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DESIGN, DOCKING, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF BENZOTRIAZOLE FUSED WITH OXADIAZOLE DERIVATIVE

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ABSTRACT

1, 3, 4-Oxadiazole derivatives exhibited significant anti-tuberculosis activity. They also showed antimicrobial, anti-inflammatory, analgesic, antifungal, antipyretic, antidepressant, anti - convulsant, anticholinesterase, antihypertensive, antidiabetic, antitumor/anticancer, anti-HIV, antioxidant, etc. Benzotriazole is a nitrogen-containing heterocyclic compound characterized by the presence of three nitrogen atoms at the 1st, 2nd, and 3rd positions of its five-membered ring. Benzotriazole derivatives that exhibit antimicrobial, antibacterial, antifungal, antiviral, antitubercular, anticancer, anti-inflammatory, anticonvulsant, analgesic, and antioxidant activities. The basis nucleus of the research work is oxadiazole. A series of novel benzotriazole fused with oxadiazole derivative were designed, Synthesized, and biologically evaluated. The synthesized compounds were characterized using various spectroscopic techniques. Molecular docking studies were performed to predict the binding affinity and mode of interaction with target proteins. The anti-tubercular activity of these compounds was evaluated using the Microplate Alamar Blue Assay (MABA). The results revealed promising anti-tubercular activity against Mycobacterium tuberculosis.

KEYWORDS: Anti-tuberculosis, Anti-cancer, Anti-microbial, Anti-pyretic, Anti-oxidant, Oxadiazole and Benzotriazole.

1. INTRODUCTION

The quest for new and effective therapeutic agents has led to the exploration of various heterocyclic compounds, including benzotriazoles and oxadiazoles. Oxadiazole have recognized for their antimicrobial, anti-pyretic, ant-oxidant, anti-cancer and anti-tuberculosis activity making them attractive candidates for the development of novel therapeutics. Benzotriazoles, in particular, have garnered significant attention due to their wide-ranging biological activities, including anti-microbial, anti-viral, and ant-cancer properties. The incorporation of benzotriazole moieties into pharmaceutical agents has been show to enhance their efficacy and reduce toxicity. The benzotriazole fused with oxadiazole moieties may leads to the creation of new compounds with enhanced biological activities, improved and reduced side effects. The structure-activity relationship (SAR) of benzotriazole fused with oxadiazole derivatives has been explored to identify the key structural features responsible for their biological activities. The SAR studies have revealed that the presence of certain functional groups, such as hydroxyl, amino, or alkyl groups, can significantly impact the biological activities of these compounds.

In-Silico Studies for Lead Identification

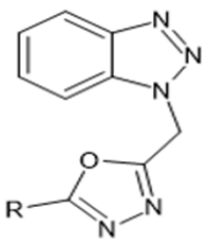
The basis of structure-based drug design lies in understanding the intricate molecular interactions between active site groups and ligands. This approach has become a cornerstone of modern drug discovery. Historically, combinatorial chemistry and high-throughput screening dominated lead identification, while computational methods focused on lead optimization. However, the rapid increase in available 3D protein structures and advances in computational tools have transformed structure-based drug design into a powerful method for both lead generation and optimization.

Computational approaches offer several advantages over traditional high-throughput screening, including reduced storage and handling requirements, minimized false positives, and the ability to identify low-molecular-weight leads. Target and lead discovery are crucial components of early pharmaceutical research. Target discovery involves identifying and validating potential drug targets, while lead discovery focuses on finding novel molecules that interact with those targets.

In silico models simulating biopharmaceutical, pharmacokinetic, and physiological properties have become invaluable tools for streamlining the drug development process. These models help predict absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties, reducing the need for experimental trials and minimizing the risk of failure during development. By leveraging this software, medicinal chemists can rapidly assess the biological impact of structural modifications and design optimized analogues with enhanced physicochemical properties.

2. DESIGN AND MOLECULAR DOCKING STUDIES

The design of benzotriazole fused with oxadiazole derivatives was performed using Molinspiration, ChemSketch, Swiss ADME, and Protox 3.0 software. Molinspiration was used to predict the bioactivity scores of the designed compounds, while ChemSketch was employed to draw and optimize the chemical structures. Swiss ADME was utilized to predict the pharmacokinetic properties, such as lipophilicity, solubility, and permeability, of the designed compounds. Protox 3.0 was used to predict the toxicity profiles of the designed compounds. The designed compound adheres to the Lipinski Rule of Five, exhibiting a molecular weight of < 500 Da, lipophilicity (logP) of < 5, fewer than 5 hydrogen bond donors, fewer than 10 hydrogen bond acceptors, and a polar surface area of < 140 Å^2. This suggests that the compound is likely to possess favourable pharmacokinetic properties, including oral bioavailability.



R- Compound C1- 4-nitro benzaldehyde
Compound C2- benzaldehyde
Compound C3- 4-hydroxy benzaldehyde
Compound C4- 2-nitro benzaldehyde
Compound C5- 4- N, N dimethyl benzaldehyde
Compound C6- 2, 3 dimethyl benzaldehyde
Compound C7- 2, 3 diamino benzaldehyde
Compound C8- 2, 3 dihydroxy benzaldehyde
Compound C9- pyridine
Compound C10- quinoline

MOLECULAR STUDIES

To investigate the binding affinity and mode of interaction of our designed compounds with various protein targets, we employed molecular docking simulations using AutoDock 4.2.6.

Anti-Tuberculosis Activity: Molecular docking simulations were performed using AutoDock 4.2.6 to investigate the binding affinity of our designed compounds with the enoyl-acyl carrier protein reductase (InhA) enzyme from Mycobacterium tuberculosis (PDB ID: 6P3O).

The docking results revealed that our designed compounds exhibited significant binding affinity with the InhA enzyme, indicating their potential as anti-tuberculosis agents.

Antimicrobial Activity: The antimicrobial activity of our designed compounds was evaluated using molecular docking simulations with the bacterial DNA gyrase (PDB ID: 6H96). The docking results showed that our compounds bound strongly to the DNA gyrase, suggesting their potential as antimicrobial agents.

Anti-Cancer Activity: Molecular docking simulations were performed to investigate the binding affinity of our designed compounds with the human epidermal growth factor receptor 2 (HER2) kinase domain (PDB ID: 8OXG). The docking results indicated that our designed compounds exhibited significant binding affinity with the HER2 kinase domain, suggesting their potential as anti-cancer agents.

Anti-Oxidant Activity: The anti-oxidant activity of our designed compounds was evaluated using molecular docking simulations with the human glutathione S-transferase (PDB ID: 8PUG). The docking results revealed that our compounds bound strongly to the glutathione S-transferase, indicating their potential as anti-oxidant agents.

Anti-Pyretic Activity: Molecular docking simulations were performed to investigate the binding affinity of our designed compounds with the human cyclooxygenase-2 (COX-2) enzyme (PDB ID: 1G61). The docking results showed that our designed compounds exhibited significant binding affinity with the COX-2 enzyme, suggesting their potential as anti-pyretic agents.

Docking Parameters: All molecular docking simulations were performed using AutoDock 4.2.6 with the following parameters: grid size of 120 × 120 × 120 Å, grid spacing of 0.575 Å, 50 genetic algorithm runs, population size of 150, and a maximum of 2,500,000 energy evaluations.

3. MATERIALS AND METHODS

Chemicals: All chemicals and reagents used were of analytical/synthetic grade.

Benzotriazole (Sumison Scientific Pvt Ltd), Ethyl chloroacetate (Sumison Scientific Pvt Ltd), Acetone, Anhydrous potassium carbonate (Sumison Scientific Pvt Ltd), Hydrazine hydrate (Sumison Scientific Pvt Ltd), Ethanol, Glacial acetic acid, Chloramine T trihydrate (Sumison Scientific Pvt Ltd), 4-Nitrobenzaldehyde (Sumison Scientific Pvt Ltd), 2-Nitrobenzaldehyde

(Sumison Scientific Pvt Ltd), 4-Hydroxybenzaldehyde (Sumison Scientific Pvt Ltd), Hexane, Liquid paraffin.

The melting points of the compounds were determined using an Equiptronics digital melting point apparatus, and the values obtained were uncorrected. Thin-layer chromatography (TLC) was performed on Silica gel G plates to monitor the reaction progress, and the spots were visualized under UV light. The 1H-NMR spectra were recorded on an Agilent-NMR 400-MR DD2 spectrometer operating at 400 MHz, using chloroform as the solvent, and the chemical shifts are reported in ppm (δ) relative to TMS as an internal standard. The IR spectra were obtained using an Alpha Bruker FT-IR Spectrometer with KBr discs. The UV-Visible spectroscopy data was acquired using a JASCO V-750 spectrophotometer equipped with accessories ISV-922. The UV-Visible spectroscopy measurement was performed over a range of 800-200 nm with a data interval of 1 nm, a bandwidth of 5.0 nm, and a response time of 0.24 seconds. The scan speed was set at 400 nm/min, and the light source was automatically changed at 340 nm. The instrument used a D2/WI light source and employed filter exchange, step correction, and baseline correction to ensure accurate measurements.

General procedure for the synthesis of benzotriazole fused with 1, 3, 4-Oxadiazole derivatives (C1-C4)

Step 1: 1.19 gm [0.01mol] of benzotriazole and 1.22 gm [0.01mol] of ethyl chloroacetate were combined and heated with 60 ml of acetone and 3 gm of anhydrous potassium carbonate under reflux for 6 hours.

Then the reaction mixture poured into crushed ice, allow it to solidify and filter it. As a result, ethyl acetate intermediate produced.

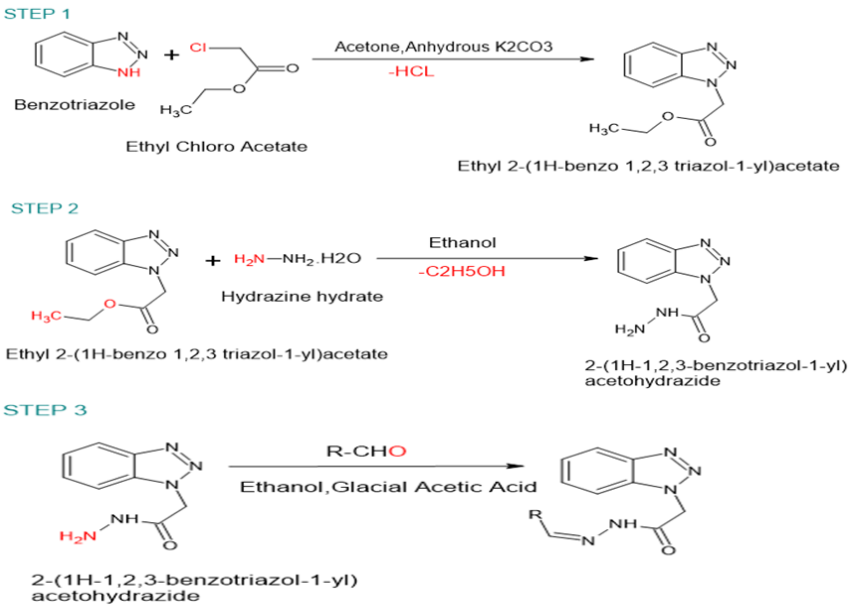
Step 2: 2.05 gm. [0.01mol] of Ethyl chloro acetate intermediate product and 1.28 gm [0.04mol] of hydrazine hydrate were combined and heated under reflux 4-5 hours with 30 ml of ethanol. Then the reaction mixture poured into crushed ice, allow it to solidify and filter it. The hydrazine intermediate product was produced.

Step 3: 1.91 gm. [0.01mol] of hydrazine hydrate intermediate product was taken and add 1.06 gm [0.01] of aromatic aldehyde. The mixture was refluxed for 5 hours with 5 ml of glacial acetic acid and 20 ml of ethanol. Then the reaction mixture poured into crushed ice, allow it to solidify and filter it. The intermediate product of aromatic aldehyde was produced.

Step 4: 2.79 gm [0.01mol] of intermediate product of aromatic aldehyde refluxed for 6 hours with 2.27 gm [0.01mol] of chloramine T and 20 ml of ethanol. Then the reaction mixture poured into crushed ice, allow it to solidify and filter it. The physical data of title compounds is given in **Table 1.**

The purity of the synthesized compounds was routinely assessed using Thin Layer Chromatography (TLC), and their melting points were determined using the open capillary tube method, with the values reported uncorrected.

SCHEME OF WORK



FT-IR spectral analysis

Infrared (IR) spectroscopy was used to confirm the presence of specific functional groups in each compound, with characteristic peaks observed in the IR spectra. The conversion of hydrazide to hydrazone (Schiff's base or imine) was confirmed by the presence of two distinctive peaks in the IR spectra, indicating the successful formation of the desired compound.

^{1}H NMR spectral analysis

The Proton NMR (1H NMR) spectra were recorded on a Bruker Avance DPX 300 instrument at a frequency of 300 MHz, using CDCl3 (deuterated chloroform) as the solvent and Tetramethyl silane (TMS) as the internal standard. The chemical shifts were recorded in parts per million (ppm) downfield from TMS. The total number of protons observed in the NMR

spectrum was in accordance with the expected number for each compound, providing useful information about the structure of the compounds. The report of the NMR spectrum for synthesised compounds (C1, C2, C3 and C4) are given in **Table 4.**

BIOLOGICAL EVALUATION

Anti-Tuberculosis activity: The anti-tuberculosis activity of the synthesized compounds was evaluated against Mycobacterium tuberculosis H37Rv strain using the microplate alamar blue assay (MABA). The results showed that some of the compounds exhibited promising anti-tuberculosis activity, with minimum inhibitory concentrations (MICs) ranging from 0.2 to 10.0 µg/mL.

4. RESULTS

In Silico ADME results: Molecular weight of each ligand was within the range of 277 to 350 D. They also had moderate to high predicted oral availability based on %ABS ranging between 50 to 82%.

Generally, a compound needs a score less than 5 for lipophilicity, the lipophilicity data suggested that the compounds were optimally were optimally lipophilic in nature ranging from 1 to 3. Most of the clinical available drugs have Log S higher than -4.00. The compounds exhibited a Log S value greater than -4.00, ranging between –3.96 to -2.74 (except compound C6). Only compound C6, exhibiting Log S value of -4.04 and one Lipinski violation.

Molecular docking results: The docking revealed that the fusion of benzotriazole with 1,3,4 oxadiazole derivatives, possessed high affinity towards 6P3O, 6H96, 8OXG, 1G61 and 8PUG. The 1, 3, 4-oxadiazole derived from benzotriazole; C1-C10 exhibited a docking score in the range of -8.93 to -12.84 Kcal/M for anti-tuberculosis activity. Compound C1 exhibits high docking score (-12.84 Kcal/M) among all the compounds. Compound C9 with the least docking score (-8.93 Kcal/M).

For anti-microbial activity, C1-C10 exhibits a docking score in the range of -8.74 to -11.13 Kcal/M. Compound C1 exhibits high docking score (-11.13 Kcal/M) and compound C9 with the least docking score (-8.74 Kcal/M). For anti-cancer activity, C1-C10 exhibits a docking score in the range of -8.2 to -10.14 Kcal/M. Compound C4 exhibits high docking score (-0.14 Kcal/M) and compound C1 with the least docking score (-8.2 Kcal/M). For anti-pyretic

activity, C1-C10 exhibits a docking score in the range of -8.56 to -10.88 Kcal/M. Compound C4 exhibits high docking score (-10.88 Kcal/M) and compound C9 with the least docking score (-8.56 Kcal/M). For anti-oxidant activity, C1-C10 exhibits a docking score in the range of -5.55 to -11.94 Kcal/M. Compound C1 exhibits high docking score (-11.94 Kcal/M) and compound C9 with the least docking score (-5.55 Kcal/M).

BIOLOGICAL EVALUATION

Anti-tuberculosis activity: The result of MABA test reveals that the synthesized compound (C1,C2, C3&C4) good anti-tuberculosis activity. The compound C1,C2,C3 & C4 sensitive at (100µg/ml, 50µg/ml), compound C2, C3 &C4 sensitive at (25µg/ml, 12.5 µg/ml,6.25 µg/ml,3.12 µg/ml, compound C3 & C4 sensitive at (1.6 µg/ml).

5. SUMMARY AND CONCLUSION

This research aimed to design and develop novel benzotriazole fused with 1, 3, 4-oxadiazole derivatives as anti-tuberculosis, anti-cancer, anti-pyretic, anti-oxidant and antimicrobial agents. The 1, 3, 4-oxadiazole scaffold is a biologically important lead molecule, exhibiting various activities, including analgesic, anti-inflammatory, bactericidal, antifungal, anticonvulsant, and psychotropic effects. The benzotriazole derivatives that exhibit antimicrobial, antibacterial, antifungal, antiviral, antitubercular, anticancer, anti-inflammatory, anticonvulsant, analgesic, and antioxidant activities. To develop an ideal anti-tuberculosis, anti-cancer, anti-pyretic, anti-oxidant and anti-microbial agent, this study employed a rational approach, involving in silico screening of novel benzotriazole fused with 1,3,4-oxadiazole analogues using Molinspiration software. Compounds obeying the Lipinski rule of five were selected for synthesis. This approach ensured that the designed compounds possessed optimal physicochemical properties, increasing their potential for successful drug development.

Ten analogues (C1-C10) were designed and molecular docking studies were done autodock 4.2.6 and the only four compound (C1,C2,C3&C4) were synthesized and their purity was ascertained by melting point, Rf value, and spectroscopic studies (UV, FT-IR and 1H NMR). The synthesized compounds (C1, C2, C3 &C4) were then characterized, and their structural elucidation was confirmed by spectroscopic analysis.

Ten analogues were screened for anti-tuberculosis, anti-microbial, anti-cancer, anti-pyretic and anti-oxidant activity with compounds C1, C2, C3&C4 showing promising results at

molecular docking studies. These compounds demonstrated significant anti-tuberculosis, anti-microbial, anti-cancer, anti-pyretic and anti-oxidant effect, indicating their potential as lead molecules for further optimization.

The synthesized compounds (C1, C2, C3 & C4) were biologically evaluated for anti-tuberculosis activity. The results revealed that certain compounds exhibited notable anti-tuberculosis effects, highlighting their potential as therapeutic agents against mycobacterium tuberculosis. Acute toxicity studies revealed that the analogues were safe with low toxicity, making them potential leads for future anti-tuberculosis, anti-microbial, anti-cancer, anti-pyretic and anti-oxidant activity. Overall, this study demonstrates the potential of benzotriazole fused with 1, 3, 4-oxadiazole derivatives as novel therapeutic agents, warranting further investigation and optimization.

Predicted ADME, Lipinski parameters and molecular properties of the synthesized compounds.

Table No. 1

Code	M.W	H-bond acceptor	H-bond donar	Log P	M.R (Cm^3/mol)	No. of criteria
Rule	**<500**	**<10**	**<5**	**<5**	**<150**	**Atleast-3**
Compound C1	322.28	7	0	2.07	85.39	ALL
Compound C2	277.28	5	0	2.58	76.57	ALL
Compound C3	293.28	6	1	2.07	78.59	ALL
Compound C4	322.28	7	0	1.73	85.39	ALL
Compound C5	320.35	5	0	2.69	90.78	ALL
Compound C6	305.33	5	0	2.98	80.50	ALL
Compound C7	307.31	5	2	1.61	85.38	ALL
Compound C8	309.28	7	2	1.53	80.61	ALL
Compound C9	278.27	6	0	2.20	74.36	ALL
Compound C10	328.33	6	0	2.55	91.87	ALL

Table No. 2

Code	Solubility (log s)	GI absorption	BBB permeant	Synthetic Accessibility
Compound C1	-3.49	High	No	2.97
Compound C2	-3.48	High	Yes	2.95
Compound C3	-3.32	High	No	2.98
Compound C4	-3.49	High	No	3.10
Compound C5	-3.67	High	Yes	3.09
Compound C6	-4.04	High	Yes	3.28
Compound C7	-2.74	High	No	3.13
Compound C8	-3.17	High	No	3.06
Compound C9	-2.81	High	No	2.88
Compound C10	-3.96	High	No	3.03

DOCKING SCORE OF 1, 3, 4- OXADIAZOLE DERIVATIVE C1-C10 TOWARDS RECEPTOR (6p3o, 6h96, 8oxg, 1g61 and 8pug).

Table No. 3

COMPOUNDS	BINDING ENERGY				
	ANTI-TUBERCULOSIS	ANTI-MICROBIAL	ANTI-CANCER	ANTI-PYRETIC	ANTI-OXIDANT
C1	-12.84	-11.13	-8.2	-8.79	-11.94
C2	-10.94	-9.28	-8.52	-9.04	-9.74
C3	-11.99	-10.63	-9.03	-10.59	-10.65
C4	-12.24	-11.6	-10.14	-10.88	-11.66
C5	-11.66	-10.76	-9.23	-9.88	-11.29
C6	-11.44	-8.83	-9.93	-10.04	-9.9
C7	-10.32	-8.63	-8.96	-9.56	-5.76
C8	-10.57	-8.93	-9.4	-9.47	-5.96
C9	-8.93	-8.74	-8.35	-8.56	-5.55
C10	-10.38	-8.97	-9.86	-9.07	-6.4

HIGH DOCKING SCORES

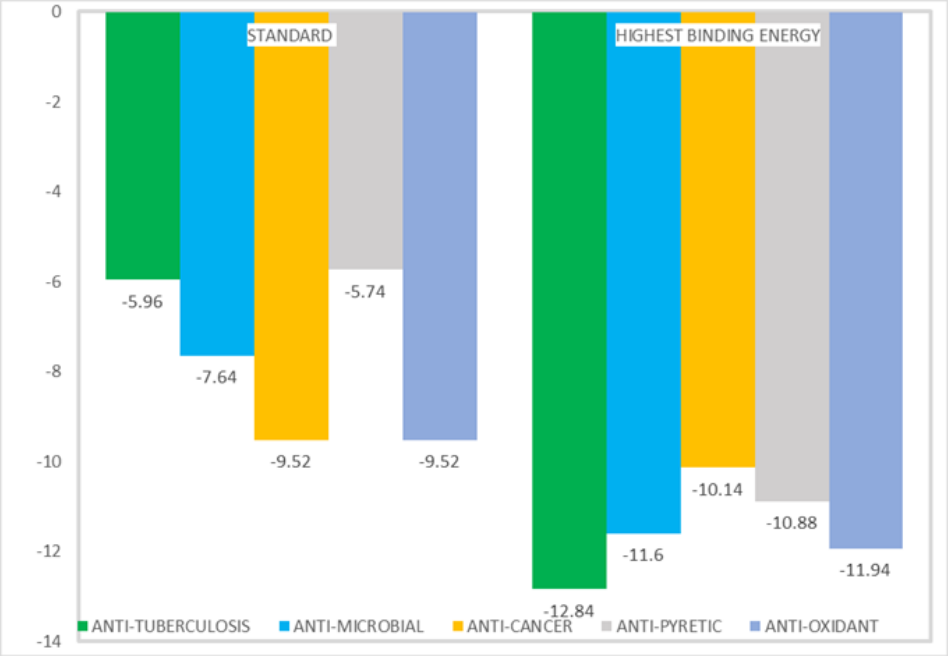


Figure No. 1

APPEARANCE, % YEILD, MELTING POINT AND RF- VALUE

Table No. 4

Code	Appearance	Yeild (%)	Melting Point	Solubility	Rf Value
Compound C1	Brown colour powder	81.09%	277-280^{o}C	Chloroform	0.52
Compound C2	Dark Brown colour powder	76.04%	271-274^{o}C	Chloroform	0.68
Compound C3	Brown colour powder	84.67%	270-274^{o}C	Chloroform	0.57
Compound C4	Brown colour powder	83.27%	265-268^{o}C	Chloroform	0.65

FT-IR DATA

Table No. 5

S.NO	CODE	FUNCTIONAL GROUP	OBSERVED VALUE IN 1/cm
1.	Compound C1	O-H str C=O str C=O str N-H bend N-O asymmetric str C-C str (in-ring) C-N str, N-O symmetric str C-N str C-N str	3246.57 1745.26 1676.8 1595.81 1502.28 1446.35 1279.54 1220.72 1090.55
2.	Compound C2	N-H str, O-H str N-H str, O-H str O-H str C-H str (aromatic) C-H str (alkanes) -C=C- str N-O asymmetric str C-H bend C-C str (in-ring) C-N str C-O str C-N str =C-H bend	3355.53 3257.18 3184.88 3059.51 2977.55 1671.98 1487.81 1453.1 1415.49 1272.79 1220.72 1089.58 948.806
3.	Compound C3	O-H str, H bonded N-H str N-H str C-H str (aromatic) C-H str(alkanes) C=O str C-C str(in-ring) N-O asymmetric str N-O symmetric str C- str C-H “oop”	3353.6 3254.29 3075.9 2921.63 1695.12 1591.95 1520.6 1337.39 1088.62 1012.45 811.885
4.	Compound C4	C-H str (aromatic) C-H str (alkanes) C=O str N-H bend N-O asymmetric str N-O symmetric str C-O str C-N str C-H “oop”	3076.87 2832.92 1691.27 1586.16 1509.99 1335.46 1239.04 1128.15 739.567

UV- VISIBLE SPECTRUM DATA

Table No. 6

CODE	WAVELENGTH	MAXIMUM ABSORBANCE
Compound C1	337.0000	0.87637
Compound C2	279.0000	0.79684
Compound C3	408.0000	0.96219
Compound C4	366.0000	0.87381

NMR SPECTRUM DATA

Table no. 7

CODE	OBSERVED VALUE (in ppm) & TYPE OF PROTONS
Compound C1	7.810, 7.789 (d, 4H, Ar-H), 7.305-7.264 (m, 5H, Ar-H), 5.436,5.007 (C=C-H), 1.289-1.255 (–CH_3, –CH_2–, CH–)
Compound C2	8.092-7.653 (d, 4H, Ar-h), 7.530-6.048 (m, 5H, Ar-H), 5.947-5.148 (C=C-H), 4.266, 4.250 (C-O), 1.257 (-CH3, -CH2-, -CH-)
Compound C3	8.400-7.782 (d, 4H, Ar-H), 7.423-7.273 (m, 5H, Ar-H), 5.434, 5.114 (C=C-H), 1,287-1.256 (-CH3, -CH2-,-CH-)
Compound C4	9.566 (-CHO), 8.408-7.793 (d, 4H, Ar-H), 7.52-6.010 (m, 5H, Ar-H), 4.887 (C-O)

ANTI-TB ACTIVITY OF SYNTHESIZED ACTIVITY

Table No. 8

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	0.4 µg/ml	0.2 µg/ml
01	01	S	S	R	R	R	R	R	R	R	R
02	02	S	S	S	S	S	S	R	R	R	R
03	03	S	S	S	S	S	S	S	R	R	R
04	04	S	S	S	S	S	S	S	R	R	R

Note: S- sensitive; R- resistant

The compound C1,C2,C3 & C4 sensitive at (100µg/ml, 50µg/ml), compound C2, C3 &C4 sensitive at (25µg/ml, 12.5 µg/ml,6.25 µg/ml,3.12 µg/ml, compound C3 & C4 sensitive at (1.6 µg/ml).

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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