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Research Article

Design, synthesis, characterization and biological evaluation of some novel 1, 3, 4 oxadiazole derivatives as anti-tubercular agents targeting L, D transpeptidase 2

Ayyadurai Jerad Suresh*, Nallappan Vidhyashree and Parakkot Ramakrishnan Surya

Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-600003, India

QR Code

***Correspondence Info:**
Dr. A. Jerad Suresh
Principal, Professor & Head
Department of Pharmaceutical Chemistry
College of Pharmacy, Madras Medical College,
Chennai-600 003, Tamil Nadu, India

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Abstract

Tuberculosis is a major disease causing 1.8 million deaths worldwide, every year. It represents the leading cause of mortality resulting from a bacterial infection. This point to an urgent need for new promising drug candidates to combat the drug resistance and control the disease. Recent studies reveal the ability of 1, 3, 4 Oxadiazole derivatives to produce antibacterial, anti-tubercular anticancer and anti-inflammatory activity. In the present research work a series of 4-(1, 3, 4-Oxadiazol-2-yl) pyridine based 1, 3, 4 Oxadiazole derivatives were designed and docked against MTB enzyme target L, d transpeptidase 2. The selected molecules were synthesized and repeatedly recrystallized to attain the expected purity. All the purified compounds were characterized by various spectral analytical techniques and evaluated for anti- mycobacterial activity against tuberculosis H37RV strain by Microplate Alamar Blue Assay (MABA) method. The experimental results showed that Compounds SA, VS4 and VS5 possesses anti-tubercular activity in the range of 12.5mcg/mL while Compounds VS1 and VS2 showed antitubercular activity with an MIC value of 6.25mcg/mL and compound NA exhibited moderate activity.

Keywords: Docking, Oxadiazole derivatives, Synthesis, MABA, Anti-tubercular.

1. Introduction

Tuberculosis is a major disease causing 1.8 million deaths worldwide, every year. It represents the leading cause of mortality resulting from a mycobacterial infection.

According to the World Health Organization (WHO) about one-third of the World's population has latent TB. The WHO also declared that TB is only second to HIV/AIDS as the most dreaded disease. TB incidence has fallen by an average of 1.5% per year since 2000. This needs to accelerate to a 4-5% annual decline to reach the 2020 milestone of the "END TB STRATEGY". Ending the TB epidemic by 2030 is among the health targets of the newly adopted Sustainable Development Goals. [1]

The transpeptidase Ltd_{Mt2} catalyzes the formation of the (3–3) cross-links characteristic of the peptidoglycan layer in the *Mycobacterium tuberculosis* cell wall. This enzyme is an excellent drug target because it is essential, is accessible from the periplasm, and has no equivalent in mammalian cells. DD-transpeptidase is the target protein of the famous β-lactam antibiotics. This research work will leads to structure-guided discovery of L, D-transpeptidase 2 inhibitors as novel antituberculosis drugs against MTB[2].

Oxadiazole derivatives have gained importance in medicinal chemistry and pharmaceutical fields due to a broad spectrum of biological activities. Recent studies reveal the potential of Oxadiazole derivatives as antifungal, anti-tubercular, anticancer and anti-inflammatory activity. Oxadiazole derivatives are the compounds, containing the imine or azomethine (–C=N–) functional group. Among

them the compounds bearing 1, 3, 4-oxadiazole and pyrazole nucleus have wide applications in medicinal chemistry and also have been reported to have significant antitubercular activity.[3]

2. Materials and Methods

2.1 Docking

Docking program is used to fit the ligand molecules into the target structure in a variety of positions, conformations and orientations in order to identify the most favourable energetical pose. Docking mode is known as pose. A process of design and discovery of new chemical entities using an automated docking program GLIDE, Auto Dock and Argus Lab is called Docking**.** Docking searches for Molecules (ligands) that have maximum favourable interactions with receptors usually a protein. Docking is done by using Argus Lab software. Argus Lab 4.0 software is distributed freely and available for windows platforms by plannaria software. [4]

2.2 Insilco Screening of Drug Likeness

Drug likeness is a qualitative concept used in drug design for how "drug like" a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested. A drug like molecule has properties such as: hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and course presence of various pharmacophoric features influence the behavior of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others.[5]

2.3 Toxicity Risk Assessment

Toxicity is one of the major criteria to be considered for a molecule to be a successful clinical candidate in pharmaceutical research. About 20-40% of drug failure comes under this category.[6]

Toxicity screening is done insilico using OSIRIS® Property Explorer. The OSIRIS® Property Explorer lets us to draw chemical structures and calculates on-the-fly various drug-relevant properties, whenever a structure is valid. Prediction results are valued and color coded. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red. Whereas a green color indicates drug-conform behavior.[7] Such of those molecules which show good drug like properties, favorable docking score, favorable interactions and no toxicity were taken up for synthesis.

2.4 Synthetic Scheme

pyridine-4-carbohydrazide

2.4.1 Procedure

A mixture of 0.01 mole of pyridine-4-carbohydrazide and 0.01 mole of aromatic acid is dissolved in phosphorus oxychloride and refluxed for 18-22 hr. The reaction mixture is slowly poured over crushed ice and kept overnight. The solid mass thus separated is filtered, dried, and purified by recrystallization from ethanol.[8]

2.4.2 Acids used

- Salicylic Acid
- Nicotinic Acid
- 4-Chloro benzoic Acid
- Benzoic Acid
- 3-Chloro benzoic Acid

2.5 Justification of Purity

2.5.1 Melting Point

Melting points were determined using one end open capillary method. The melting points were found to be sharp.

2.5.2. Thin Layer Chromatography

The compounds were checked for homogeneity by TLC on silica gel G. Solutions of the reactants and products were prepared by dissolving them inethanol. A single spot not corresponding to the parent compound was noticed hence the purity of the synthesized compounds was justified.

2.6 Biological Evaluation:

Method: Micro plate Alamar Blue Assay [MABA]

Preparation of inoculums: 100µl of the Middle brook 7H9 broth.

Requirements: 96 wells plate, Para film [all are sterilized by dry heat].

Nutrient medium: 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80

Working Procedure:

Stock solutions of the synthesized compounds and standard drug used were prepared in sterile deionized water

and taken in the concentration of 0.1 to 100µl/ml.200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100µl of the Middle brook 7H9 broth and serial dilutions of compounds were made directly on plate. The final drug concentrations were tested were 100 to 0.2µg/ml. Plates were covered and sealed with Para film and incubated at 37°c 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 24hrs. 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**.** [9,10]

3. Results

3.1 Docking Study Results

The designed molecules were docked against the selected target l, d transpeptidase 2. The best docked pose was selected based on the docking score and the multiple interactions. Doking score for the synthesized compounds are as follows- SA: -9.310 kcal/mol, NA: -8.351 kcal/mol, VS1:-9.796 kcal/mol, VS2:-9.672kcal/mol, VS4:-9.541, VS5:-9.452 kcal/mol.

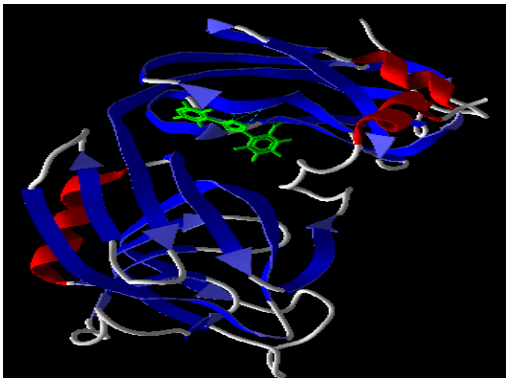


Fig 1: SA

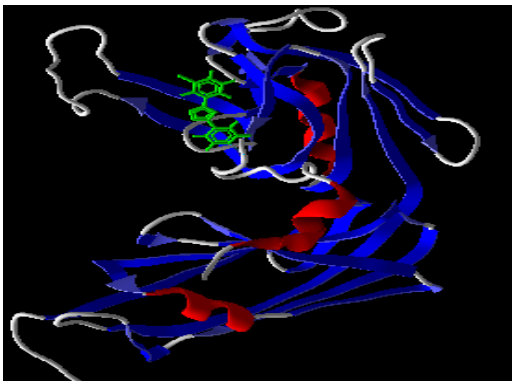
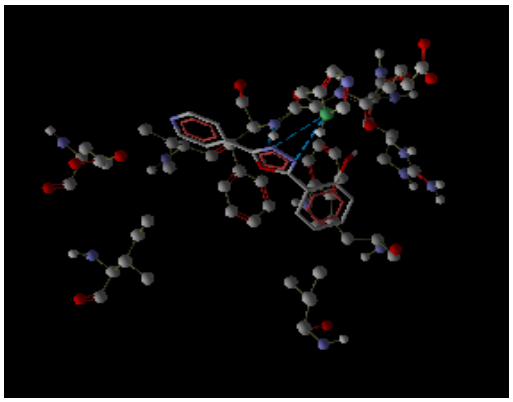


Fig 2: VS4

Fig No: 1, 2: Compounds SA, VS4 Docked against the Protein L, D Transpeptidase-2 [Docking View]



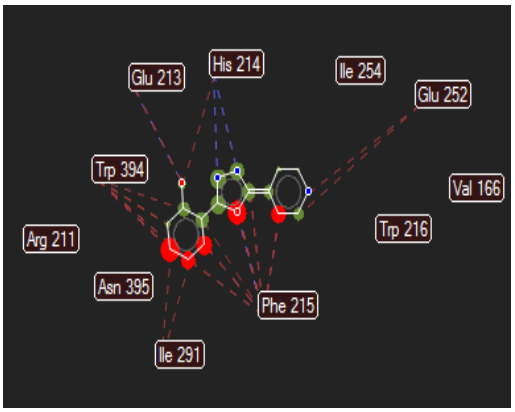
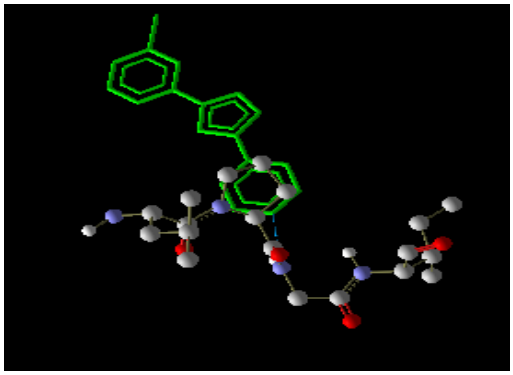


Fig 3: SA Hydrogen bond interactions and their interaction with amino acids



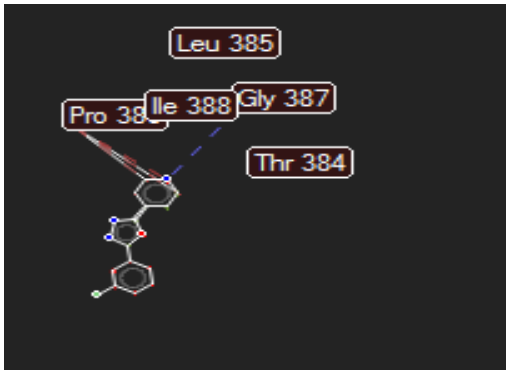


Fig 4: VS4 Hydrogen bond interactions and their interaction with amino acids

3.2 Toxicity Prediction

Prediction results are color coded in which the **red** color shows **high** risks with undesired effects like mutagenicity, tumorigenic, irritant and reproductive effects or a poor intestinal absorption and **yellow** color shows **moderate** risks with undesired effects and **green** color indicates drug-conform behavior.

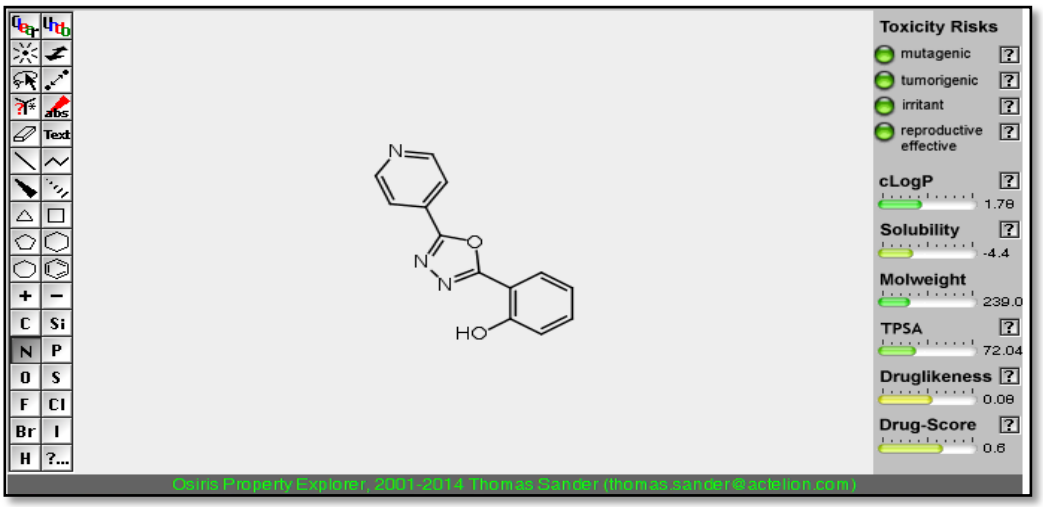


Fig 5: SA

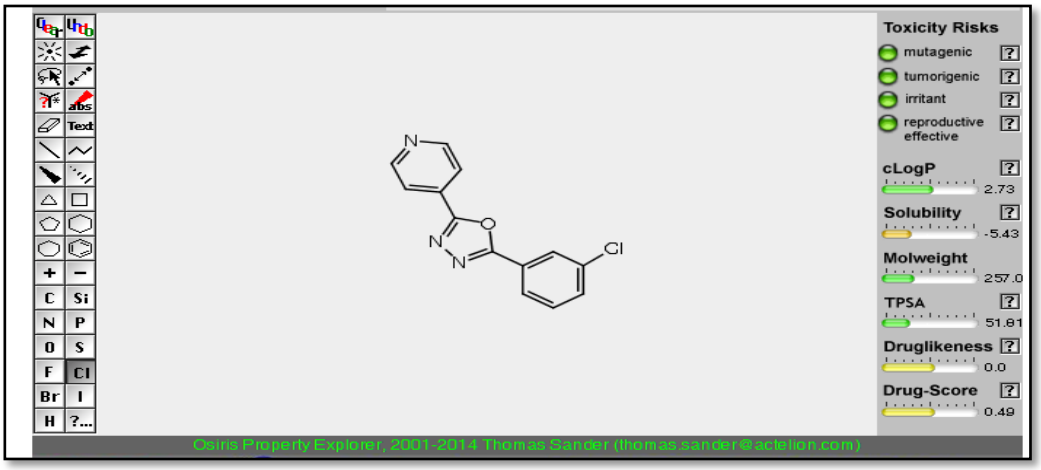


Fig 6: VS4

3.3 Spectral Analysis

Compound SA: 2-[5-(pyridin-4-yl)-1, 3, 4-oxadiazol-2-yl] phenol. IR (CM^{-1}): 3078 [Ar- CH str],1257[C-O-C str],1573[Ar C=C str], 1697[C=N str],3433[OH str] H1NMR:7.7-7.9 (8H, s, Ar-H), 8.96(1H, s, OH); MASS (g/mol): **Actual mass:239.22 g/mole, Expected mass: 239.18 g/mole.**

Compound NA: 4, 4'-(1, 3, 4-oxadiazole-2, 5-diyl) dipyridine. IR (CM^{-1}): 3031[Ar- CH str],1296[C-O-C str], 1596[Ar C=C str],1704[C=N str] H1NMR: 7.6-9.3[8H, m, Ar-H]**Actual mass: 224.21 g/mole, Expected mass: 224.09 g/mole.**

Compound VS1:4-[5-(4-chlorophenyl)-1, 3, 4-oxadiazol-2-yl] pyridine. IR (CM^{-1}): 3055[Ar- CH str], 1288[C-O-C str],1596[Ar C=C str],1689 [C=N str], 753[C-Cl str].H1NMR: 7.6-8.9[8H, d, Ar -H]. **Actual mass: 257.67g/mole, Expected mass: 257.20g/mole.**

Compound VS2: 4-(5-phenyl-1, 3, 4-oxadiazol-2-yl) pyridine. IR (CM^{-1}): 3062[Ar- CH str], 1288[C-O-C str], 1542[Ar C=C str], 1606[C=N str]. H1NMR: 7.53-7.57[1H, t, Ar- H], 7.65-7.7[4H, d, Ar- H], 7.9-8.2[4H, t, Ar- H]. **Actual mass: 223.23 g/mole, Expected mass: 223.09 g/mole.**

Compound VS4: 4-[5-(3-chlorophenyl)-1, 3, 4-oxadiazol-2-yl] pyridine.IR (CM^{-1}): 3078[Ar CH str],1257[C-O-C str],1573[Ar C=C str],1697[C=N str], 748[C-Cl str].H1NMR:7.53-7.57(3H, t, Ar- H),7.89-7.91(4H, m, Ar-H),8.88-8.89(2H, d, Ar- H).**Actual mass: 257.67 g/mole, Expected mass: 257.15 g/mole.**

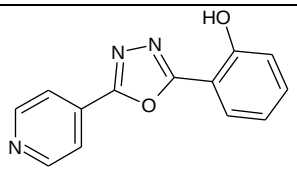
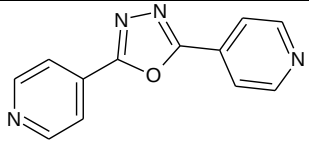
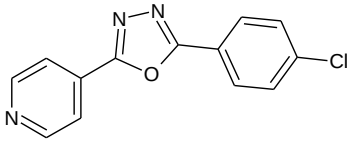
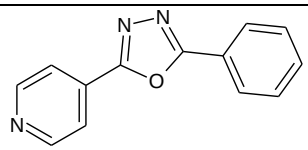
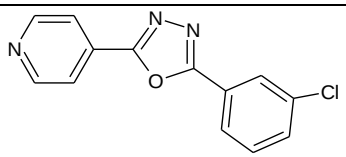
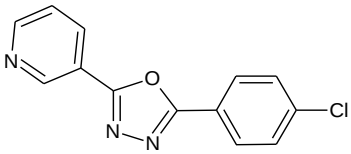
Compound VS5: 3-[5-(4-chlorophenyl)-1, 3, 4-oxadiazol-2-yl] pyridine. IR (CM^{-1}): 3055[Ar- CH str], 1288[C-O-C str], 1596[Ar C=C str], 1689[C=N str], 753[C-Cl str]. H1NMR: 6.64-6.66(1H, d, Ar-H), 7.17-8.85(7H, m, Ar H). **Actual mass: 257.67 g/mole, Expected mass: 257.08 g/mole.**

3.4 Properties and Activities of the Synthesized Compounds:

Table 1: Physical Properties

Code	Molecular weight(g/mol)	Melting point (°C)	Molecular Formula	Solubility	Yield	Molar refractivity
SA	239.22	196	$C_{13}H_9N_3O_2$	Ethanol/ DMSO	80%	63.89 ± 0.3 cm^3
NA	224.21	157	$C_{13}H_8N_4O$	Ethanol/ DMSO	72%	60.10 ± 0.3 cm^3
VS1	257.67	166	$C_{13}H_8ClN_3O$	Ethanol/ DMSO	70%	66.90 ± 0.3 cm^3
VS2	223.23	92	$C_{13}H_9N_3O$	Ethanol/ DMSO	75%	62.01 ± 0.3 cm3
VS4	257.67	108	$C_{13}H_8ClN_3O$	Ethanol/ DMSO	82%	66.90 ± 0.3 cm^3
VS5	257.67	165	$C_{13}H_8ClN_3O$	Ethanol/ DMSO	74%	66.90 ± 0.3 cm^3

Table 2: Docking Score with MIC Value

Compound	Synthesized Compounds	Docking Score(kcal/mol)	MIC Value Mcg/ml
SA		-9.310	12.5
NA		-8.351	100
VS1		-9.796	25
VS2		-9.672	25
VS4		-9.541	12.5
VS5		-9.452	12.5

3.5 *In vitro* activity of the Synthesized Compounds

Table 3: MABA Report

Sample Code	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
SA	S	S	S	S	R	R	R	R
NA	S	R	R	R	R	R	R	R
VS1	S	S	S	R	R	R	R	R
VS2	S	S	S	R	R	R	R	R
VS4	S	S	S	S	R	R	R	R
VS5	S	S	S	S	R	R	R	R

Note: S- Sensitive, R- Resistant, Strain used: - M. tuberculosis [H37RV]: ATCC NO-27294.

3.6 Toxicity Prediction Reports Of Synthesized Compounds

Toxicity predictions of synthesized compounds were performed by Osiris property explorer and its toxicity characteristics were observed.

Table 4: Toxicity Prediction:

Samples	SA	NA	VS1	VS2	VS4	VS5
Mutagenic	+	+	+	+	+	+
Tumorigenic	+	+	+	+	+	+
Irritant	+	+	+	+	+	+
Reproductive Effect	+	+	+	+	+	+

[+] indicates absence of toxicity; [-] indicates Presence of toxicity.

4. Discussion

Compounds SA, NA, VS1, VS2, VS4, and VS5 have docking score -9.31,-8.35,-9.79,-9.67,-9.54, and -9.45 Kcal/mol respectively against tuberculosis specific targets and were synthesized in an appropriate manner .The purity of the compounds were determined by the sharp melting point and single spot obtained in the TLC.

Of the six compounds, five of them were obtained at 98% purity. It was confirmed by GC-MS analysis (obtaining a single peak) and molecular weight also obtained at ± 1 variation. The functional group determination was confirmed from FT-IR spectra.

The biological evaluation of the compounds indicated that the specific organism H37RV was sensitive at 12.5 mcg/ml and showed better activity compared to the standard drugs.

The toxicity assessment of the compounds also showed that all the 6 compounds are non-toxic.

5.Conclusion

A series of 1, 3,4 Oxadiazole derivatives were designed and docked against Mtb enzyme target l, d transpeptidase 2 which is crucial enzyme for the lipid biosynthetic pathway. The selected molecules were synthesized and repeatedly recrystallized to attain the expected purity. All the purified compounds were characterized by IR, NMR, and GCMS spectral data and evaluated for anti- mycobacterial activity against tuberculosis H37RV strain by Microplate Alamar Blue Assay (MABA) method.

The experimental results shown that Compounds VS4, VS5, SA possess anti-tubercular activity with an MIC value of 12.5 mcg/ml while Compound VS1,VS2 showed antitubercular activity with an MIC Value 25mcg/ml. while compound NA possesses moderate activity.

Reference

[1]. WHO, Tuberculosis. Available from URL http://www.who.int/mediacentre/factsheets/fs104/en/

[2]. Sabri B Erdemli: Targeting the Cell wall of Mycobacterium Tuberculosis: Structure and Mechanism of L, D-Transpeptidase 2. *Journal of Author Manuscript* 2012; 20(12):2103-15.

[3]. De Oliveira, C.S.; Lira, B.F.; Barbosa-Filho, J.M.; Lorenzo, J.G.F.; de Athayde-Filho, P.F. Synthetic Approaches and Pharmacological Activity of 1,3,4-Oxadiazoles: A Review of the Literature from 2000–2012. *Molecules* 2012; 17: 10192-10231.

[4]. Kitchen DB, Decornez H, Furr J R, Bajorath J: Docking and scoring in virtual screening for drug discovery. *Nature Reviews Drug Discovery*, 2004; 3(11): 935-49.

[5]. Joy S, Nair PS, Hariharan R, Pillai MR. Detailed comparison of the protein-ligand docking efficiencies of GOLD, a commercial package and Argus Lab, a licensable freeware. *In Silico Biol*. 2006; 6(6):601-5.

[6]. http://e.wikipedia.org/wiki/lipinski%27s_rule_of_five 2014.

[7]. Lin J, Sahakian DC, De Morais SM: The Role of Absorption, Distribution, Metabolism, Excretion and Toxicity in Drug Discovery. *Curr Top Med Chem* 2003; 3(10): 1125-1154.

[8]. Shashikant R Pattan, Rabara PA, Jayashri S Pattan, Bukitagar AA: Synthesis and evaluation of some novel substituted 1, 3, 4-Oxadiazole and pyrazole derivatives for antitubercular activity. *Indian Journal of Chemistry* 2009; 48(B): 1553-1456.

[9]. Brian Leonard, Jorge Coronel, Mark Siedner, Louis Grand Jean: Inter and Intra- Assay Reproducibility of Micro plate Alamar blue Assay Results for Isoniazid, Rifambicin, Ethambutol, Streptomycin, ciprofloxacin and Capreomycin Drug susceptibility Testing of mycobacterium tuberculosis. *Journal of Clinical Microbiology* 2008; 3526-3529.

[10]. Vanitha JD, Paramasivan CN: Evaluation of Micro plate Alamar blue assay for drug susceptibility testing of *Mycobacterium avium* complex isolates. *Diagnostic Microbiology and Infectious Disease*. 2004; 49: 179-182.