SYNTHESIS CHARACTERIZATION AND IN-SILICO SCREENING OF 1,3,4-OXADIAZOLE DERIVATIVES FOR ANTIBACTERIAL ACTIVITY

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Abstract: Eight 1,3,4-oxadiazole derivatives were designed and synthesized. The aim of the study was to verify the effectiveness of synthesized oxadiazole derivatives against bacterial activity. Assessment of their antibacterial activity exposed that the newly synthesized compounds showed significant growth inhibition of a wide spectrum of Gram-positive bacteria and Gram-negative bacteria. The majority of the compounds displayed greater antibacterial activity than the reference drugs. Docking studies of all the compounds were performed in order to explore their binding mode at the DHFR protein. The combine analysis could be very useful in designing better novel potent antimalarial agents.

Key words: 1, 3, 4-oxadiazole, invitro anti-bacterial, molecular docking, zone of inhibition, disc-diffusion method.

I.INTRODUCTION

Bacterial infection is one of the most blooming problems of modern-day medicine, especially in developing countries where increased prevalence of infectious disease is the key cause of population slump [1]. Antimicrobials are the most frequent drugs to treat these attacks but from the last decade, the incidence of microbe's infection has caressed alarming levels on accounts of antimicrobial resistance [2]. Only a few medical drugs are available to cure these diseases nevertheless they have received their own limitations like complicated structures, multiple synthetic steps and atrocious side effects [3]. DHFR catalyses the NADPH dependent reduction of dihydrofolate to tetrahydrofolate and is vital for the biosynthesis concering purines, thymidylate and several amino acids. DHFR is a well identified target in several therapeutic areas including cancer in addition to anti-infective where it can also be used as antibacterial, antifungal and antiparasitic agents. In the past, most antibacterial DHFR agents are based on the 5-benzyl-2, 4-diaminopyrimidine scaffold credited its excellent potency and selectivity for bacterial versus mammalian DHFR. The most widely used DHFR antiseptic agent is trimethoprim (TMP), which has been used for decades to take care of a variety of bacterial diseases [3]. DHFR is frequently used as representations in molecular docking studies to forecast the hypothetical protein-ligand binding mode, which acts a significant part in structural based drug design and structure activity relationship[4],[5].

Amongst all, heterocyclic molecules that contain nitrogen and oxygen possess most potent pharmacological activities [6]. With this context, 1, 3, 4-oxadiazoles are important five-membered heterocyclic motifs, which are associated with great research interest credited to their potential application in pharmaceutical chemistry.

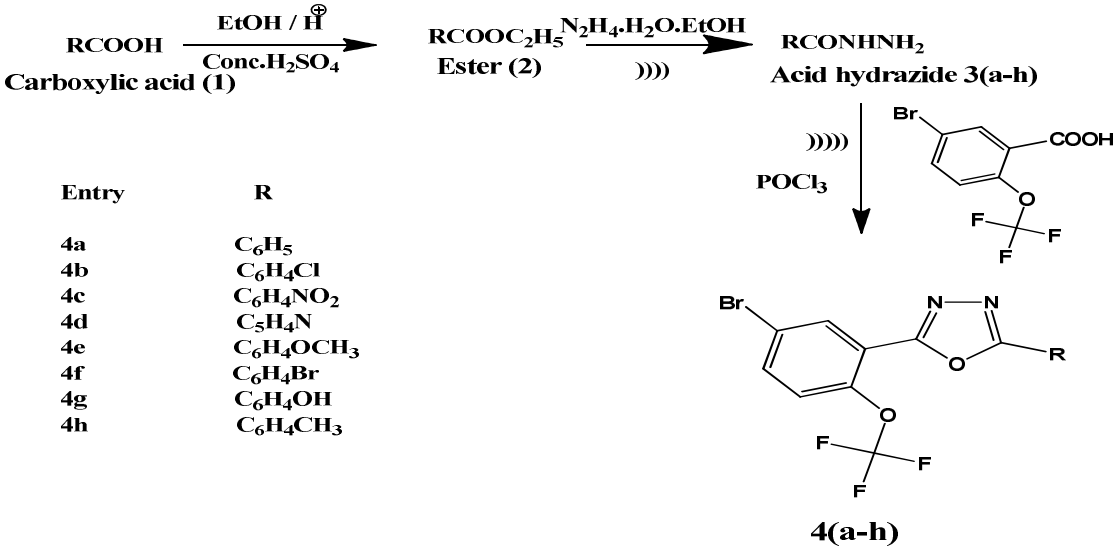
It follows through the literature that depending on the type of substituent, the analogues of 1, 3, 4-Oxadiazole have got a high possibility of a variety of biological activities such as anti-HIV [7], analgesic, anti-inflammatory[8], anti-cancer[9], antimalarial [10], antimicrobial [11] and anti-tuberculosis[12].

Fluorine and fluorine containing compounds has become an essential tool in novel drug discovery as incorporation of fluorine atom or fluorinated group into organic molecules are responsible to coexisting modulation of steric, electric and lipophilic parameters, these parameters have vital effect on the pharmacodynamics and pharmacokinetic properties of such derivatives. Fluorine is much more lipophilic than hydrogen and this improved lipophilicity may lead to easier assimilation and transportation of compounds within the biological systems. The inherent properties of fluorine atom, such as electronegativity, size, omniphobicity/ lipophilicity and electrostatic interactions can drastically influence chemical reactivity lead to a significant development in the biological process of fluorinated molecules[13]-[16]. Hence, by blending fluorine with the title compounds, we may expect some increment in the biological assets of medicine derivatives, helping them selectively latch on to their molecular targets, and wade through the surrounding cells of fatty membranes which cause substantial improvement in efficacy [17].

Considering the above literature findings and in connection with the ongoing studies towards the activity of novel bioactive molecules, we have taken out the synthesis of 1, 3, 4-oxadiazole derivatives associated trifluoro moiety. All the synthesized compounds were characterized by various spectroscopic methods and after that evaluated for 1n- vitro antibacterial activities. Many structural modifications were recommended inside the parent molecule to be able to derive more efficient oxadiazole compounds. The molecular interactions associated with the selected synthesized derivatives were performed by the usage of in silico docking scientific studies with AutuDock 4. 2 docking software provided by pyRx to determine the actual molecular target plus analyze the binding attributes of various proteins along with oxadiazole derivatives.

II.MATERIALS AND METHODS

All solvents and chemicals were purchased from commercial sources (Sigma–Aldrich and Fisher Scientific) and were used without additional purification. The melting point of oxadiazoles was calculated in open capillaries and is uncorrected. FT-IR spectrum was obtained by using an SHIMADZU Fourier transformed infrared (FT-IR) spectrometer using KBr (pellet form). The NMR spectra were measured on a Bruker instrument in DMSO-d_6 solution. The chemical shifts were measured relative to TMS.



Entry	R
4a	C_6H_5
4b	C_6H_4Cl
4c	$C_6H_4NO_2$
4d	C_5H_4N
4e	$C_6H_4OCH_3$
4f	C_6H_4Br
4g	C_6H_4OH
4h	$C_6H_4CH_3$

Scheme 1 Synthesis of 1,3,4-oxadiazoles 4a-h

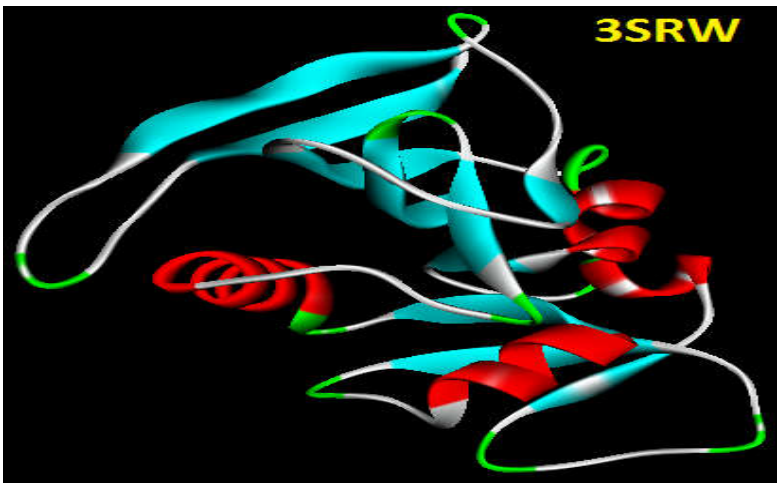


Fig. 1 3D image of 3SRW of Staphylococcus aureus

For AutoDock 4.2, ligand molecules were drawn in Chem Bio Draw Ultra 12.0 and converted to their three-dimensional structures in Chem Bio3D Ultra 12.0 and saved as in pdb format. The prepared ligands were used as input files for Auto Dock in the next step. For docking simulations, Lamarckian genetic algorithm method was employed. The standard docking procedure was used for a rigid protein and a flexible ligand whose torsion angles were identified (for 10 independent runs per ligand). A grid of 60, 60, and 60 points in x, y, and z directions was built with a grid spacing of 0.375 A˚ and a distance dependent function of the dielectric constant were used for the calculation of the energetic map. The default settings were used for all other parameters. At the end of docking, the best poses were analyzed for hydrogen bonding/π–π interactions and root mean square deviation (RMSD) calculations using Discovery Studio Visualizer 4.2 (Accelrys Software Inc.) and Pymol (The PyMOL Molecular Graphics System) programs.

Antibacterial Activity

The newly prepared compounds were screened for their antibacterial activity against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa Streptococcus pyogenes and Bacillus Subtilis bacterial strains by disc-diffusion method [18],[19]. A standard was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. By using Whatman no.1 filter paper ,the discs measuring 6.25 mm in diameter were prepared and sterilized by heat at 140 °C for 1 hour. The sterile discs previously soaked with the test compound solution in DMSO of specific concentration 100 µg/disc were carefully placed on the agar culture plates. The plates were incubated at 37 °C and the diameter of the growth inhibition zones were measured after 24 hrs. The plates were inverted and incubated for 24 hrs at 37°C. Ciprofloxacin was used as a standard drug, Inhibition zones were measured and compared with the Ciprofloxacin.

Synthesis of ester:

The compounds **2a-h** was prepared according to the procedure given in literature with a little modification [20]. Carboxylic acid (0.1mol), ethanol (60 ml) and conc. H_2SO_4 (1.4 ml) were placed in a 250 ml round-bottom and were irradiated for 1 hr on a ultrasonic cleaning bath. The reaction mixture was concentrated on a rotatory evaporator. It was filtered and collected.

Synthesis of acid hydrazide 3a-h

The compounds **3a-h** was prepared according to the procedure given in literature with a little modification [20]. Ester and hydrazine hydrate in 1:1 portion and ethanol (30 ml) was placed in a round-bottom flask. The mixture was irradiated for 30 min. The reaction mixture was concentrated on a rotatory evaporator. It was filtered and collected.

Synthesis of 2- (5-bromo-2- (trifluoromethoxy) phenyl) -5- aryl - 1,3,4 oxadiazole 4a-h.

A mixture of acid hydrazide (0.01 mol) and 5-bromo-2-(trifluoromethoxy)benzoic acid (0.01mol) in $POCl_3$ (5ml) was irradiated on ultrasonic cleaning bath for 2 hrs. Then the reaction mixture was cooled and transferred to crushed ice. It was neutralized with sodium bicarbonate solution and the resulting solid was filtered, dried and washed with water and recrystallized from ethanol to give 2,5-disubstituted-1,3,4-Oxadiazole 4(a-h). The Synthetic procedure is shown in **Scheme 1**.

2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-phenyl-1,3,4-oxadiazole (4a):

Pale Yellow solid; Yield 69%., M.P: 193-195°C, MF: $C_{15}H_8BrF_3N_2O_2$; IR (KBr): 3078 cm^{-1} (C-H Ar str); 1598 cm^{-1} (C=N str); 503 cm^{-1} (C-Br str); 1165 cm^{-1} (C-F str); 1080 cm^{-1} (N-N str). ^{1}H-NMR (400 MH_Z, DMSO-d_6): 7.09-7.93 δ (8H, Aromatic protons); ^{13}C-NMR (400 MH_Z, DMSO-d_6): 114.02-133.35 δ (Aromatic carbon); 163.11 δ (C of 1,3,4-Oxadiazole ring); 151.66 δ (C-O).

2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-(4-chlorophenyl)-1,3,4-oxadiazole (4b)

Pale Yellow solid; Yield 72%., M.P: 132-135°C, MF: $C_{15}H_7BrClF_3N_2O_2$; IR (KBr): 3076 cm^{-1} (C-H Ar str); 1602 cm^{-1} (C=N str); 516 cm^{-1} (C-Br str); 1165 cm^{-1} (C-F str); 1062 cm^{-1} (N-N str); 736 cm^{-1} (C-Cl str). ^{1}H-NMR (400 MH_Z, DMSO-d_6): 6.86-7.44 δ (7H, Aromatic protons); ^{13}C-NMR (400 MH_Z, DMSO-d_6): 117.16-138.77 δ (Aromatic carbon); 161.72 δ (C of 1,3,4-Oxadiazole ring); 152.18 δ (C-O).

2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (4c)

Dark brown solid; Yield 65%., M.P: 152-155°C, MF: $C_{15}H_7BrF_3N_3O_4$; IR (KBr): 3066 cm^{-1} (C-H Ar str); 1602 cm^{-1} (C=N str); 505 cm^{-1} (C-Br str); 1166 cm^{-1} (C-F str); 1060 cm^{-1} (N-N str). ^{1}H-NMR (400 MH_Z, DMSO-d_6): 7.10-7.94 δ (7H, Aromatic protons); ^{13}C-NMR (400 MH_Z, DMSO-d_6): 114.11-134.58 δ (Aromatic carbon); 166.76 δ (C of 1,3,4-Oxadiazole ring); 152.11 δ (C-O).

4-(5-(5-bromo-2-(trifluoromethoxy)phenyl)-1,3,4-oxadiazol-2-yl)pyridine (4d)

Pale Yellow solid; Yield 66%., M.P: 146-147°C, MF: $C_{14}H_7BrF_3N_3O_2$; IR (KBr): 3064 cm^{-1} (C-H Ar str); 1612 cm^{-1} (C=N str); 513 cm^{-1} (C-Br str); 1161 cm^{-1} (C-F str); 1060 cm^{-1} (N-N str). ^{1}H-NMR (400 MH_Z, DMSO-d_6): 7.06-7.90 δ (7H, Aromatic protons); ^{13}C-NMR (400 MH_Z, DMSO-d_6): 119.03-134.86 δ (Aromatic carbon); 156.95 δ (C of 1,3,4-Oxadiazole ring); 156.08 δ (C-O).

2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-(4-methoxyphenyl)-1,3,4- oxadiazole (4e)

Pale Yellow solid; Yield 75%., M.P: 124-126°C, MF: $C_{16}H_{10}BrF_3N_2O_3$; IR (KBr): 3080 cm^{-1} (C-H Ar str); 2941 cm^{-1} (C-H Aliphatic str); 1579 cm^{-1} (C=N str); 536 cm^{-1} (C-Br str); 1166 cm^{-1} (C-F str); 1064 cm^{-1} (N-N str). ^{1}H-NMR (400 MH_Z, DMSO-d_6): 7.04-7.91 δ (7H, Aromatic protons); 3.82 δ (3H, OCH_3 group). ^{13}C-NMR (400 MH_Z, DMSO-d_6): 113.65-139.95 δ (Aromatic carbon);166.27 δ (C of 1, 3, 4-Oxadiazole ring); 55.32 δ (OCH_3 group); 151.65 δ (C-O).

2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-(4-bromophenyl)-1,3,4-oxadiazole (4f)

Pale Yellow solid; Yield 63%., M.P: 162-165°C, MF: $C_{15}H_7Br_2F_3N_2O_2$; IR (KBr): 3070 cm^{-1} (C-H Ar str); 1606 cm^{-1} (C=N str); 528 cm^{-1} (C-Br str); 1062 cm^{-1} (C-F str); 1178 cm^{-1} (N-N str). ^{1}H-NMR (400 MH_Z, DMSO-d_6): 7.04-7.91 δ (7H, Aromatic protons); ^{13}C-NMR (400 MH_Z, DMSO-d_6): 114.56-135.88 δ (Aromatic carbon); 166.76 δ (C of 1,3,4-Oxadiazole ring); 152.15 δ (C-O).

4-(5-(5-bromo-2-(trifluoromethoxy)phenyl)-1,3,4-oxadiazol-2-yl)phenol (4g)

Pale Yellow solid; Yield 71%., M.P: 122-124°C, MF: $C_{15}H_8BrF_3N_2O_3$; IR (KBr): 3083 cm^{-1} (C-H Ar str); 1598 cm^{-1} (C=N str); 503 cm^{-1} (C-Br str); 1168 cm^{-1} (C-F str); 1062 cm^{-1} (N-N str). ^{1}H-NMR (400 MH_Z, DMSO-d_6): 7.03-7.84 δ (7H, Aromatic protons); 10.12 δ (1H, OH group) ^{13}C-NMR (400 MH_Z, DMSO-d_6): 111.94-136.36 δ (Aromatic carbon); 165.31 δ (C of 1,3,4-Oxadiazole ring); 158.36 δ (C-O).

2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-*p*-tolyl-1,3,4-oxadiazole (4h)

Pale Yellow solid; Yield 67%., M.P: 116-119°C, MF: $C_{16}H_{10}BrF_3N_2O_2$; IR (KBr): 3072 cm^{-1} (C-H Ar str); 2945 cm^{-1} (C-H Aliphatic str); 1608 cm^{-1} (C=N str); 495 cm^{-1} (C-Br str); 1168 cm^{-1} (C-F str); 1060 cm^{-1} (N-N str). ^{1}H-NMR (400 MH_Z, DMSO-d_6): 7.16-7.98 δ (7H, Aromatic protons); 2.49 δ (3H, CH_3 group). ^{13}C-NMR (400 MH_Z, DMSO-d_6): 117.14-135.90 δ (Aromatic carbon); 164.77 δ (C of 1,3,4-Oxadiazole ring); 26.10 δ (CH_3 group); 152.17 δ (C-O).

III.RESULTS AND DISCUSSION

The synthesis of compounds **4a-h** necessitated the preparation of suitably modified aromatic hydrazide. As outlined in **Scheme 1**, esterification of carboxylic acids produced the corresponding carboxylic esters **2a-h** and ester produced the corresponding aromatic hydrazides **3a-h.** 2-(5-bromo-2-(trifluoromethoxy) phenyl)-5-aryl-1,3,4-oxadiazole **4a-h**, have been synthesized and their chemical structure was confirmed by means of FT-IR, ^{1}H and ^{13}C NMR spectral techniques.

Table 1 Antibacterial activity of synthesized 1,3,4-oxadiazole derivatives 4a-h

S. No.	Bacteria	Ciprofloxacin	Zone of inhibition mm in diameter							
			1	2	3	4	5	6	7	8
1	*Bacillus subtilis*	24	-	-	16	-	14	11	13	15
2	*Escherichia coli*	30	17	-	-	18	23	13	12	15
3	*Pseudomonas aeruginosa*	31	-	17	-	18	-	11	11	11
4	*Staphylococcus aureus*	30	17	15	-	-	09	14	12	13
5	*Streptococcus pyogenes*	32	12	18	-	-	14	11	-	14

Antibacterial Activity

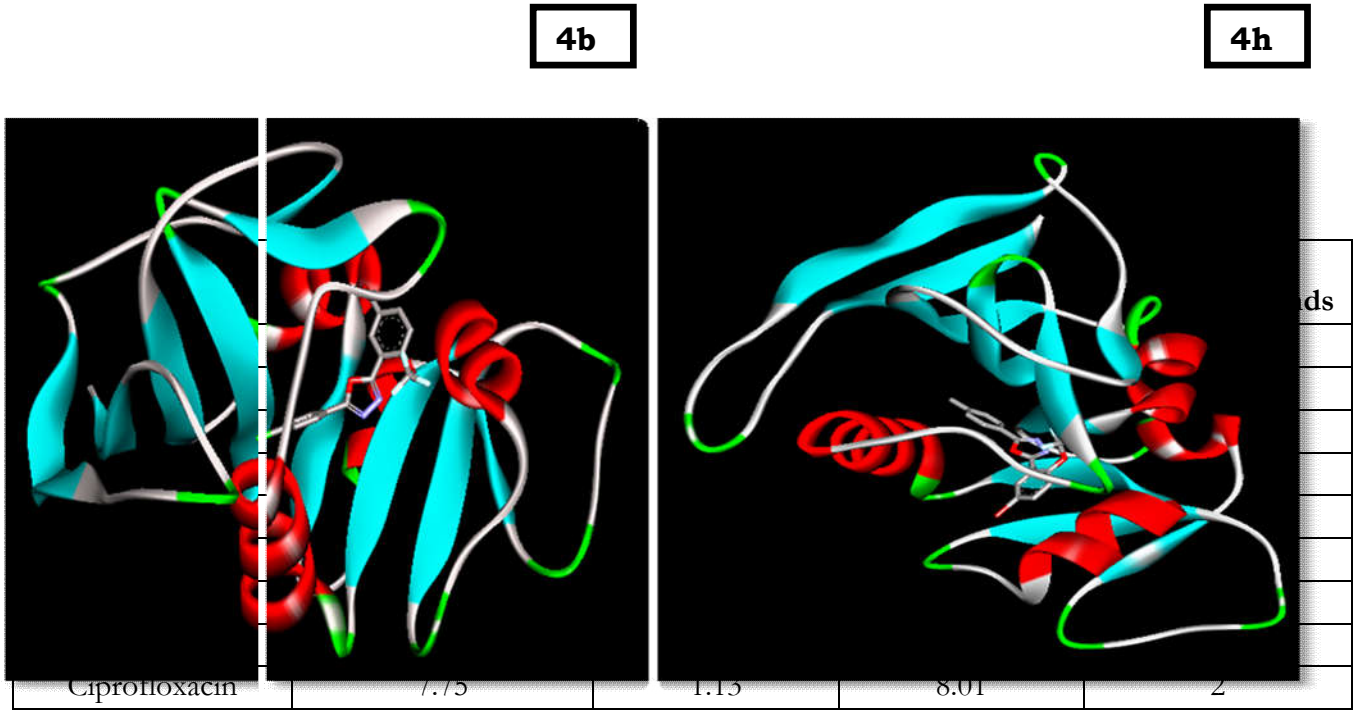
The compounds described were evaluated by measuring *in vitro* antibacterial activity against gram positive organisms (*Staphylococcus aureus, Bacillus subtilis* and *Streptococcus pyogenes)* and gram negative organisms *(Escherichia coli* and *Pseudomonas aeruginosa).* Results are summarized in **Table 1** along with standard drug. These results have been validated by studying the inhibition efficiency of standard drug *Ciprofloxacin*. **Table 1** shows that all the compounds exhibit a varied range 9-23 mm of antibacterial potency against the tested bacterial strains. Compound **4a, 4b and 4d** are inactive against B.subtilis, but remaining compounds active against the same strain. Compounds **4f** and **4g** exhibited poor activity against E.Coli, but the introduction of methoxy group at phenyl (compound **4e**) exhibited excellent activity against E.Coli strain, whereas unsubstituted compound (compound **4a**) shows good activity.

Compounds **4a, 4c** and **4e** are fails to inhibit the Pseudomonas aeruginosa bacterial strain, whereas the remaining synthesized compounds exhibited inhibition in the range 11-18 mm. Compounds **4c** and **4d** are inactive against S.aureus and S.pyogenes, whereas remaining compounds shows good activity.

Docking studies

In the variable strategies available, molecular docking is developed as a possible instinctively tempting methodology predicting the binding affirmation of the ligand with various target proteins to be able to develop really reliable QSAR models [21],[22]. Ample structural information accessible in the protein data bank allows a detailed investigation of the bioactive motifs as bacterial targets. From the in vitro validation study, it was absolutely seen that all the produced compounds 4a-4h was active analogs which is confirmed by additional studies through the docking simulations. Compounds were docked with DHFR protein to evaluate their affinity to bacterial proteins that usually are known targets for many antibiotics set up. The synthesized explications may play their job either by the inhibition of cell wall synthesis, proteins synthesis or nucleic acid solution synthesis.

Table: 2 Docking validation results for 1,3,4-Oxadiazole 4a-4h docked into DHFR proteins Oxadiazoles 4a-4h had been docked into active site of DHFR receptors regarding antimicrobial proteins obtained through Protein Data Bank (PDB) using AutoDock 4. 2 software. Docking study performed with compound 4b that it is truly bound to proteins with a range of amino acids and the binding site with higher binding affinities of around -8. 30. H-bonding interactions together with the biological target an influential role to get a closer understanding into the associations and design of molecules along with improved biological profile. These types of interactions have been calculated and the calculated connection lengths are about 2. 23 A ° which are found to be well within the range associated with hydrogen bonding interactions.



Ciprofloxacin	7.75	1.13	8.01	2

Fig: 2 The binding of compound 4b and 4h in the active sites of DHFR proteins

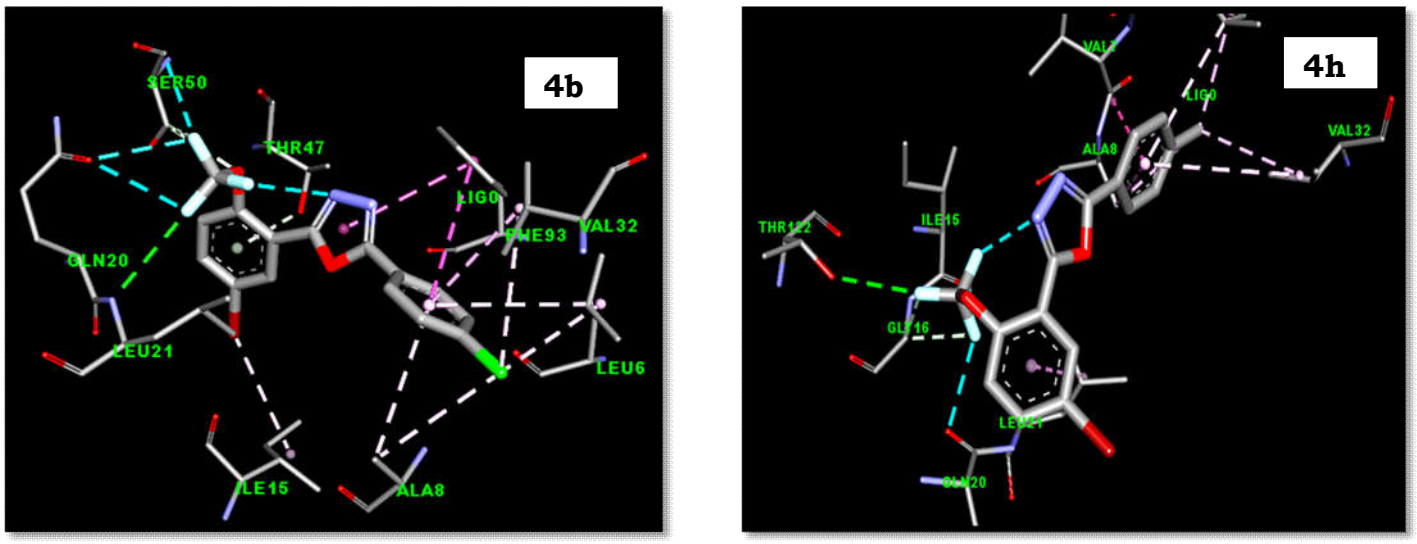


Fig : 3 The binding modes and molecular interactions of compound 4b and 4h in the active sites of DHFR proteins

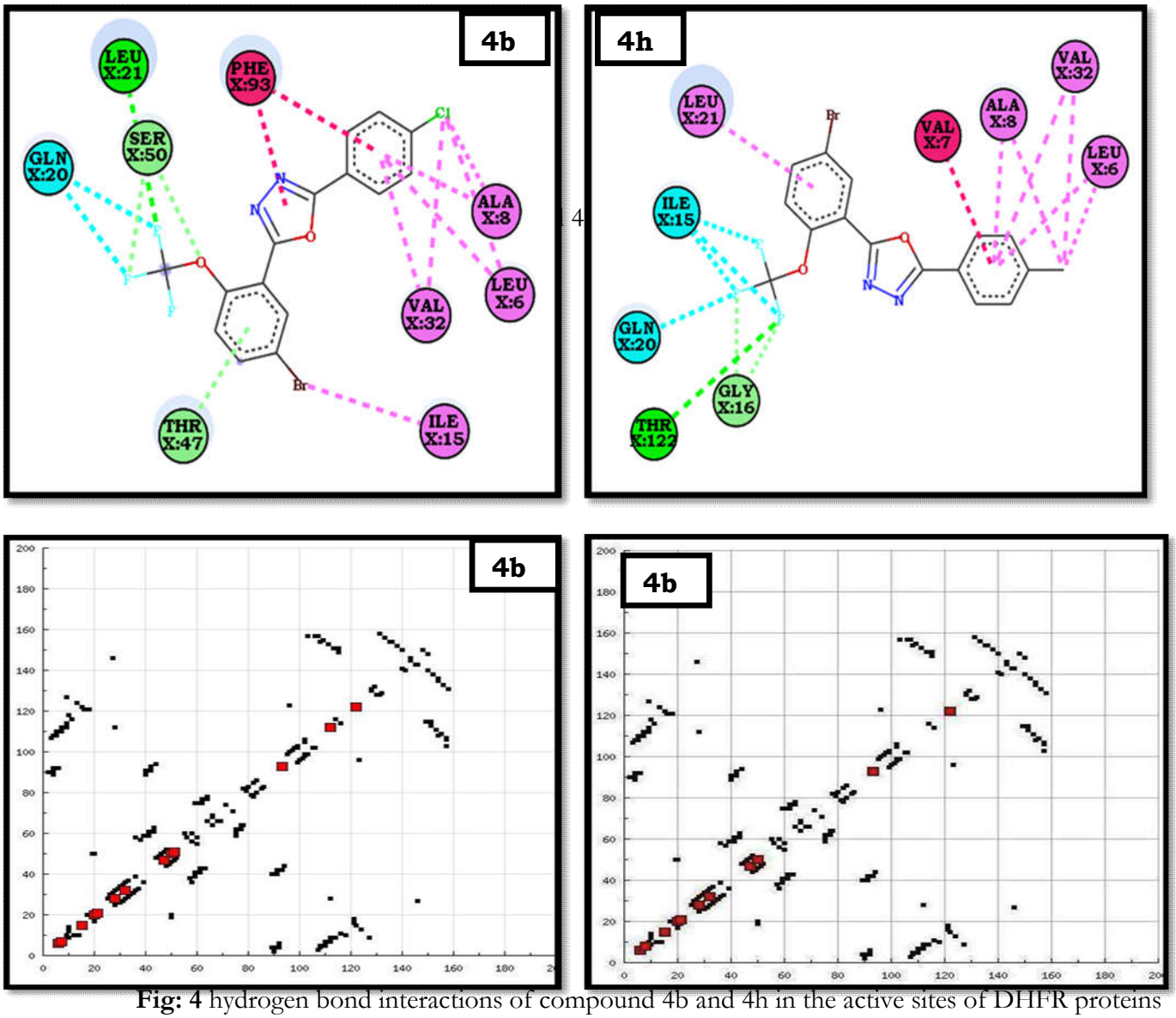


Fig: 4 hydrogen bond interactions of compound 4b and 4h in the active sites of DHFR proteins

Ligand 4b exhibited comparatively larger binding energies against the particular receptor 3srw. It was absolutely identified that all the derivatives fit well into the active site of the particular enzyme making strong connections with the available elements. In addition, methoxy oxygen of the oxadiazole moiety in ligands 4b interacts with the amide hydrogen of different active site residues via hydrogen bonds.

Table 3: Docking validation results for 1,3,4-Oxadiazole 4a-4h docked into DHFR proteins

Compound	Hydrogen bond	Halogen bond	Hydrophobic
4a	N2-PHE 93	C7-LEU 6, C6-LEU 6, C5-ALA 8 and C6-VAL 32	F1-ILE 15, BR 1- GLN-20, F2-THR 122 and F3-THR 122
4b	F3-GLN 20	CL1-VAL 7, F2-GLN 20, F3-GLN 20, CL1-ASP 28, F2-SER 50, CL1-THR 112 and BR1-THR 122	C5-LEU 6, C6-VAL 32 and C7-VAL 32
4c	O3-ARG 58	C14-LEU 21, C7-LEU 29, C8-LEU 29, C7-VAL 32, C4-ILE 51, C5-LEU 55, C4-LEU 55 and C6-LEU 55	F1-LEU 6 and BR 1-ILE 15
4d	O2-THR 47	C6-LEU 6, C4-ALA 8, C5-ALA 8, C14-ILE 15 and C13-LEU 21	F3-ILE 15, BR1-ASN 19, BR 1-GLN 20, F1-THR 122, F2-THR 122 and F3-THR 122
4e	O2-THR 47	C16-LEU 6 and C16-VAL 32	F1-ASN 19, F2-ASN 19, BR 1-ASN 19, F2-GLN 20, F1-GLN 20 and F1-SER 50
4f	N1-ILE 50 and N2-ILE 15	C8-VAL 7	F1-ASN 19, F3-ASN 19, F1-GLN 20, F3-GLN 20 and F3-SER 50
4g	-	C2-ILE 15, C4-ILE 15, C8-LEU 21, C12-VAL 32 and C13-VAL 32	BR1-VAL 7 and BR1-ASP 28
4h	N2-PHE93	F1-ILE15, F2-ILE 15, F3-ILE 15, F2-GLN 20, BR1-GLN 20 and F1-THR 122	C16-LEU 6, C7-LEU 6, C5-ALA 8, C9-LEU 21, C6-VAL 32 and C16-VAL 32,

Further, phenyl group of the oxadiazole derivative 4b and 4h is involved in p-interaction with residues ALA eight, VAL 32 and LEU 6 present in DHFR. Molecular docking study shows that all eight ligands exhibit extraneous ligand efficiency with the necessary protein DHFR at the receptor active site. From the results enlisted in Table 2 & 3, this can be concluded that the ligand 4b have the highest binding affinity into the targeted site. Based on the ligand efficiency, 1, 3 4-oxadiazole derivatives may be regarded as efficient prospects for further molecular advancement of anti-microbial agents.

IV.CONCLUSION

A series of substituted 1, 3, 4-Oxadiazole derivatives were synthesized by using an ultrasonic irradiation and were characterized using elemental analysis and spectral techniques.

Docking studies were executed with DHFR protein to predict their efficiency for antimicrobial action against Gram-positive and Gram-negative microbe strains by using AutoDock 4. 2 software. Compound 4b showed better binding energy with target protein in comparison to the other synthesized compounds and reference drug ciprofloxacin. Any time the antimicrobial investigation was done, the prediction made by docking supported the experimental results. This study will help to design structures and find better drug against bacterial activity.

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