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Synthesis and In-silico Study of Novel 1,3,4-Oxadiazole Derivatives : A Biologically active Scaffolds which induce Anti-tubercular activity by targeting Pteridine Reductase and Dihydrofolate Reductase

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ABSTRACT

Heterocyclic compounds possess diverse biological properties that have led to intense study and research of these compounds. One of these compounds is Oxadiazole which has been found to exhibit various pharmacological activities. 1,3,4-oxadiazole having heterocyclic nucleus is a novel molecule which attract the chemist to search a new therapeutic molecule. Research on 1,3,4-oxadiazole and their synthetic analogues have revealed a variety of pharmacological activities including anti-microbial, anti-tubercular and insecticidal agents. Some of these compounds have also analgesic, anti-inflammatory, anti-cancer, anti-HIV agent, anti-parkinsonian and anti-proliferative agent. It was our interested to make novel derivatives of the titled compounds and evaluate the anti-tubercular activities. 1,3,4-oxadiazole and its derivatives (4a-4e) were obtained. The current study discusses the microwave irradiation synthesis of derivatives with the goal of generating new medications with high specificity for mycobacterium tuberculosis and low harm to the human.

Keywords: 1,3,4-oxadiazole derivatives, Microwave irradiation, Mycobacterium tuberculosis Almar dye method, Anti-tubercular activity.

I. INTRODUCTION

The chemistry of heterocyclic compounds is an interesting field of study since a long time. Oxadiazole is a cyclic compound having one oxygen and two nitrogen atoms in a five-member ring[1]. 1,3,4-oxadiazole shows wide variety of activities such as virucidal, CNS depressant, genotoxic, anticonvulsant, insecticidal, anti-tubercular, Anti-HIV, herbicidal, anti-inflammatory. It is also known to exhibit anti-malarial, Muscle relaxants, anti-tumour, lipid peroxidation inhibitor, antimicrobial, and remarkable analgesic, anti-convulsant, diuretic, hypnotic and sedative properties. There for 1, 3, 4-oxadiazole is commonly used in the area of new drug development[2]. We can distinguish several isomeric forms of oxadiazole, which occur in the structure of many drugs, e.g., anticancer zibotentan, antimicrobial furamizole, antiviral raltegravir, ataluren for Duchenne muscular dystrophy[3]. At the end of the

nineteenth century, the first derivatives of 1,3,4-oxadiazole were synthesized. The methods of obtaining the new structures were multidirectional, including reactions of appropriate hydrazides and phosgene, thermal cyclization of 1-acylsemicarbazides or cyclization of 1,2-diacylhydrazines by the action of dehydrating agents[3]. In the design of new compounds, development of hybrid molecules through the combination of different pharmacophores in one structure may lead to compounds with increased antimicrobial activity. Therefore, these observations prompted us to synthesize new 1,3,4-oxadiazole derivatives which were attached with s-triazine ring through a sulphur bridge[4].

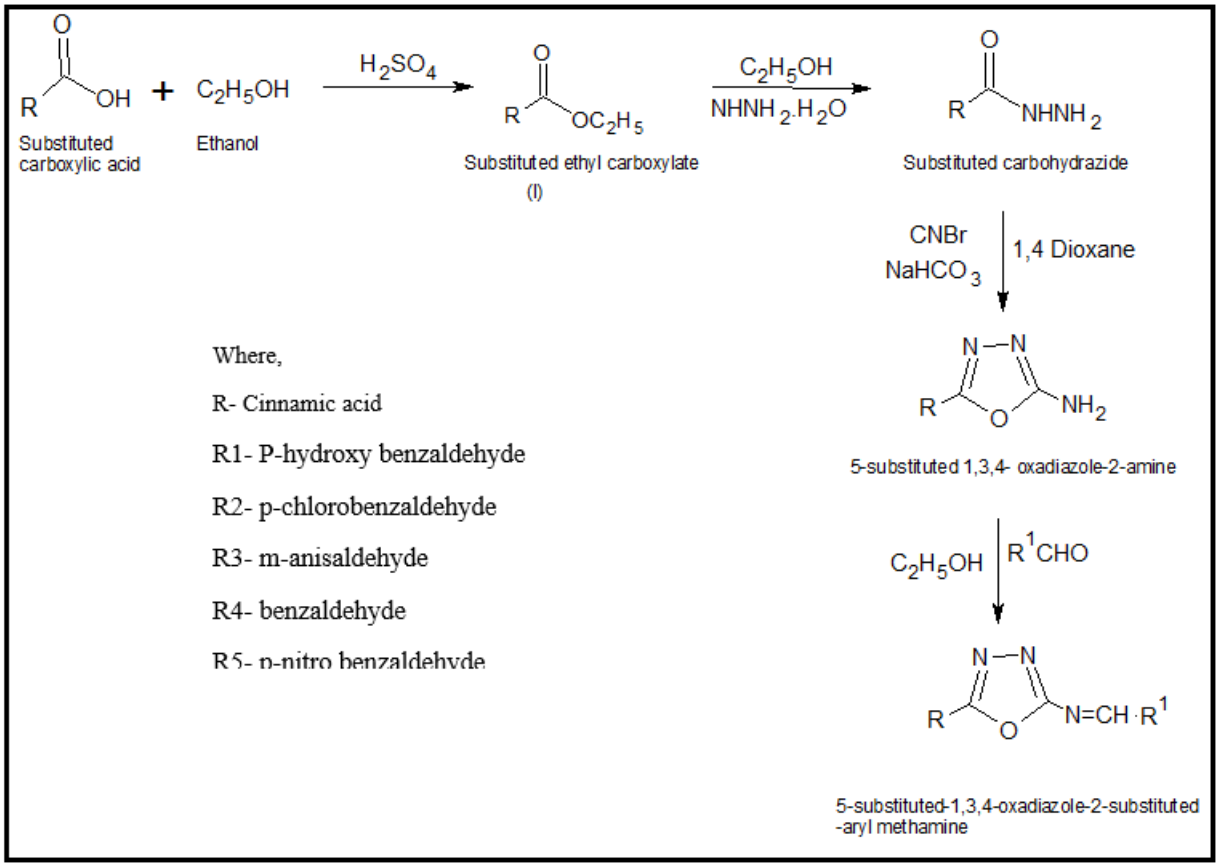
Tuberculosis (TB) is one of the peril diseases. It is caused by a pathogenic bacteria Mycobacterium tuberculosis (Mtb). Tuberculosis (TB) is the world's oldest known infectious disease and the one that kills the most people today, about two million a year. The urgency to develop new anti-tubercular drug is rapidly growing as strains that resist the current medications are spreading across the globe quite fast. Recently the profile of TB has been raised dramatically because of the growing problem of TB and HIV co-infection. The World Health Organization estimates that approximately 300,000 cases of multi-drug resistant TB emerge each year, enhanced by the parallel spread of HIV, which weakens the immune system and encourages other diseases[5]. Life threatening infectious disease caused by multidrug-resistant pathogenic bacteria (Gram-positive/Gram-negative) increased an alarming level around the world. Owing to this increased microbial resistance, new classes of antimicrobial agents with novel mechanisms are today's need to fight against the multidrug-resistant infections[6].

Oxadiazoles are important classes of compounds which have long attracted attention, owing to their remarkable biological and pharmacological properties, such as antitubercular, antibacterial, antiviral, anti-inflammatory, antifungal and insecticidal activities. Also, the azole group of heterocyclic compounds possess significant pharmacokinetic property, lipophilicity that influence the ability of drug to reach the target by transmembrane diffusion and show promising activity against resistant TB by inhibiting the biosynthesis of lipids. Most of the antitubercular drugs (isoniazid, pyrazinamide, etc.) are inhibitors of mycobacterial cell wall synthesis by inhibiting fatty acid synthetase. In the present investigation it has been tried to design and synthesized such novel compounds which inhibit the pteridine reductase and dihydrofolate reductase enzyme[6].

II. MATERIALS AND METHODS

Chemistry

All chemicals and solvents were obtained from commercial sources, and whenever necessary, they were purified and dried using guidelines from the literature. Regents were purchased from S.D fine, Research laboratory Mumbai, and MARCK laboratory Mumbai, as well as the Rajarambapu College of Pharmacy's store department in Kasegaon. The open capillary tube method was used to calculate the melting points of the synthesised chemical (^{0}C), and the results are uncorrected. The purity of the intermediate and final compounds was verified using thin layer chromatography on a single spot-on TLC plate (silica gel G) using a variety of solvents, including toluene, acetone, and ethanol system. In a chamber filled with iodine, the TLC plates were examined.



General Procedure for Synthesis of substituted ethyl carboxylate:

2-hydroxy cinnamic acid (0.15 mole) in absolute ethanol (36 ml), con. Sulphuric acid (38 ml) was added slowly under stirring. It was refluxed on steam bath for 7-8 h, cooled and poured onto crushed ice under stirring. The pH was adjusted to 7 using 10% $NaHCO_3$ and the mixture was then extracted with 5 x 25 ml of diethyl ether. Combined ethereal extracts were dried over anhydrous $MgSO_4$ and solvent was removed under reduced pressure. Reaction is observed in TL chromatography.

General Procedure for Synthesis of substituted carbohydrazide:

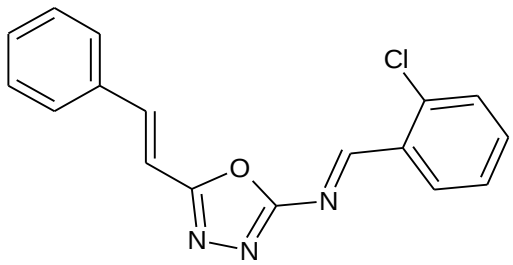
The solution of (1) in absolute ethanol (40 ml) 98% hydrazine hydrate (7.5 gm) was added. Reflux it for 7-8 hr. cool it. Then diluted with sufficient ice-cold water. White coloured precipitate was formed, filtered, washed with ice cold water, dried and recrystallize with ethanol. Completion of reaction is checked with thin layer chromatography.

General Procedure for Synthesis of 5- substituted 1,3,4-oxadiazole-2amine:

The mixture (2) in 1,4 dioxane (47 ml) was added. Cyanogen bromide (1.08 gm) was then added to resulting mixture and stirred for 4 hrs. at room temperature. Diluted with water, filtered to obtain white solid then recrystallize it with ethanol. Determination of complete reaction is observed by thin layer chromatography.

General Procedure for Synthesis of 5-substituted-1,3,4-oxadiazole-2-substituted-aryl methamine:

Obtained mixture (3) added in equimolar of aldehyde (0.005 mole). Dissolve in ethanol (0.025 mole). Reflux it for 4-5 hrs. on water bath cooled to room temperature and neutralize with ice cold water. Filter, wash with cool water. Completion of reaction is carried out by TLC method. Recrystallize with ethanol.



4B

Molecular Docking Studies:

The molecular modelling programme (VlifeMDS) version 4.3 was used for all molecular docking operations. It offered the ability to dock several ligands in the user-selected protein binding sites. The molecules can be docked in rigid (neither a protein nor a ligand has torsional flexibility) or flexible (a ligand has torsional flexibility with a rigid protein) ways using VlifeMDS. The target or receptor was theoretically created using homology modelling or knowledge-based protein modelling, or it was experimentally known[7]. The goal of the molecular docking tool was to find the optimal geometry of contact between ligand-receptor complexes with the lowest possible interaction energy using three separate scoring functions: the dock score alone, electrostatics, and the combination of steric and electrostatic forces (parameters from the force field). We were able to screen a number of compounds using this tool in order to improve leads[8-9]. To reduce the energy of the interaction between the ligand and receptor protein, VlifeMDS employs the Piecewise Linear Pair Wise Potential (PLP), genetic algorithms, and grid algorithms. BioPredicta generates 85% of binding models and the least incorrect postures from native co-crystallized structures. Strong inhibitors of pteridine reductase and dihydrofolate reductase enzyme were used for the docking investigations to signalling in the mycobacterium tuberculosis cell wall. preparation of ligands the 1,3,4-oxadiazole derivatives' 2D structures were shown using the Chemsketch programme. We cleaned and 3D optimised every structure. Using the Merck molecular force field (MMFF) with a distance-dependent dielectric function and an energy gradient of 0.01 kcal/mol A over 10,000 cycles, all of the 3D structures were optimised. All the conformers were produced, and the low energy conformer of each compound was chosen and used for further research. In order to gather knowledge of the binding modalities of 1,3,4-oxadiazole, the binding free energies of the inhibitors against the 7opj receptor were assessed using the VlifeMDS 4.3 BioPredicta too[10-12].

Selection and preparation of ligands and target protein crystal structures

X-ray diffraction techniques have resolved that potent inhibitor of breast cancer mute hormone signalling NUDT5 (PDB Code-5NWH) for anti-cancer activity with resolution 2.60A°. This must include all hydrogens that are close to the binding location and have the correct charge declarations. In order to give such a precise preparation of protein for chemical correctness and the improved protein structure, a Protein Complex PDB model for Structural Bioinformatics (SBBI) operates jointly without hydrogen and residue, in certain load conditions. PDB structure files only contain specific multimeric structures that may include metal ions and co-crystallized ligands, water molecules, and cofactors, so they are not suitable for molecular modelling calculations that need to be used right away. PDB structures might overlook the connectivity information that must be assigned in addition to formal charge and bond orders[13-15]. The protein preparation unit was mounted for docking using this programme. Because of this, the co-crystallized complex has been accomplished with minimum impact minimization, and the side chain hydroxyl group has been refocused utilising the MMFF force field[16-17].

Identification of cavities

The cavities in the receptor were mapped in order to designate an acceptable active site. The key techniques used to map the cavities were surface

mapping of the receptor, geometric void identification, scaling of the void for its hydrophobic properties, and the VLife MDS analysis tool. As a result, all receptor holes are identified and categorised based on their size and hydrophobic surface area[18].

Run of docking study

Using the V Life MDS 4.3 software and usual operating protocols, the conformers of each were aligned with the cavity's active site before being docked using the genetic algorithm (GA). The complexes were energy lowered using the MMFF method until they reached an rms gradient of 0.1 kcal/mol. The binding energy in kcal/mol or the ligand-receptor interaction energy can be expressed as follows once the ligands have been docked into the enzyme active site:

E= InterEq + InterEvdW + IntraEq +s IntraEtor.

Where,

InterEq = Intermolecular electrostatic energy of complex.

InterEvdW stands for the intermolecular vdW energy of the complex,

IntraEq for the ligand's intramolecular electrostatic energy,

IntraEvdW for the ligand's intramolecular vdW energy, and

IntraEtor for the ligand's intramolecular torsion energy.

All conformers were synthesized, and the low energy conformer for each drug was chosen and used for more research[19-20].

Anti TB evaluation

Microplate Alamar Blue Dye Method

Procedure:

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. 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.

Standard Strain used:

Mycobacteria tuberculosis (Vaccine strain, H37 RV strain): ATCC No- 27294.

Standard values for the Anti-Tb test which was performed.

Isoniazid – 1.6 µg/ml

Ethambutol – 1.6 µg/ml P

Pyrazinamide- 3.125µg/ml

Rifampicin – 0.8 µg/ml

Streptomycin- 0.8µg/ml[21-22].

III. RESULTS AND DISCUSSION

Chemistry

Different substituted 1,3,4-oxadiazole compounds were synthesised in the current study using both conventional and microwave assistance, and their anticancer efficacy was then tested. High yields are produced quickly using the microwave aided technique of synthesis.

Step 1:

a) Synthesis of Substituted Carbohydrazide.

Step 2:

b) Synthesis of 5- Substituted 1,3,4-oxadiazole-2-amine.

Step 3:

c) Synthesis of 5- Substituted-1,3,4- oxadiazole-2 – substituted aryl methamine(4a-4j) derivatives.

The synthesis by using microwave assisted method gives high yields in short time.

The compounds giving high percentage yield by conventional are 4a=87% , 4b=90%, 4c=82% ,4d=78%, and 4e=78%.

Molecular docking study

The dock score for compound 4[a, b, c, d, e] is shown in the table, and compound 4b's dock score of -55.33 makes it the compound with the lowest dock score overall. When compared to the literature, this docking score shows that the indicated medicines have a strong affinity for binding to receptor-potent inhibitors pteridine reductase and dihydrofolate reductase, which suppresscell wall synthesis of mycobacterium tuberculosis (PDB Code- 7opj). Figure 3 displays the optimal pose derived from docking data and depicts the primary interaction between the ligand and the receptor. According to the 2D representation diagram, all proposed compounds showed a remarkably comparable conformation at the binding pocket, demonstrating Vander Waals binding with the molecules ASN67A, ASN65C, SER66C, ASN67C, THR115C, GLU117C, THR118C, and ALA121C(fig 2). combine the drug's illustration with the diagram's receptor (fig 4). Isoniazid a widely used drug, has a dock score of -63.65.

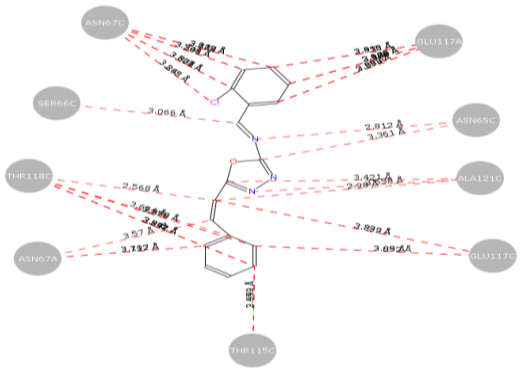


Figure 1: 2D Representation of Docking Poses of Compound 4b

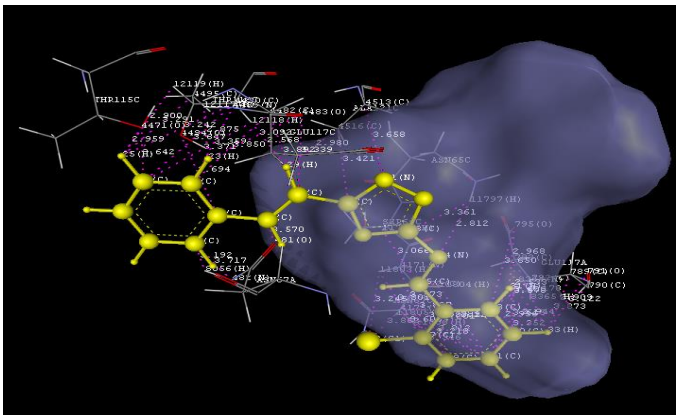


Figure 2: 3D Representation of Docking Poses of Compound 4b

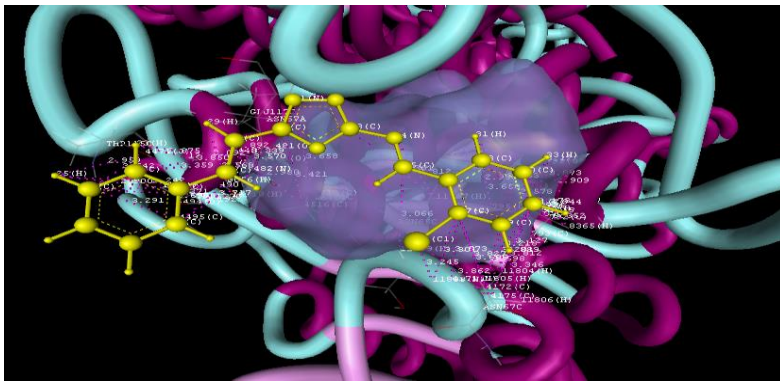


Figure 3: Superimpose Image Representation of Docking Poses of Compound 4b

Anti TB activity:

Exhaustive pharmacological studies have been conducted with the 1,3,4-oxadiazole derivative. The 2-position and 5-position is an extremely important site of molecular modification, which play a dominant role in determining the pharmacological activities of 1,3,4- oxadiazole derivatives. The synthesized compounds were screened in-vitro anti-tubercular activity with mycobacterium tuberculosis, which is cause for TB. Few compounds like compound 4B were shows good anti-tubercular activity against standard.

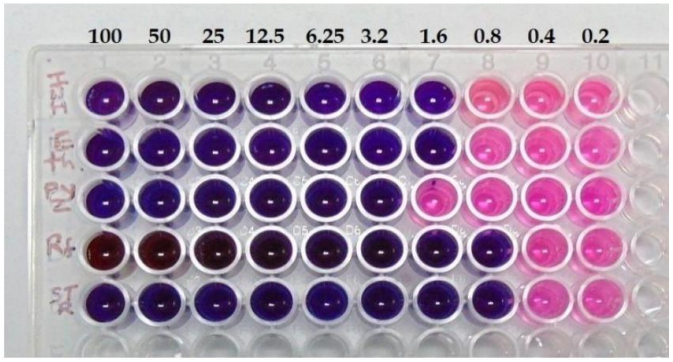


Fig 4: Standard drug

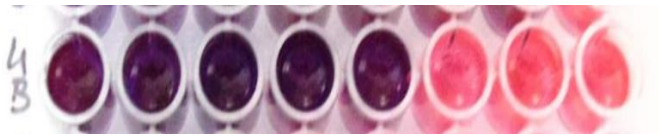


Fig 5: Test compound (4B)

Table no. 1: Anti-Tb activity of synthesized compound

Sr. 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
1	4B	S	S	S	S	S	R	R	R

Note:

S – Sensitive

R- Resistant

IV. CONCLUSION

A series of 1,3,4-oxadiazole derivatives was synthesized and assessed for antimycobacterial activity. Among the newer derivatives, it is conceivable that some of the derivatives that displayed promising anti-mycobacterial activity can be further modelled to exhibit better potency than the standard drugs.

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