Original Article

In-Silico Drug Design And Adme Studies Of Substituted 1, 3, 4-Oxadiazole Analogues As Potent Mycobacterial Dpre1 Enzyme Inhibitors

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Abstract

Mycobacterial infections and multidrug-resistant (MDR) strains of Mycobacterium generate high mortality, this has triggered the scientific community to search for novel, effective, and safer therapeutics. A series of 4, 5-disubstituted-1, 3, 4-oxadiazole derivatives were screened for mycobacterial activity against H37Rv, MDR and XDR strains. To recognize the mechanism of action of these compounds and to identify their supposed drug target, molecular docking and dynamics studies were employed against **DprE1** mycobacterial enzyme, which is reported to be an essential enzyme for mycobacterial growth and survival. The ADME and drug-likeness properties revealed that all the compounds have good pharmacokinetic properties. All the compounds have high affinity towards the enzyme. The newly synthesized derivatives were confirmed through FT-IR, 1 H-NMR, and LCMS. In-vitro microplate alamar blue assay (MABA) to determine the MIC (minimum inhibitory concentration) values against Mycobacterium tuberculosis H37Rv was performed for the synthesized compounds. The synthesized compounds **2A, 3A, 4A, 5A** and **6A** exhibited promising activity against Mycobacterium tuberculosis.

Keywords: Mycobacterium tuberculosis, 4, 5-disubstituted-1,3,4-oxadiazole, Molecular modelling, MABA, DprE1, MIC.

INTRODUCTION

Mycobacterial infections and multidrug-resistant (MDR) strains of *Mycobacterium* produce high mortality rate throughout the world. *Mycobacterium tuberculosis* (MTB) is one of the leading pathogens to cause tuberculosis (TB) [1]. Several factors have contributed to the continuous health threat of TB globally. This includes the development of drug resistance such as multidrug-resistant tuberculosis (MDR-TB), extensively drug-resistant tuberculosis (XDR-TB) [2], and totally drug-resistant tuberculosis (TDR-TB) [3]**.** One third of the world's population is thought to be infected with *M. tuberculosis* [4], and new infections occur at a rate of about one per second. In 2021 approximately 10.2 million people suffered from TB and 1.6 million died. Drug resistant been a major cause for developing Drug resistant among the people, it necessary to identify a newer mechanism or developing and designing a new drug which as potential to inhibit the existing mechanism[5].

1, 3, 4-oxadiazoles derivatives are a fascinating group, that have attracted an interest in medicinal chemistry bioisosteres for a number of biological targets. As a consequence of these characteristics the 1,3,4-oxadiazole derivatives have been found to exhibit diverse biological activities such as antimicrobial[6], anti-HIV[7], antitubercular[8], anticonvulsant[9], anti-inflammatory[10], analgesic[11], antiviral[11], pesticides[11] and insecticides[11], antihypertensive[12] and anthelminthic[13].

In mtb as drug target various can be aimed like Cell wall biosynthesis (in that Mycolic acid synthase, DprE1 and etc), DNA related enzymes (like topoisomerase II, gyrase and ATP synthase), metabolic pathways, protein biosynthesis and various. Among various targets, existing potential targets are DNA related enzymes and cell wall synthase. In present research work we selected DprE1 enzyme as target for inhibition. As mutation is seen in this protein so designing new molecule is essential [14].

In the present research we aimed to locate the appropriateness and probability of novel methods for the synthesis of 4, 5-disubstituted-1, 3, 4-oxadiazole derivatives. Molecular docking is a fast and efficient computational method to predict the preferred orientation of the bioactive compounds to a specific protein, when bound to each other they form a stable complex [15].

MATERIALS AND METHODS

Drugs & Chemicals: All the chemicals were procured from Sigma-Aldrich. The compounds used were highest analytical grade.

Protein data bank (PDB)

PDB is a database for the three-dimensional structural data for large biological molecules, which includes proteins and nucleic acids. Most of the structures are determined by X-ray diffractions and NMR studies. Each structure published PDB receives a four character alphanumeric identifier called PDB ID Eg: 6HF3 [16].

Molecular docking

Docking studies were carried out to analyse the different types of biomolecular interactions and ligand receptor binding affinities. Molecular docking is achieved by means of Autodock vina. The 3D crystallographic protein structure is discovered from protein data bank (PDB ID- 6HF3). Autodock Vina is an open source program with a complete molecular viewer and graphical support for doing molecular docking. PyMOL produce a high quality 3D image of protein as well as its visualization. PyRx is for docking analysis [17]. The docking study was performed on PPARG Nuclear protein (PDB ID: 6HF3). The computational work was performed on a **HP 15s-eq0132au Laptop running on AMD Ryzen 7 3700U processor**.

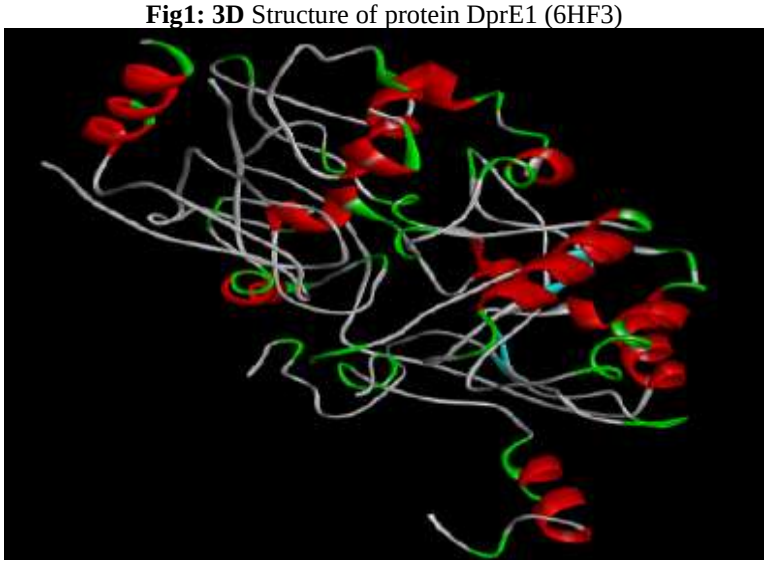
Protein preparation

DprE1 is a flavoenzyme plays a important role in synthesis of cell wall in which it convert decaprenyl phosphoryl-beta-d-ribose (DPR) to decaprenyl phosphoryl-beta-d-arabinofuranose (DPA) in presence of DprE1 enzyme and preparation of this enzymes for docking to see the affinity of nova designed molecules. Mtb DprE1 is a flavin adenine dinucleotide (FAD) - dependent enzyme the crystal structure is retrieved from protein data bank (PDB) that is 6HF3 with resolution of 2.20 Å.

In the present study rigid docking is performed. The first a binding site on 6HF3 is predicted using the software called CASTp 3.0 (Computational Atlas of Surface Topology of protein), it is based on recent theoretical and algorithm results of computational geometry.
Using Autodock vina 1.5.7 among 2 identical chains; one of the identical chains has been removed as a part of protein preparation.

The co-crystalized crystalized ligand along with drug is also deleted; the water molecule which can interrupt the docking process is also removed. Add kollman charges, here the protein selected has (3.264) and saved in PDBQT format. This is followed by additional step by adding polar contacts to find out the types of amino acid interactions during ligand-receptor binding [18]. The prepared protein is shown in (Figure 1)

Fig1: 3D Structure of protein DprE1 (6HF3)



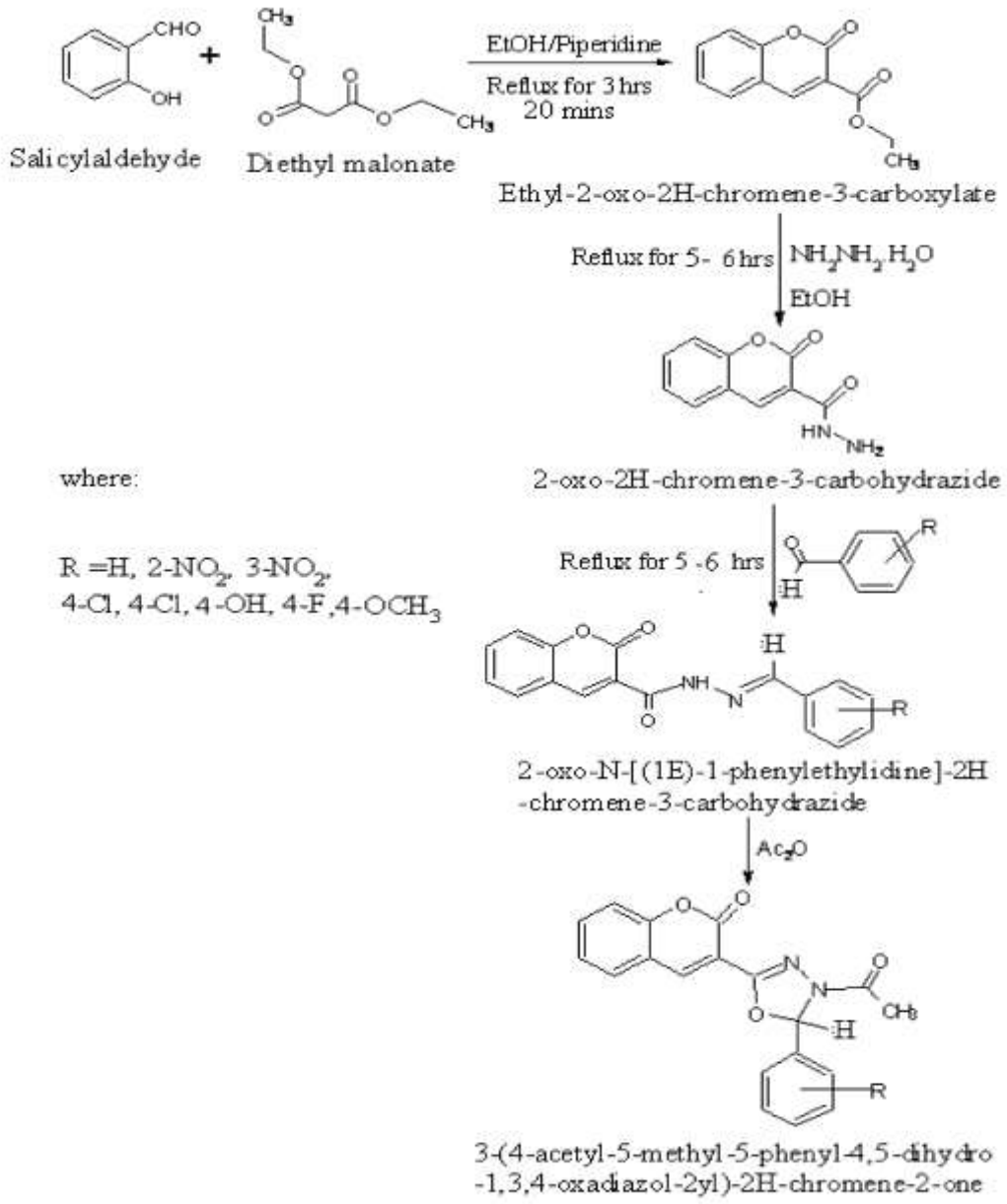
Ligand Preparation

The 2D chemical structures of ligands are drawn using ACD Lab Chemsketch ver 12.0 and generated smiles notation. The 3D structures of the ligands were downloaded from RCSB PDB (https://www.rcsb.org) and uploaded in BIOVIA Discovery Studio Visualizer-2020. Ligand minimization was done and using small molecule wizard in 'SMALL MOLECULE' wizard in BIOVIA Discovery Studio Visualizer-2020 and was saved as a cluster sdf file [19].

Fluoroquinolones

Fluoroquinolones (Moxifloxacin) is an effective, selective, noncompetitive and quick acting reversible inhibitor of decaprenylphosphoryl-D-ribose oxidase (**DprE1**) qualified for the healing of Tuberculosis (AD); and is the primary and solitary DprE1 accredited in the management of Multi resistant tuberculosis (MD-TB) [20].

SCHEME

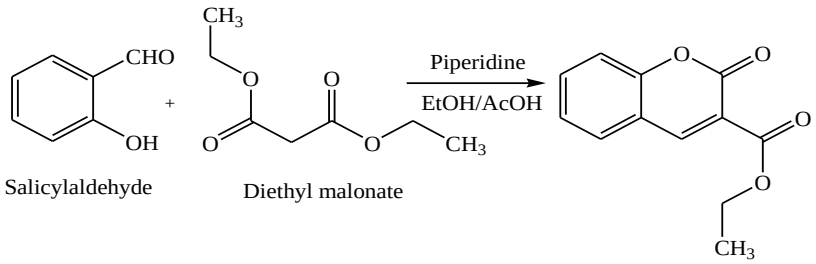


Step I: Synthesis of Ethyl-2-oxo-2H-chromene-3-carboxylate[21]

In a 500-ml RBF equipped with a reflux condenser mixture of salicylaldehyde (0.50 mole) and diethyl malonate (0.55 mole) was placed in 100 ml absolute ethanol. To this mixture 2.5 ml of piperidine was added as a catalyst and few drops of glacial acetic acid, and the solution was heated under reflux for 3 hours 20 minutes. The product crystallized readily as the solution cooled and was finally stored overnight in a refrigerator. The crystalline product was collected by filtration and washed with a solution made from 95% ethanol and water (4:6). The crude product was dried in the air and recrystallized from ethanol to give white crystals.

IR (KBr, cm^{-1}): 3059 (Ar CH str), 1768 (C=O, lactone), 1608 (C=O, ester), 1201 (C-O, coumarin), 783 (Ar CH bend).

^{1}H NMR (δ ppm): 1.42 (3H, t, CH_3), 4.44 (2H, q, CH_2), 7.27-7.69 (4H, m, Ar-H), 8.53 (1H, s, Ar-H).

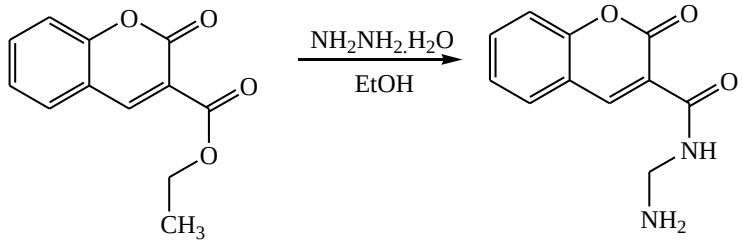


Salicylaldehyde Diethyl malonate Ethyl-2-oxo-2H-chromene-3-carboxylate

Step II: Synthesis of 2-Oxo-2H-chromene-3-carbohydrazide[22]

A mixture of 0.1 mol of ester and 0.2 mol of hydrazine hydrate were refluxed in 50ml of 95% ethanol for 5-6 hours. The resultant mixture was concentrated, cooled and poured onto crushed ice. The solid mass thus separated out was filtered, dried and purified by recrystallization from ethanol.

IR (KBr, cm^{-1}): 3381 (NH_2), 3286 (NH str), 3037 (Ar CH str), 1691 (C=O, lactone), 1616 (C=O, ketone), 1196 (C-O, coumarin) and 750 (Ar CH bend).

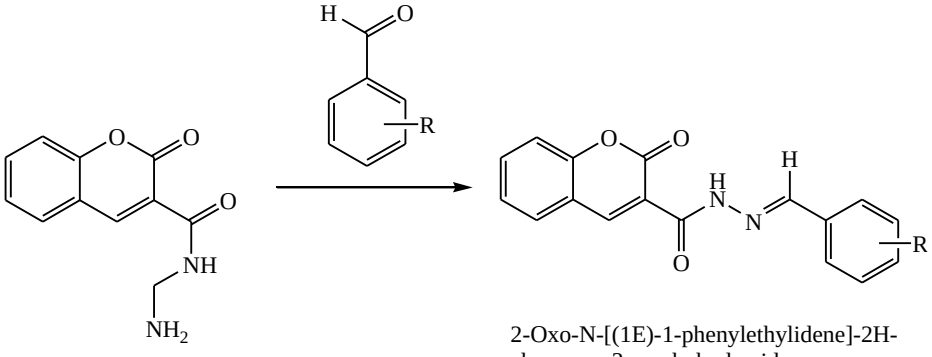


Ethyl-2-oxo-2H-chromene-3-carboxylate 2-Oxo-2H-chromene-3-carbohydrazide

Step III: Synthesis of 2-Oxo-N-[(1E)-1-phenylethylidene]-2H-chromene-3- carbohydrazide [23]

Equimolar amount of 2-oxo-2H-chromene-3-carbohydrazide and aldehydes were dissolved in ethanol and refluxed on water bath for 5-6 hours in presence of few drops of acetic acid. The reaction mixture was poured into ice-cold water and solid was filtered out. The dried solid was recrystallized from ethanol.

IR (KBr, cm^{-1}): 3055 (Ar CH str), 1701 (C=O, lactone), 1614 (C=O, ketone), 1527 (C=N), 1205 (C-O, coumarin) and 748 (Ar CH bend).

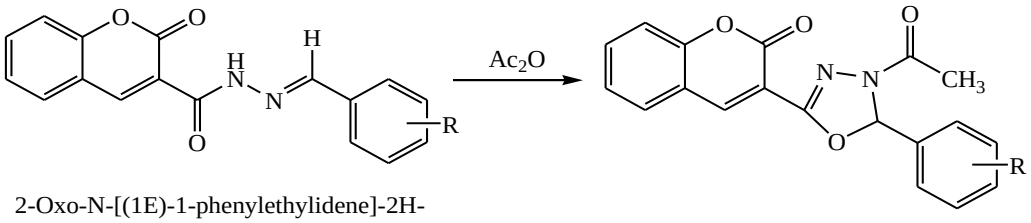


2-Oxo-2H-chromene-3-carbohydrazide 2-Oxo-N-[(1E)-1-phenylethylidene]-2H-chromene-3- carbohydrazide

Step IV: Synthesis of 3-(4-acetyl-5H-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)- 2H-chromen-2-one[24]

A mixture of 2-oxo-N-[(1E)-1-phenylethylidene]-2H-chromene-3-carbohydrazide (0.002 mol) and excess of acetic anhydride (10 mL) was refluxed for 3 hours. The excess acetic anhydride was distilled off at reduced pressure and residue was poured into ice cool water. The solid product was filtered and recrystallized from ethanol to give 3-(4-acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one.

IR (KBr, cm^{-1}): 3055 (Ar CH str), 1681 (C=O, lactone), 1618 (C=O, ketone), 1572 (C=N), 1267 (C-O-C) [oxadiazole nucleus], 1195 (C-O, coumarin), 752 (Ar CH bend).
^{1}H NMR (δ ppm): 2.38 (3H, s, $COCH_3$), 7.32-7.53 (4H, m, Ar-H), 7.8 (2H, d, Ar-H), 8.11 (2H, d, Ar-H), 8.6 (1H, s, Ar-H), 8.7 (1H, s, CH of oxadiazole).

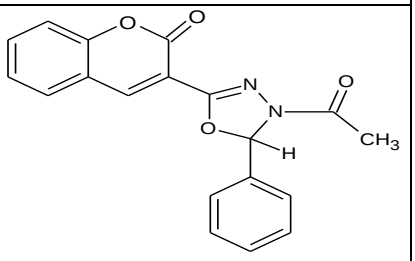
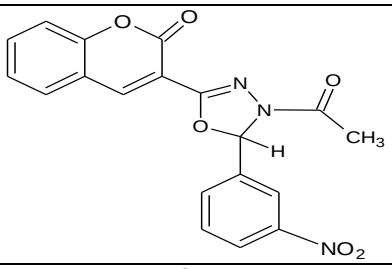
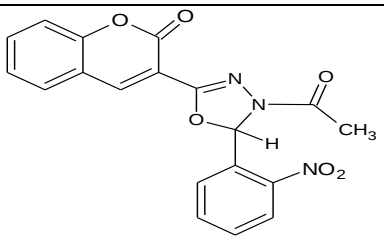
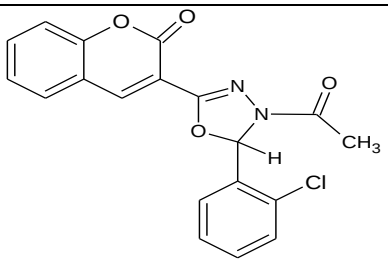
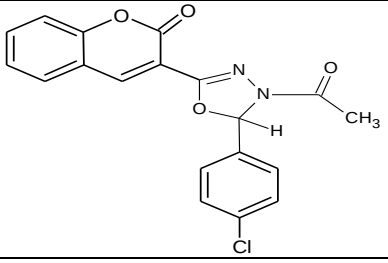


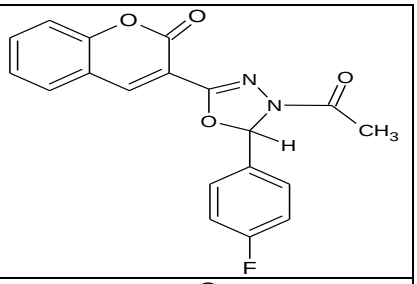
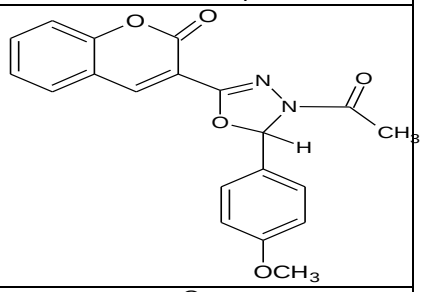
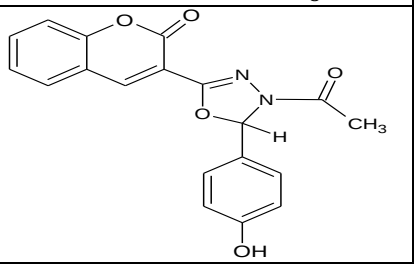
2-Oxo-N-[(1E)-1-phenylethylidene]-2H-chromene-3- carbohydrazide

3-(4-acetyl-5H-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)- 2H-chromen-2-one

1A H 2A 3-NO_2 3A 2-NO_2 4A 2-Cl 5A 4-Cl 6A 4-F 7A 4-OCH_3 8A 4-OH

List of synthesized compounds

Sl. no	Comp code	Chemical Name	Structure
01	I A	3-(4-Acetyl-5H-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one	
02	2 A	3-(4-Acetyl-5H-5-m-nitrophenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one	
03	3A	3-(4-Acetyl-5H-5-o-nitrophenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one	
04	4 A	3-(4-Acetyl-5H-5-o-chlorophenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one	
05	5 A	3-(4-Acetyl-5H-5-p-chlorophenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one	

06	6A	3-(4-Acetyl-5H-5-p-fluorophenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one	
07	7 A	3-(4-Acetyl-5H-5-p-methoxyphenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one	
08	8 A	3-(4-Acetyl-5H-5-p-hydroxyphenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one	

IN VITRO SCREENING FOR ANTITUBERCULAR ACTIVITY [25, 26]

Microplate Alamar Blue Assay (MABA) method

Four compounds namely 3-nitrophenyl, 2-nitrophenyl, 2-chlorophenyl and 4-fluorophenyl derivatives were moderately active as antibacterials, were further screened for their antitubercular activity.

Principle

The Alamar blue oxidation-reduction dye is a general indicator of cellular growth and/or viability, the blue, oxidized form; nonfluorescent dye becomes pink and fluorescent upon reduction. Growth can therefore be measured with a fluorometer or spectrophotometer or determined by a visual color change.

Preparation of different concentrations of compounds

30mg of the drug was dissolved in 30 ml DMSO (1 mg/ml or 1000µg/ml solution). From the above solution, different dilutions such as 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.125µg/ml, 1.6µg/ml, 0.8µg/ml, 0.4µg/ml and 0.2µg/ml were prepared.

Procedure for Anti-TB activity using Alamar Blue Dye

1. The anti mycobacterial activity of compounds were assessed against mycobacterium strain H37Rv using microplate alamar blue assay (MABA).
2. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method.
3. Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 48 wells plate to minimized evaporation of medium in the test wells during incubation.
4. The 48 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.
5. The final drug concentrations tested were 0.2 to 100.0 µg/ml.
6. Plates were covered and sealed with parafilm and incubated at 37º C for five days.
7. After that 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.
8. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.
9. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

Moxifoxacin:

Moxifloxacin was used as a standard and above procedure was followed to compare the antitubercular activity of different synthesized compounds with it. The MIC of moxifloxacin was found to be 3.75µg/mL.

Molecular Docking Studies [27]

Docking studies predicts the preferred orientation the binding mode of compounds **2A, 3A, 4A, 5A and 6A** to DprE1 (6HF3). Studies were carried out by using the X-ray crystal structure of *Homo sapiens* DprE1 (PDB ID: **6HF3**) [20] obtained from Protein Data Bank server (www.pdb.org).
The docking poses of the compounds **2A, 5A and 6A** are shown in (**Figures 2-3)**. By the review of literature it is recognized that moxifloxacin have high affinity to the enzyme DprE1. The moiety of substituted 1, 3, 4 oxadiazoles binds the enzyme active region by interacting with the Lys-418 amino acid. Remaining groups like 2-oxo-2H-chromene group binds to the enzyme by interacting with Tyr-415 and His-132.
By analyzing the molecular docking outcomes of **2A, 3A, 4A, 5A & 6A** proves that active binding site of these compounds are similar to that of reference standard moxifloxacin.

In-silico ADME/Pharmacokinetic Predictions [27]

It was established that the antagonistic response of inhibitors with an enzyme or a protein receptor cannot promise the suitability of an inhibitor as a potential drug. Therefore, ADME (absorption, distribution, metabolism, and excretion) including drug-likeness analysis are important in the drug discovery which helps to make a rational decision on whether inhibitors can be administered to a biological system or not. In addition, inhibitors with poor ADME properties and high toxicity effects on the biological systems are often the major cause of most failed medicines in the clinical phase of experiments. The Pfizer's rule of five also known as Rule of five (Ro5) or Lipinski's rule of five (5) by Christopher A. Lipinski in 1997 is a thumb-rule for evaluating drug-likeness and to decide if an inhibitor with a certain biological and pharmacological properties would be an orally active drug in the human body [10]. The rule states that a molecule or an inhibitor can be orally absorbed/active if two (2) or more of these thresholds; molecular weight (Mw) of molecule < 500, octanol/water partition coefficient (iLOGP) ≤ 5, number of hydrogen bond acceptors (nHBA) ≤ 10, number of hydrogen bond donors (nHBD) ≤ 5, and topological polar surface area (TPSA) < 40 $Å^2$) are not violated. From the output of some ADME and drug-likeness properties shown in **Table 1**, it was observed that 8 molecules have zero violations of the Lipinski's rule. The drug-likeness parameters are related to aqueous solubility and intestinal permeability which determines the first step of oral bioavailability. The results also showed good pharmacokinetic properties in which all molecules have high gastrointestinal absorption, non- substrate to P-glycoprotein and 1A & 5A possess blood–brain barrier BBB permeant.

RESULTS AND DISCUSSION

All the compounds prepared during the present exploration have been authentically established by various spectroscopic methods.
The compound **Ethyl-2-oxo-2H-chromene-3-carboxylate** was prepared by treating salicyaldehyde with diethyl malonate and few drops of glacial acetic acid using piperidine as catalyst and ethanol as solvent**.** IR spectrum and showed its characteristic peak at 3059 cm^{-1} due to Ar CH str, 1768 cm^{-1} due to C=O, lactone, 1608 cm^{-1} due to C=O, ester, 1201 cm^{-1} due to C-O, coumarin, 783 cm^{-1} is due Ar CH bend. Further the conformation of the compound done by ^{1}H NMR**.** The presence of signals at δ gave 1.42 (3H, t, CH_3), 4.44 (2H, q, CH_2), 7.27-7.69 (4H, m, Ar-H), 8.53 (1H, s, Ar-H).
This compound is further treated with hydrazine hydrate to get **2-oxo-2H-chromene-3-carbohydrazide,** the formation is indicated by peaks at 3381 cm^{-1} is due to NH_2, 3286 $cm^{-1}$is due to NH str, 3037 cm^{-1} is due Ar CH str, 1691 cm^{-1} C=O, lactoe peak, 1616 $cm^{-1}$is due to C=O, ketone, 1196 cm^{-1} is due to C-O, coumarin and 750 cm^{-1} is Ar CH bend peak, than followed by treating with aldehyde, ethanol and few drops of glacial acetic acid to get carbohydrazide derivatives i.e. **2-Oxo-N-[(1E)-1-phenylethylidene]-2H-chromene-3-carbohydrazide**, there are characterized by the IR peaks. The substituted 1, 3, 4 oxadiazole derivatives are prepared by treating carbohydrazide derivatives with acetic anhydride, which are further characterized by IR peaks at 3055 cm^{-1} Ar CH str, 1681 cm^{-1} C=O, lactone, 1618 cm^{-1}C=O, ketone, 1572 cm^{-1} C=N, 1267 cm^{-1} C-O-C due to oxadiazole nucleus, 1195 cm^{-1} C-O, coumarin, 752 cm^{-1} Ar CH bend **(Table 4)**. Our aim was to actually isolate this compound**,** which was purified by column chromatography and confirmed by ^{1}H NMR with signals at δ 2.38 (3H, s, $COCH_3$), 7.32-7.53 (4H, m, Ar-H), 7.8 (2H, d, Ar-H), 8.11 (2H, d, Ar-H), 8.6 (1H, s, Ar-H), 8.7 (1H, s, CH of oxadiazole) (**Table 5)**.

A series of derivatives was synthesized by replacement **R** on benzene moiety by different electron donationg and electron withdrawing groups. In chemexper data + sign indicate good drug and – sign indicate bad drug and moleinspiration shows viceversa.

In vitro antimycobacterial activity was carried out by using microplate alamar blue assay (MABA) method. The Compounds were randomly selected for activity against reference standard moxifloxacin. The most excellent result in terms of minimum inhibitory activity (MIC), were shown by five derivatives **2A, 3A, 4A, 5A & 6A (Table 6).** The MIC values of the compound **2A, 4A** was 3.55µg/mL and compound **3A, 5A and 6A** was 3.55µg/mL, 3.70µg/mL and 3.72µg/mL respectively. All the above mentioned compounds were almost close to standard moxifloxacin 3.75 µg/mL (**Table 6)**.
In Substituted 1, 3, 4 oxadiazole derivatives with electron withdrawing group on benzene ring demonstrated very good activity anti-mycobacterium activity. However, electron releasing groups at the same position, exhibited lesser activity. This could be possibly being attributed to steric hinderance.

According to the docking poses **2A, 3A, 4A, 5A & 6A** compounds have five common interactions and there docking

scores were almost near to standard reference moxifloxacin i.e. **-10.5** (**Table 6)**. Moxifloxacin is a known inhibitor (standard drug) interacts with the active pocket of the receptor (PDB ID: **6HF3**) to form three (3) H-bonds with Tyr-415, His-132, Lys-418. The hydrophobic interactions of chromene with 3 amino acid residues Pro-116, Val-121 and Arg-58 in the active pocket of the target having no any hydrogen bond as shown in **Fig 2-4** and the interaction is π-alkyl, alkyl. While the delocalized π-electron of the benzene ring and 1, 3, 4 oxadiazole ring moiety in the molecule also interact to form another hydrophobic interactions with amino acid residue of Lys 418.

In addition, the results of the ADME and drug-likeness properties revealed that the 8 newly designed molecules have good pharmacokinetic properties which are predicted to have good orally bioavailability with minimal toxicity and good absorption.

CONCLUSION

DprE1 (6HF3) enzyme plays a key role in tuberculosis. In order to ameliorate DprE1 inhibitory activity, a wide range of electron withdrawing group (NO_2, Cl & F) at *m* and *p* position on the phenyl ring in case of substituted 1, 3, 4 oxadiazole derivatives need to be investigated as pharmacophore development for these classes of molecules. The supplementary lead optimization should be carried out for the better anti-mycobacterial activity.

Table No. 1 ADME and Drug Likeness Parameters of synthesized compounds

CC	SA	GI	BBB	PgP	MW	BS	N HBA	N HBD	TPSA	I LogP	W Log	nLV
1A	4.13	High	Y	N	334.33	0.55	5	0	72.11	3.08	1.95	0
2A	4.17	High	N	N	379.32	0.55	7	0	117.93	2.64	1.85	0
3A	4.24	High	N	N	379.32	0.55	7	0	117.93	2.66	1.85	0
4A	4.14	High	N	N	368.77	0.55	5	0	72.11	3.27	2.6	0
5A	4.1	High	Y	N	368.77	0.55	5	0	72.11	3.21	2.6	0
6A	4.11	High	N	N	352.32	0.55	6	0	72.11	3.27	2.51	0
7A	4.19	High	N	N	364.35	0.55	6	0	81.34	3.24	1.95	0

SA synthetic accessibility, GI gastrointestinal absorption, BBB blood–brain barrier permeant, Pgp P-glycoprotein substrate, MW molecular weight, n HBD number of hydrogen bond donor,n HBA number of H- bond acceptor, BS Bio--availability Score, TPSA topological polar surface area, WLOGP water partition co-efficient, nLV number of Lipinski violation.

Table No. 2 Predicted data of synthesized compounds

Sl. no.	Compound code	Calculated % of element (C, H, N)	Clog p	Drug likeness	Drug score
01	1A	45.85, 2.20, 15.28	3.36	-6.11	0.11
02	2A	45.10, 3.79, 15.03	1.18	-6.51	0.11
03	3A	40.84, 1.71, 17.01	1.18	-6.51	0.11
04	4A	51.79, 3.86, 16.78	3.99	-5.87	0.11
05	5A	54.93, 4.37, 16.86	3.99	-5.87	0.11
06	6A	53.01, 4.45, 19.52	3.77	-6.15	0.11
07	7A	56.73, 3.33, 16.54	3.4	-6.31	0.15
08	8A	56.60, 3.09, 13.20	2.87	-6.46	0.11

Table No. 3 Predicted Moleinspiration data of synthesized compounds

Sl. no	Compound code	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand
01	1A	-0.16	-0.06	-0.11	-0.94
02	2A	-0.17	0.02	-0.19	-1.00
03	3A	-0.19	-0.08	-0.13	-0. 98
04	4A	-0.18	-0.07	-0.04	-0.81
05	5A	-0.09	-0.04	-0.11	-0.75
06	6A	-0.03	0.08	0.02	-0.76
07	7A	-0.19	-0.05	0.02	-0.92
08	8A	-0.16	-0.03	-0.20	-0.83

Table No. 4 Physical properties of synthesized compounds

Sl. No	Compound code	Molecular formula	Mol. weight	Melting Point (°C)	% yield	R_f value	Mobile phase
01	1A	$C_{19}H_{14}N_2O_4$	334.33	175-177	58	0.43	n-H:EA 2:1
02	2A	$C_{19}H_{13}N_3O_6$	379.32	188-190	60	0.24	n-H:EA 2:1
03	3A	$C_{19}H_{13}N_3O_6$	379.32	189-192	66	0.44	n-H:EA 2:1
04	4A	$C_{19}H_{13}ClN_2O_4$	368.77	184-186	62	0.28	n-H:EA 2:1
05	5A	$C_{19}H_{13}ClN_2O_4$	368.77	183-186	80	0.50	n-H:EA 2:1
06	6A	$C_{19}H_{13}FN_2O_4$	352.32	200-203	57	0.54	n-H:EA 2:1
07	7A	$C_{20}H_{16}N_2O_5$	364.35	162-165	70	0.34	n-H:EA 2:1
08	8A	$C_{19}H_{14}N_2O_5$	350.32	170-172	74	0.40	n-H:EA 2:1

Table No.5 Infra Red spectral study of the synthesized compounds

Compound code	Molecular nature and Spectral peaks (cm^{-1})
1A	3045 (Ar CH str), 1684 (C=O, lactone), 1628 (C=O, ketone), 1576 (C=N), 1195 (C-O, coumarin) and 752 (Ar CH bend).
2A	3065 (Ar CH str), 1681 (C=O, lactone), 1618 (C=O, ketone), 1554 (Ar-NO_2), 1572 (C=N), 1267 (C-O, coumarin) and 752 (Ar CH bend).
3A	3065 (Ar CH str), 1681 (C=O, lactone), 1618 (C=O, ketone), 1554 (Ar-NO_2), 1572 (C=N), 1267 (C-O, coumarin) and 752 (Ar CH bend).
4A	3065 (Ar CH str), 1681 (C=O, lactone), 1618 (C=O, ketone), 1186 (C-Cl, aromatic), 1572 (C=N), 1267 (C-O, coumarin) and 752 (Ar CH bend).
5A	3065 (Ar CH str), 1681 (C=O, lactone), 1618 (C=O, ketone), 1186 (C-Cl, aromatic), 1572 (C=N), 1267 (C-O, coumarin) and 752 (Ar CH bend).
6A	3065 (Ar CH str), 1681 (C=O, lactone), 1618 (C=O, ketone), 1186 (C-F, aromatic), 1572 (C=N), 1267 (C-O, coumarin) and 752 (Ar CH bend).
7A	3065 (Ar CH str), 2860 (C-H str, aromatic), 1681 (C=O, lactone), 1618 (C=O, ketone), 1572 (C=N), 1267 (C-O, coumarin) and 752 (Ar CH bend).
8A	3550 (Ar CH str), 2860 (C-H str, aromatic), 1681 (C=O, lactone), 1618 (C=O, ketone), 1572 (C=N), 1267 (C-O, coumarin) and 752 (Ar CH bend).

Table No. 6 ^{1}H NMR Spectral data of synthesized compounds

Compound code	Chemical shift value δ and Proton nature
5A	(400MHz; DMSO-d_6/TMS, ppm): 1.42 (3H, t, CH_3), 4.44 (2H, q, CH_2), 7.27-7.69 (4H, m, Ar-H), 8.53 (1H, s, Ar-H).
7A	(200MHz; $CDCl_3$/TMS, ppm): 2.38 (3H, s, $COCH_3$), 7.32-7.53 (4H, m, Ar-H), 7.8 (2H, d, Ar-H), 8.11 (2H, d, Ar-H), 8.6 (1H, s, Ar-H), 8.7 (1H, s, CH of oxadiazole).

Table No. 7 Docking results of substituted 1, 3, 4 oxadiazole derivatives

Sl. no	Compound Code	Docking score	Amino acid residue	MIC (µg/mL)
1	1A	-9.9	Tyr-415,His-132,Lys-418,Cys-387,Pro-116,Val-121	2.75
2	2A	-9.8	Tyr-415,His-132,Lys-418,Cys-387,Pro-116,Val-365	3.55
3	3A	-10.2	Thr-118,Lys-418,Gly-117,Val-365	3.45
4	4A	-10.2	Lys-418,Val-365,Pro-116,Arg-58	3.55
5	5A	-10.0	His-132,Tyr-415,Lys-418, Pro-116,Arg-58,Gln-336,Vla-365	3.70
6	6A	-10.1	Tyr-415, His-132, Lys-418,Val-365,Gly-114,Pro-116	3.72
7	7A	-9.9	Tyr-415,Lys-418,Val-365,Cys-38,Pro-116	2.15
8	8A	-9.9	Tyr-415,His-132,Lys-418,Val-365,Cys-387, Pro-116	1.75
9	Moxifloxacin	-10.5	Tyr-415,His-132,Lys-418,Cys-387,Pro 116,Val-121	3.75

MIC = minimal drug concentration required to stop the growth of Mycobacterial tuberculosis H37Rv

Figure No. 2: The docking pose of compound 2A with DprE1 (6HF3).

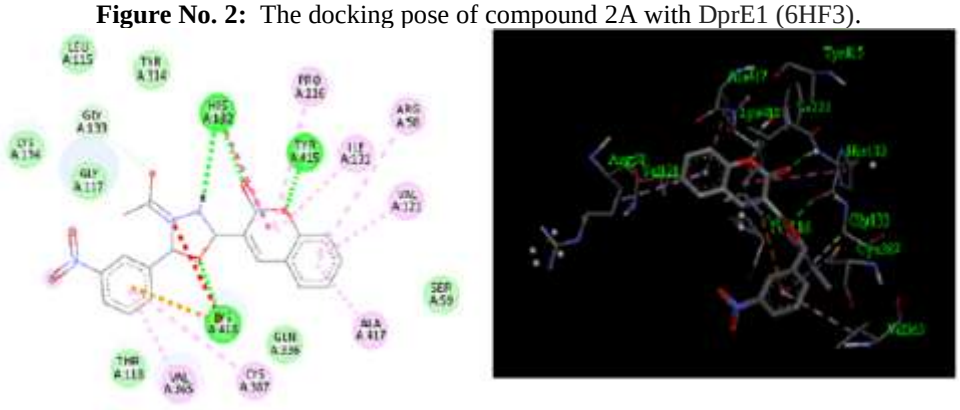


Figure No. 3: The docking pose of compound 5A with DprE1 (6HF3).

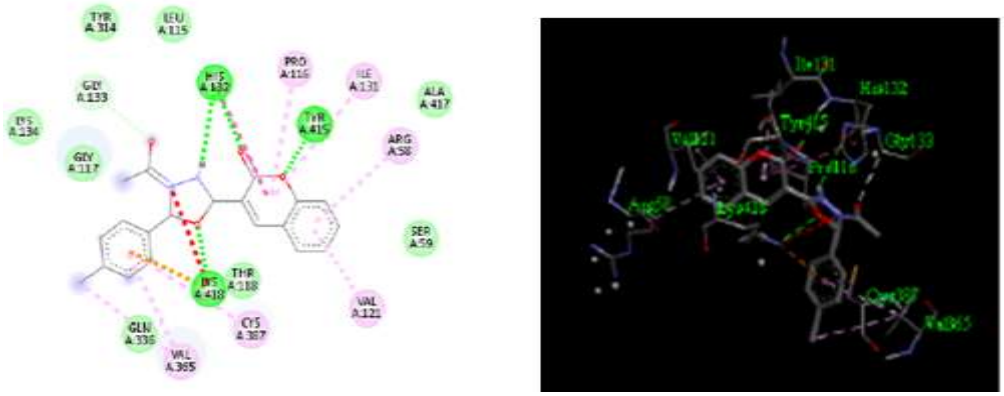
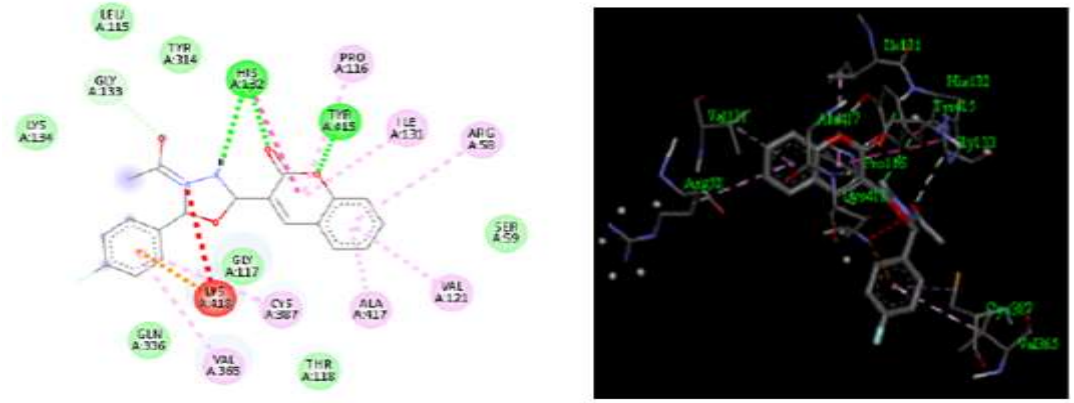


Figure No. 4: The docking pose of compound 6A with DprE1 (6HF3).



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