glutathione, and redox flux through these pools is maintained by NAD(P)H. Pyridine was produced from coal tar and a by-product of the coal gasification. Pyridine is miscible with water and virtually all organic solvents it is weakly basic and with hydrochloric acid it forms a crystalline hydrochloride salt, melts at 145–147 °C[3-8]. In organic reactions pyridine behaves both as a tertiary amine undergoing protonation, alkylation, acylation, and N-oxidation at the nitrogen atom and as an aromatic compound, undergoing nucleophilic substitutions. The name pyridine is derived from the Greek word and is the combination of two words “pyr” means fire and “idine” is used for aromatic bases. The first pyridine base was isolated in 1846 by Anderson, picoline . After quit a long time its structure was determined by Wilhelm Korner in 1869 and James Dewar in 1871 independently. It was suggested that the structure of pyridine might be analogous to quinoline and naphthalene. It was concluded that pyridine has been derived from benzene and its structure might be obtained by replacing a CH moiety with a nitrogen atom. In the year 1876, William Ramsay synthesized this compound by combining acetylene and hydrogen cyanide, a red-hot iron-tube furnace was used to carry out the reaction. It was the ever first synthesis of a hetero-aromatic compound. Pyridine became an interesting target in 1930 with the importance of niacin for the treatment of dermatitis and dementia.



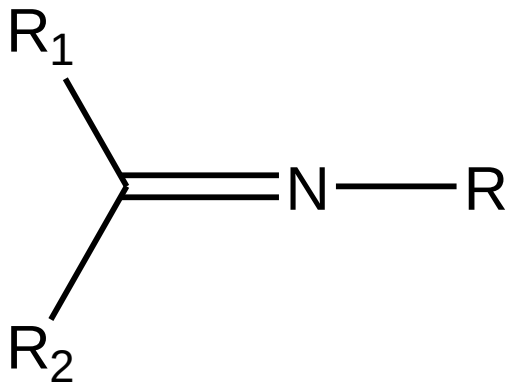


Pyridine derivatives: Picoline and Niacin.

Electrophilic substitutions;
Many electrophilic substitutions on pyridine either do not proceed or proceed only partially,however, the heteroaromatic character can be activated by electron-donating functionalization. Common alkylations and acylations, such as Friedel–Crafts alkylation or acylation, usually fail for pyridine because they lead only to the addition at the nitrogen atom. Substitutions usually occur at the 3-position, which is the most electron-rich carbon atom in the ring and is, therefore, more susceptible to an electrophilic addition.

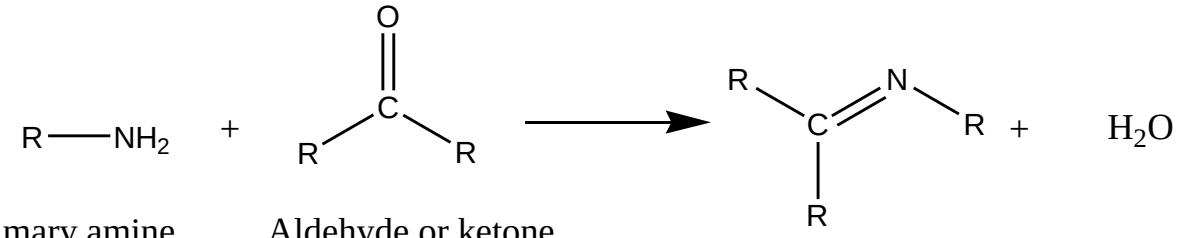
- Nitrogen containing six membered aromatic pyridine and its derivatives abundantly exist in nature and they play a vital role in the field of heterocyclic chemistry ,Such compounds are widely used for many **applications in medicinal science as below,**
- Pyridine is used as a precursor to agrochemicals and pharmaceuticals and also act as an important solvent and reagent. Pyridine is used in the in vitro synthesis of DNA, in the synthesis of sulfa pyridine is a drug against bacterial and viral infections, antihistamicdrugs tripelennamine and mepyramine, bactericides, and herbicides as well as water repellents.
- Pyridine derivatives are an important class of azaheterocycle found in many natural products, active pharmaceuticals, and functional materials.Pyridine derivatives of different heterocyclic nucleus have shown potent pharmacological properties like, antitubercular, antifungal antibacterial, antimicrobial, insecticidal etc.
- Organic reactions promoted by a solid heterogeneous catalyst have attracted wide spread interest and are advantageous because of operational simplicity, high selectivity and clean separation of the product. Metal oxides impregnated over silica has been recognized as a remarkably useful green heterogeneous catalyst to promote a wide range of organic reactions .
- Rapid and green approach to achieve highly substituted Azetidinones with pyridine in excellent yields in the presence of catalytic amount of SiO2/Fe2O3 under controlled. Pyridine derivatives of different heterocyclic nucleus have shown very important pharmacological properties like antifungal, antitubercular, antibacterial, antimicrobial and insecticidal etc. Azetidinone moieties at 4-position of pyridine nucleus with a hope to develop better antifungal agents. These compounds have been screened for their antifungal activity,Such biological activities include antimicrobial, anti tubercular, anti inflammatory, anticonvulsant, local anaesthetics and also hypoglycaemic agents[9-10].

SCHIFF BASE:
The condensation of primary amines with carbonyl compounds was first reported Schiff and the condensation product are often referred to as Schiff bases. The general structure of these bases is given below ,Where R, R1 and R2 are H, alkyl, cyclohexyl, aryl or heterocyclic readicals, which may be variously substituted.



Schiff bases are also known anils, imines or azomethines. Various studies have been shown that the >C=N group has considerable biological importance, This can be substantiated by the fact that the possibility of having a lone pair of electrons in either a π or SP2 hybridized orbital on trigonally hybridized nitrogen in the >C=N group. Schiff bases appear to be an important intermediate in a number of enzymatic reactions involving interaction of an enzyme with an amino or a carbonyl group of the substrate. One of the most important types of catalytic mechanism is the biochemical process which involves the condensation of a primary amine in an enzyme usually that of a lysine residue, with a carbonyl group of the substrate to form an imine, or Schiff base, synthesis of a new series of substituted 2-aminopyridine derivativesis a pyridine derivatives continue to attract great interest due to the wide variety of interesting biological activities observed in these compounds, such as anticancer, analgesic, antimicrobial, and anti depressant,activities.

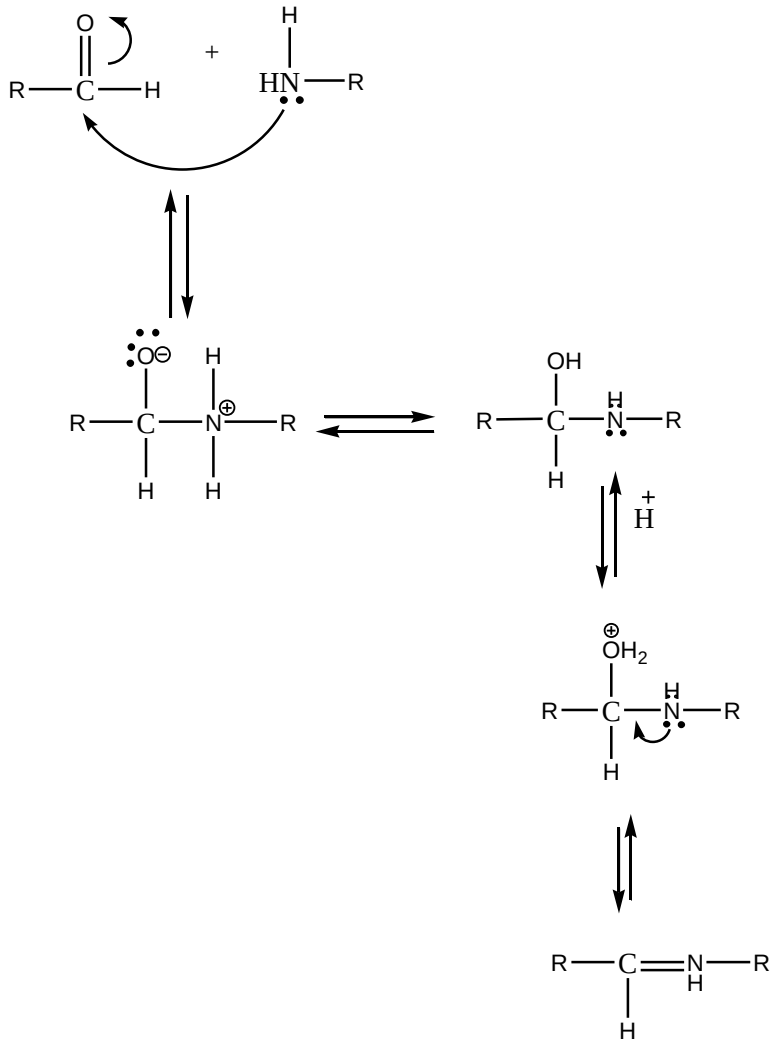
A Schiff base is a nitrogen analog of an aldehyde or ketone in which the C=O group is replaced by C=N-R group. It is usually formed by condensation of an aldehyde or ketone with a primary amine according to the following scheme:



Primary amine Aldehyde or ketone Schiff base

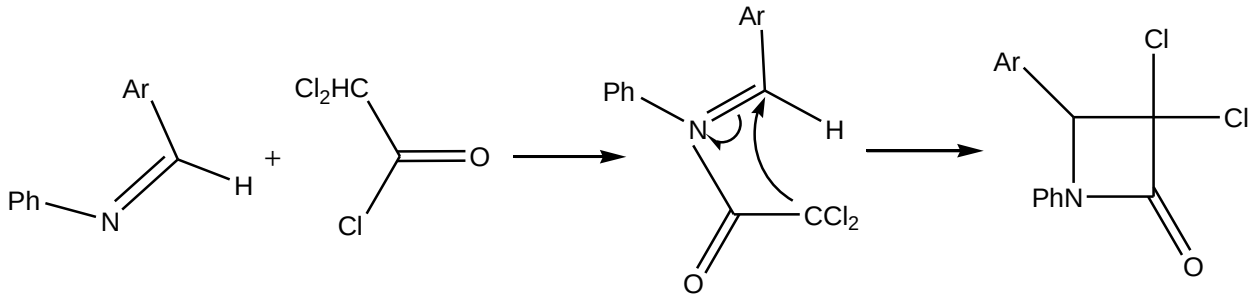
Where R, may be an alkyl or an aryl group. Schiff bases that contain aryl substituents are substantially more stable and more readily synthesized, while those which contain alkyl substituents are relatively unstable. Schiff bases of aliphatic aldehydes are relatively unstable and readily polymerizable1,2 while those of aromatic aldehydes having effective conjugation are more stable.

The mechanism of Schiff base formation is another variation on the theme of neucleophilic addition to the carbonyl group. In this case, the neucleophile is the amine. In the first part of the mechanism, the amine reacts with the aldehyde or ketone to give an unstable addition compound called carbinolamine.The carbinolamine loses water by either acid or base catalyzed pathways. Since the carbinolamine is an alcohol, it undergoes acid catalyzed dehydration.



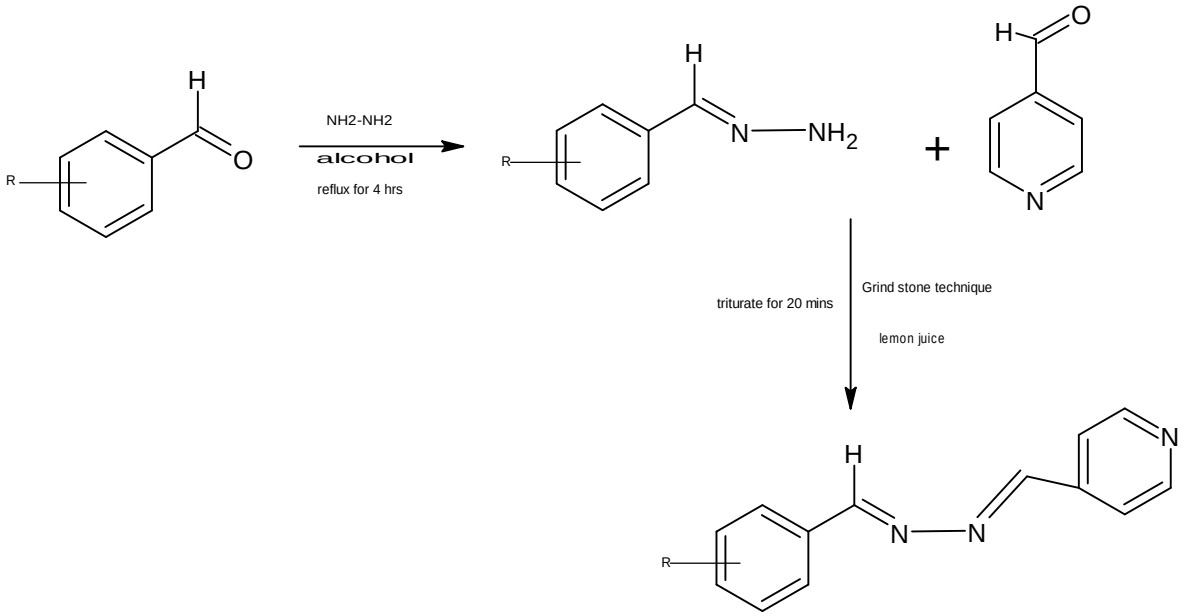
Schiff bases have a large number of synthetic uses in organic chemistry. Acylation of Schiff bases by acid anhydrides, acid chlorides and acyl cyanides is initiated by attack at the nitrogen atom and leads to net addition of the acylating agent to the carbon-nitrogen double bond. Reactions of this type have been put to good use in natural product synthesis.

Also the base catalyzed condensation of acetyl chlorides (bearing an electron withdrawing group and at least one hydrogen atom at the α-position) with *N*-arylaldimines occurs by initial acylation at the nitrogen atom and leads to β-lactams of interest in penicillin chemistry[11-15].



Experimental work

Scheme



Schiff base pyridine derivatives(AZ-1-AZ-6)

Table no. 1

SL.NO.	COMPOUND CODE	R
1	AZ-1	-H
2	AZ-2	*P*-Cl
3	AZ-3	*O*-OH
4	AZ-4	3-OCH_3, 4-OH
5	AZ-5	*P*-NO_2
6.	AZ-5	*o*-CH3

Procedure

1. Equimolar concentrations of benzaldehyde and hydrazine hydride are taken in RBF. To this add 25mL of alcohol and reflux mixture for 4hrs. The resultant mixture was concentrated ,cooled and poured in crushed ice.The solid mass thus seperated out was filtered ,dried and recrystallised from methanol.Yield -77%,Melting point-152-154.
2. The Schiff based pyridine was prepared by taking equimolar mixture of intermediate with pyridine-4-carbaldehyde. Transfer into motar and pestle add 2 mL of lemon juice and triturate for 20 mins. The formed final derivatives are filtered dried and recrystalized by alcohol.

Biological evaluation
Invitro anti-TB activity

MICROPLATE ALAMAR BLUE ASSAY:
The anti mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA).This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method. Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation

of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days.After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. Finally the readings were noted based on the visual colour change. Pink colour in the well indicates growth of the bacteria and blue colour indicates no bacterial growth.

The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.The minimum inhibitory concentrations of the synthesized derivatives against Mycobacterium tuberculosis obtained through Microplate Alamar Blue Assay method were represented below:

2. RESULTS AND DISCUSSIONS

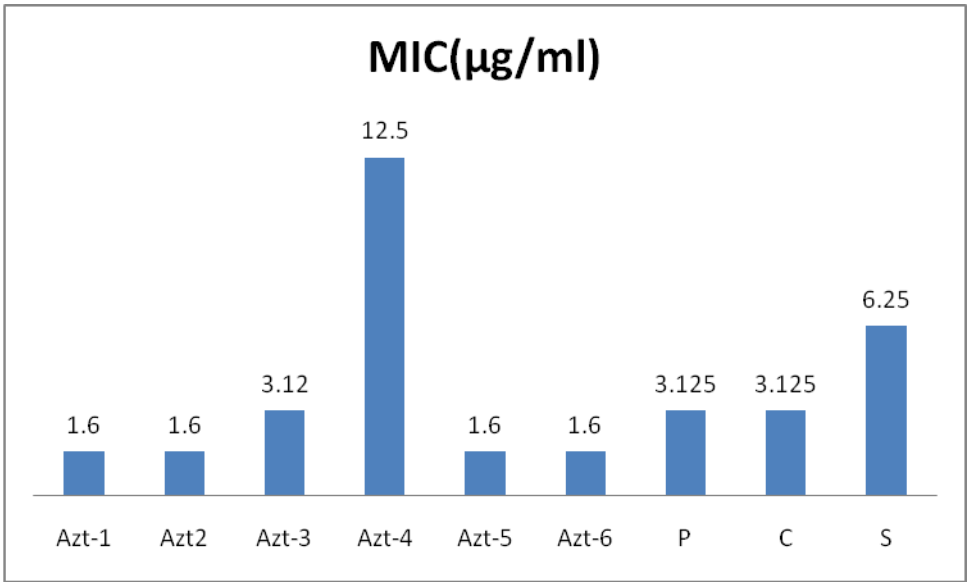
IN VITRO ANTI-TUBERCULAR ASSAY

The anti-tubercular activity of the synthesized derivatives was performed by Microplate Alamar Blue Assay. The results of the assay were as follows:

Table no.2

Sl. No.	samples	**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**
1	AZ-1	S	S	S	S	S	S	S	R
2	AZ-2	S	S	S	S	S	S	S	R
3	AZ-3	S	S	S	S	S	S	R	R
4	AZ-4	S	S	S	S	R	R	R	R
5	AZ-5	S	S	S	S	S	S	S	R
6	AZ-6	S	S	S	S	S	S	S	R

Where
S-Sensitive
R-Resistance
For anti-TB standard drugs are performed the values are as follows
Pyrazinamide **3.12 µg/ml** Streptomycin **6.25 µg/ml**
Isoniazid **3.12 µg/ml**



P=Pyrazinamide , C=Ciprofloxacine, S=Streptomycine.

Figure 1: M. tuberculosis MIC values obtained for the synthesized derivatives through MABA

3. DISCUSSION

In the present research work, based on the wide literature survey, novel derivatives of pyridine containing azetidinone derivatives were synthesized in simple two-step facile procedure
All the six derivatives were screened for their *in-vitro* anti-tubercular activity using Microplate Alamar Blue Assay method

The anti-tubercular activity of the synthesized derivatives, Azt1-6 was carried out using MABA (Microplate Alamar Blue Assay) against *Mycobacterium tuberculosis* (H37 RV strain) at various concentrations from 100 to 0.8 µg/ml (100, 50, 25, 12.5, 6.25, 3.12, 1.6, and 0.8 µg/ml). Out of all six derivatives Azt1, Azt2 and Azt5 shown more potent activity than standards. Azt3,Azt4 shown equipotent activity when compared to standard. good to excellent activities with similar minimum inhibitory concentrations compared to that of the standard drugs pyrazinamide, streptomycin, and ciprofloxacin.

4. CONCLUSION

Novel derivatives of pyridine containing azetidinone derivatives were synthesized using grindstone technique and conventional methods. All the synthesized compounds were identified by performing their melting point and TLC check thenovel pyridine containing azetidinone derivatives and characterized by IR spectroscopy, ^{1}H-NMR spectroscopy, and Mass spectrometry. Later all the derivatives were screened for their *in-vitro* anti-tubercular activity using Microplate Alamar Blue Assay method, anti-bacterial and anti-fungal by agar well diffusion method.

In vitro anti-tubercular assay revealed that the derivatives Azt-1, Azt-2 and Azt-5 ,Azt-6 showed more potent anti-tubercular activity and posses with lower minimum inhibitory concentration than the standard drug pyrazinamide, The minimum inhibitory concentrations of derivatives Azt-3 &Azt-4 are shown equipotent activity than standards .

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