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DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL HETEROCYCLIC DERIVATIVES AS ANTI-TUBERCULAR AGENTS

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ABSTRACT:

Heterocyclic structures are found to have specific properties and biological importance in research and development. (1) Imidazole, a fused diazole heterocyclic structure and its derivatives have occupied a unique place in the field of medicinal chemistry (2).The high therapeutic properties of the imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents (3). Recent studies report that benzimidazole based Schiff bases possess antibacterial, antimicrobial, anti-tubercular, and anti-inflammatory activities. (17) Tuberculosis is a serious threat to public health throughout the world.A series of benzimidazolebased molecules were designed and docked against crucial mtb enzyme target Cyclopropanemycolic acid synthase2. The molecules with good docking-score and multiple interactions were chosen for synthesis.Synthesis is carried out using microwave irradiation technique; the compounds are characterized by spectrometric analysis.Anti-tubercular activity was carried out by usingMicroplateAlamar Blue Assay (MABA) method.

KEY WORDS: Imidazole, Mycolic acid, Anti-tubercular,MicroplateAlamar Blue Assay

INTRODUCTION:

Mycobacterium tuberculosis (MTB) or Tubercle bacillus (TB) is the organism that is the causative agent for tuberculosis. [5]The cell wall is a major virulence factor of Mycobacterium tuberculosis and contributes to its intrinsic drug resistance.[16] Identification of the components of the uptake and secretion machinery across this membrane is critical for understanding the physiology and pathogenicity of Tuberculosis and for the development of better anti-tuberculosis drugs.[6]

The cyclopropane synthase CmaA2 and other enzyme are the part of FASII pathway for the biosynthesis of mycolic acids in mycobacteria and Cyclopropane synthases as an attractive target for the new drug development.This makes the prospect of a single drug effectiveness against multiple targets is highly possible, so that the chance for the development of drug resistance is less. [7]

Imidazole is a planer five-member heterocyclic ring with 3C and 2N atom and in ring N is present in 1st and 3rd positions. The imidazole ring is a constituent of several important natural products, including purine, histamine, histidine and nucleic acid. [8]Fig: 1

Benzimidazole is a bicyclic compound consists of the fusion of benzene and imidazole. The most prominent benzimidazole compound nature is *N*-ribosyl-dimethylbenzimidazole, which serves as an axial ligand for cobalt in vitamin B_{12}. [9] Fig.2

On the basis of various literature surveys Imidazole and Benzimidazole derivatives showed various pharmacological activities.[21],Anti-tubercular activity. [22], Anti-fungal and Anti-bacterial activity, Anti-inflammatory activity and analgesic activity, Anti-depressant activity,and Anti-cancer activity. [23]

MATERIALS AND METHODS:

The drug design, synthesis, characterization and Biological evaluation of the synthesized molecules were carried out.

Drug design

Argus lab 4.0 is distributed freely available for windows platforms by Planaria Software. It is an introductory molecular modeling package with academics. [10]

Drug likeness

Druglikeness is a qualitative concept used in drug design for how "druglike" a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested. [11]

Lipinski's rule of five also known as the **Pfizer's rule of five** or simply the **Rule of five** (RO5) is to evaluate druglikeness or determine if a chemical compound with acertain

pharmacological or biological activity has properties that would make it alikely orally active drug in humans.[12]Fig.3

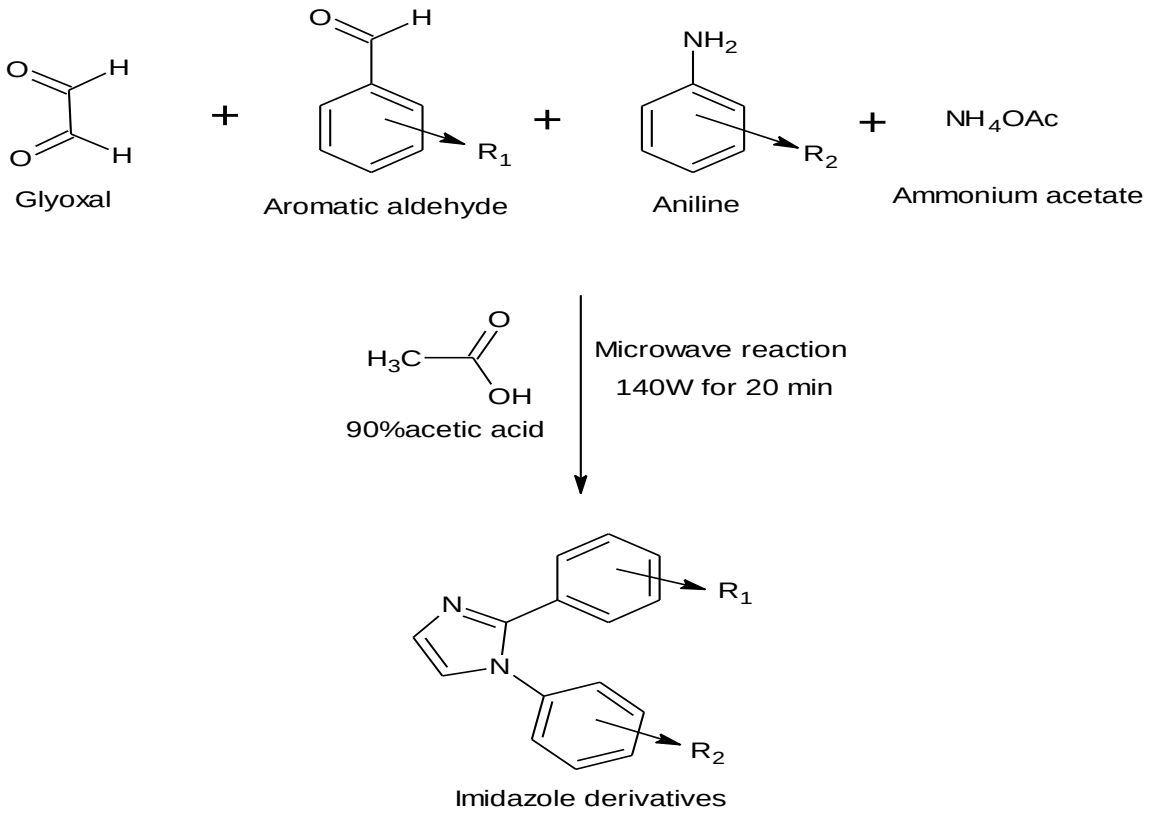
Toxicity risk assessment

All the docked molecules are subjected to the toxicity risk assessment by using Osiris program, which is available online.Prediction results are color coded in which the red color shows high risks with undesired effects like mutagenicity or a poor intestinal absorption and green color indicates drug-conform behavior.[13]

EXPERIMENTAL PROCEDURE:

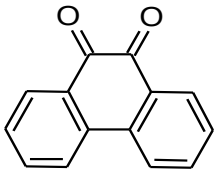
SCHEME1:

A Mixture of 1 mol of Glyoxal, 1 mol of Substituted aromatic aldehyde and 1 mol of primary amine and 0.1 mol of Ammonium acetate is taken in an Erlenmeyer flask and it is subjected to microwave irradiation. On completion of reaction was monitored by TLC at an interval of 30 seconds. The sticky mass transferred to acetone: water (6:4) mixture. The obtained Precipitated was filtered, dried and recrystallized. Finally purified by using columnchromatography.[14]



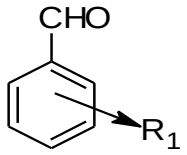
SCHEME2:

A Mixture of 1 mol of Phenanthrene 9, 10-dione, 1 mol of Substituted aromatic aldehyde and 2 mol of Ammonium acetate is taken in a round bottom flask and Poly ethylene glycol act as a Catalyst and they subjected to reflux condenser. Completion of reaction was monitored by TLC at an interval of 30 minutes. The sticky mass transferred to acetone: water (6:4) mixture. The obtained Precipitated was filtered, dried and recrystallized. Finally purified by column choromatography.[53]



Phenanthrene 9,10 Quinone

+

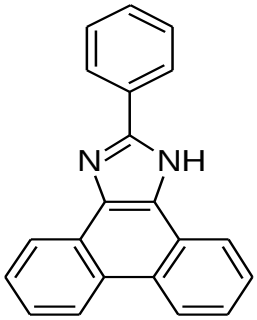


Benzaldehyde derivatives

+ NH_4OAc

Ammonium acetate

PEG | MICROWAVE REACTION 5 to 8 min



Imidazole derivative

SPECTRAL ANALYSIS:

4-[1H-Phenanthro (9,10-d) imidazole-2-yl] Phenol(B1): IR- using KBR pellet technique : 3409.90cm^{-1}(-OHstr),1257.50Cm^{-1}(-C=Nstr),1650.94Cm^{-1}(C=Cstr),1257.50Cm^{-1}(C-Cstr).^{1}HNMR:δ(1.2ppm,singlet,4H)(3.61-3.63ppm,multiplet,3H),(6.93-6.95ppm,doublet,3H),(7.76-7.78ppm,multiplet,3H),(9.84ppm,singlet,1H).MASS:m/z 311.05(M+).Fig.4

2-(4-methoxy phenyl)-1H-Phenanthro (9,10-d) imidazole(B2):IR-3409.90cm^{-1}(-OH str), 1296.07cm^{-1}(N-H str), 1627.80cm^{-1} (-C=C- str), 1249.78cm^{-1}(-C-C- str), 2368.41cm-^{1}(-C=N-

str).NMR: δ(3.4-3.8 ppm,multiplet,6H),(4.2 ppm,singlet,1H),(6.7- .7ppm,multiplet,9H).MASS: m/z 325.02(M+).Fig.5

3,5-dichloro-4-[2-(2-nitrophenyl)-1H-imidazol-1-yl]pyridine(R4):IR-1272.93cm^{-1}(-C-N-Str),1620.09cm^{-1} (-C=C- Str), 1195.78cm^{-1}(-C-C-Str), 3109.02cm^{-1}(-C-H Str (aromatic), 2360.70cm^{-1}(-C=N- Str), 786.90cm^{-1}(C-Cl (Halogens).NMR:δ(,5.22ppm,Singlet,1H),(7.25-8.24, Multiplet,6H),(10.49, Singlet,1H).MASS:336.02(M+)

4-(2-cyclohexyl-1H-imidazol-1-yl)pyridine(R2): IR-1272.93cm^{-1}(-C-N- Str), 1620.09cm^{-1}(-C=C-Str), 1195.78cm^{-1}(-C-C- Str), 2360.70cm^{-1}(-C=N-Str), 3109.02cm^{-1}(-C-H Str (aromatic), 2923.87cm^{-1}(-C-H Str (aliphatic).MASS:228.01(M+).

1-{4-[2-(4-methoxyphenyl)-1H-imidazol-1-yl]phenyl}methanamine(R1a):IR-3271.03cm^{-1},(-NH_2Str(1□ amine), 1242.07cm^{-1}(-C-N- Str), 1612.37cm^{-1}(-C=C- Str), 1188.06cm^{-1}(-C-C- Str), 2314.41cm^{-1}(-C=N- Str), 3078.16cm^{-1}(-C-H Str (aromatic).NMR:δ (2.0ppm,singlet,1H), (3.8ppm,singlet,2H), (4.8ppm,singlet,6H),(6.8-8.1ppm,multiplet,9H).MASS:279.28(M+).

IN-VITRO ANTI-TB ACTIVITY USING ALAMAR BLUE DYE:

PROCEDURE [18],[19]

The anti-mycobacterial activity of compounds were assessed against M.tuberculosis using microplateAlamar Blue assay (MABA).This methodology is non-toxic, uses a thermally stable reagent and shows Good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrooks 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 Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

RESULT AND DISCUSSION:

The present study described a (Radziszewski reaction) condensation between a aromatic aldehyde and primary amine by using experimental protocol as shown in scheme 1.

The structures of all the final derivatives were established on the basis of spectral analysis. IR spectrum of the **4-[1H-Phenanthro (9,10-d) imidazole-2-yl] Phenol(B1):** showed a strong absorption band at 3409 cm–1 corresponding to OH and absorption at 1257 Cm^{-1} and 1296 cm-1 corresponding to C=N,NH in imidazole. The 1HNMR spectra showedthe singlet peak at d 1.2, was assigned to NH. The singlet peak was at δ 9.8 corresponding toimidazole NH. The doublet for vicinal protons has beenseen along with the aromatic multiplet between δ 6.3and δ 6.9 ppm. Mass spectrum of compound B1 revealed the molecularion peak [M+] at m/z 311.05 corresponding to the molecularmass of the compound.

In–vitro anti-tubercular activity

All the compounds showed good and moderate activity against mycobacterium tuberculosis, The inhibition of growth of bacteria was measured in microgram (µg) and nanogram (ng). Out of the synthesized derivatives R4, R1a, shows good activity at the range of 0.8µg and B1, B2 and R2 shows activity at 12.5µg.Table.2,3,4.

CONCLUSION:

The present study concludes that, the synthesize of novel imidazole and benzimidazole derivatives by both conventional and microwave assisted method. Synthesized molecules were effective in inhibiting enzyme CyclopropaneMycolic acid Synthase-2 (1KPI), which is important forthe growth of Mycobacterium tuberculosis Cell wall. There is correlation between the score and activities of all the compounds which were tested and compared with the standard drugs. This goes to prove that CyclopropaneMycolic acid Synthase-2 (1KPI) is a critical enzyme for anti-mycobacterial activity. Further structural refinement to the structure of the synthesized compounds is expected to yield promising molecules against the pathogen Mycobacterium tuberculosis.

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Table .1: It shows physical properties of compounds:

S.I No	MolWt	Melting point	M.Formula	Solubility	Colour	Yield	Molar refractivity
B1	310.34	308 □C	$C_{21}H_{14}N_2O$	Chloroform, Ethanol	Dark Brown	85%	98.77±0.3cm3
B2	324.16	86 □C	$C_{22}H_{16}N_2O$	Chloroform, Ethyl acetate	Light Brown	81%	103.57±0.3cm 3
R4	335.14 g/mol	118 □C	$C_{14}H_8Cl_2N_4O_2$	Chloroform, ethyl acetate	Dark brown	80%	84.34±0.5cm3
R2	227.27	68 □C	$C_{14}H_{17}N_3$	Chloroform, Ethyl acetate	Dark brown	60%	69.48±0.5cm3
R1a	279.33 6g/mol	52_C	C17H17N3O	Chloroform.	Brown.	65%	83.37±0.5cm3

Table.2: It shows Anti-microbial activity of compounds

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	0.2 µg/ml
1	B1	S	S	S	S	R	R	R	R	R
2	B2	S	S	S	S	R	R	R	R	R
3	R4	S	S	S	S	S	S	S	S	R
4	R2	S	S	S	S	R	R	R	R	R
5	R1a	S	S	S	S	S	S	S	S	R

Note:

S-SensitiveR-Resistant

- ✓ **Strain used: M.tuberculosis (H**
- ✓ **37 RV strain).**
- ✓ Here are the **standard values** for the Anti-Tb test which was performed.
- ✓ Pyrazinamide- 3.125µg/ml.
- ✓ Streptomycin- 6.25µg/ml.
- ✓ Ciprofloxacin- 3.125µg/ml.

Table.3: It shows sample drug photograph

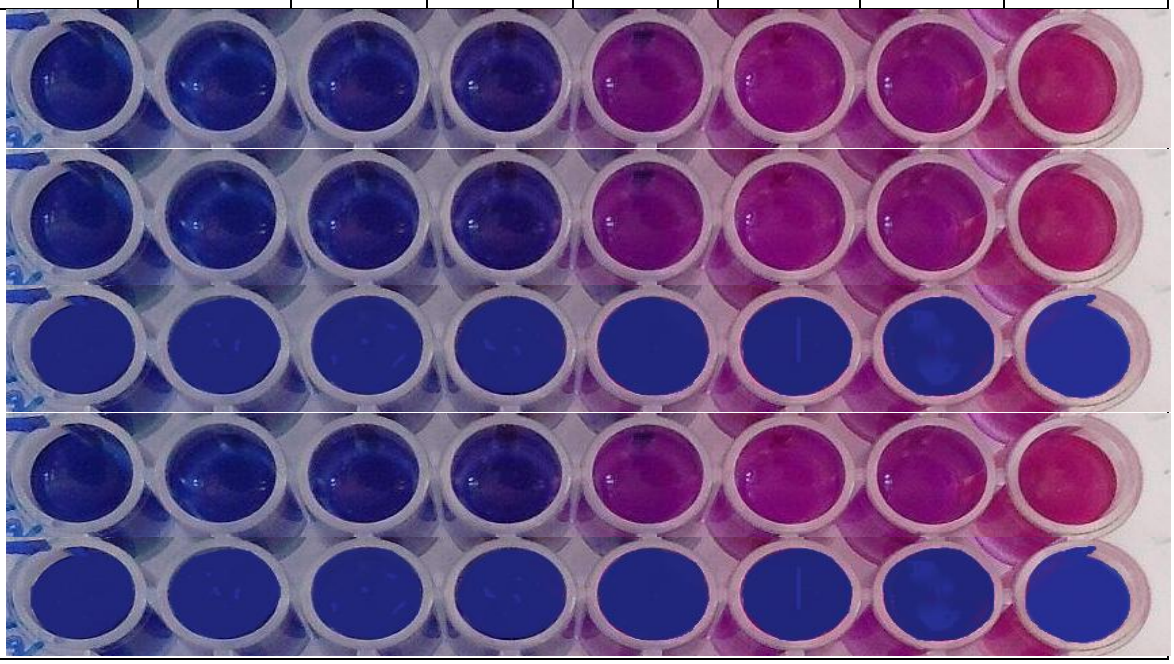
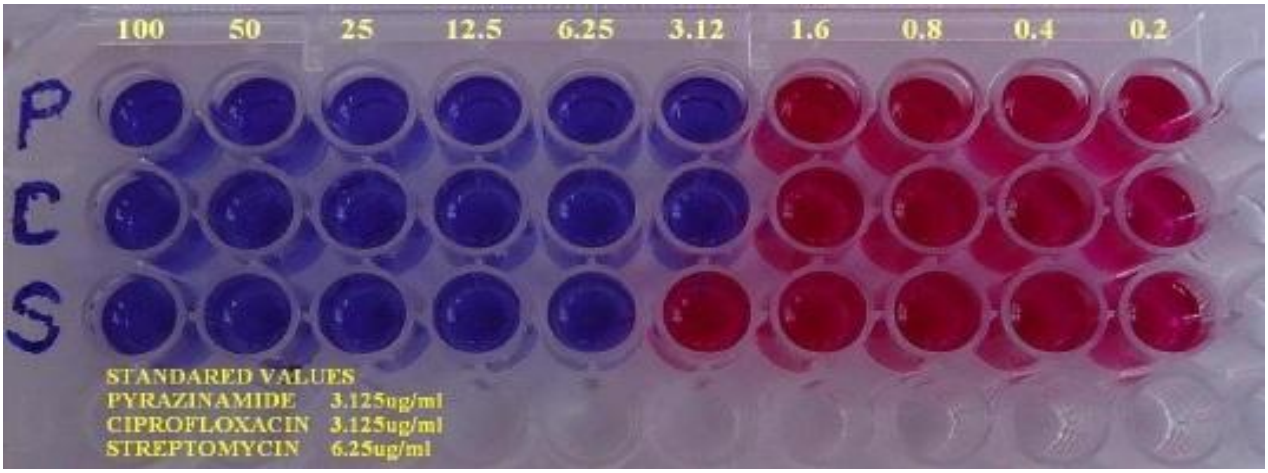
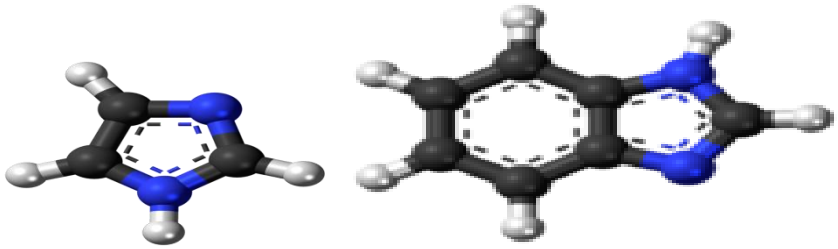
Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
B1								
B2								
R4								
R2								
R1a								

Table.4: It showsstandard drug photograph



FIGURES

Figure.1, 2: It shows parent nucleus of synthesized compounds.



IMIDAZOLE BENZIMIDAZOLE

Figure.3: It shows Lipinski rule of 5.

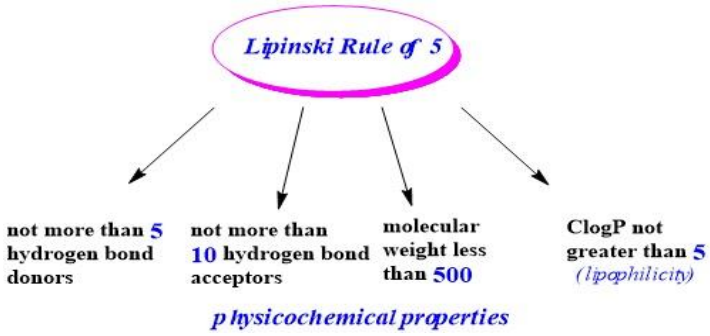


Figure.4: It shows NMR spectrum of B1

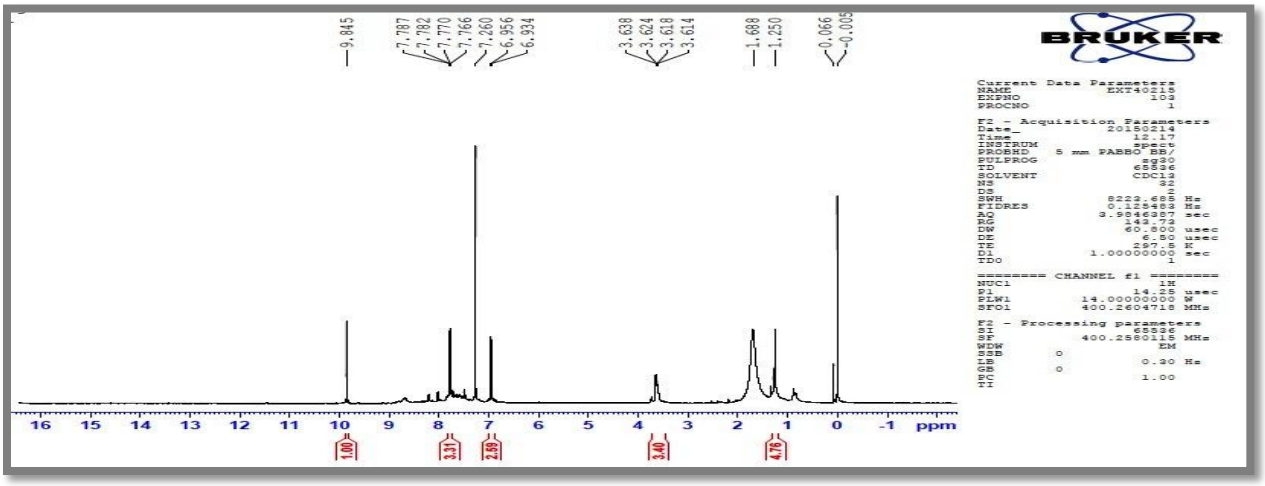
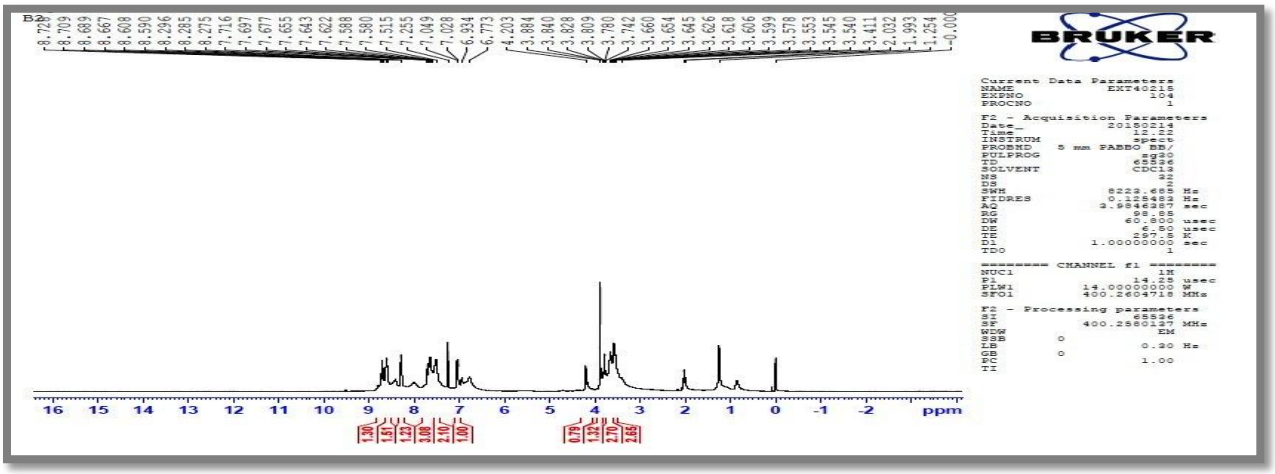


Figure.5:It shows NMR spectrum of B2



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