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Antibacterial activity of 1, 3, 4- oxadiazole derivatives and inhibition against β-ketoacyl-ACP synthase

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Abstract

A new series of 2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-aryl-1,3,4-oxadiazole **4a-h** (aryl = C_6H_5, *p*-ClC_6H_4, *p*-$NO_2C_6H_5$, C_5H_4N, *p*-$OCH_3C_6H_4$, *p*-BrC_6H_4, *p*-OHC_6H_4, *p*-$CH_3OC_6H_4$) were prepared from the condensation of acid hydrazide,5-bromo-2-(trifluoromethoxy)benzoic acid in $POCl_3$. The Oxadiazole derivatives have been characterized by basis of FT-IR, ^1H and ^{13}C NMR spectra data. The aim of study to assess all compounds for in-vitro antibacterial inhibition and the results were compared with the standard drug Ciprofloxacin to prove the antibacterial potency compared to the existing one.Compound **4e** was found good active against *E.Coli* bacterial strain used for the present study. Additionally, molecular docking increased our understanding of their receptor-ligand binding. These results demonstrated that Compound **4e** derivative from Oxadiazole was potential β-ketoacyl-ACP inhibitors.

Keywords:*Oxadiazole,in-vitroantibacterial, molecular docking, β-ketoacyl-ACP inhibitors.*

1. Introduction

Heterocyclic compounds containing the five-membered oxadiazole nucleus possess a diversity of useful therapeutic agents (Sahu et al.,2011; SumanBala et al., 2010,; Singh et al.,2013; Joshi et al.,2013). Oxadiazole ring is considered to be derived from furan by replacement of two methane (-CH=) groups by two pyridine type nitrogen atoms (-N=).Oxadiazoles are cyclic compounds containing one oxygen and two nitrogen atoms in a five-membered ring. There are four known isomers of this five-membered heterocycle including 1,2,4-, 1,2,3-, 1,2,5-, and 1,3,4-oxadiazole (Redhu and Kharb,2013;de Oliveira et al.,2012).

However, 1,3,4- oxadiazole is more important because of its remarkable biological activities and occupied a specific placein the field of medicinal chemistry due to its wide range of activities. Compounds containing 1,3,4-oxadiazole structure possess various pharmacological effects including antibacterial, antifungal, antitubercular, anticonvulsant, anti-allergic, anti-inflammatory, cytotoxic, and insecticidal activities (Bhat et al.,2011; Azzawi et al.,2016;Malhotra et al.,2012;Singh and Kumar,2015; Muhammad Akramet al.,2018)

Antibiotic resistance is all the time more recognized as a serious and permanent public health concern and is usually considered to be a consequence of the wide usage and misuse of antibiotics. The emergence of bacterial resistance to most of all antibiotics poses a threat to health care, and novel therapeutics are needed. Recently, the research has been focused towards the development of new antibacterial agents with a novel target. A promising target is the fatty acid synthase (FAS) pathway in bacteria(Soares daet al.,2017). Fatty acid biosynthesis (FAB) is an essential metabolic process for prokaryotic organisms and is required for cell viability and growth.

β-Ketoacyl-acyl carrier protein (ACP) synthase III, also known as FabH or KAS III, plays an essential and regulatory role in bacterial FAB(Zhang et al.,2012; Jeffrey et al.,2015). The enzyme initiates the fatty acid elongation cycles(Peng-Cheng et al.,2009) and is involved in the feedback regulation of the biosynthetic pathway via product inhibition (Xiaoning et al.,2011). Therefore, it represents a promising target for the design of novel antimicrobial drugs. Because of this, various kinds of compounds were screened by enzymatic assays to generate leads

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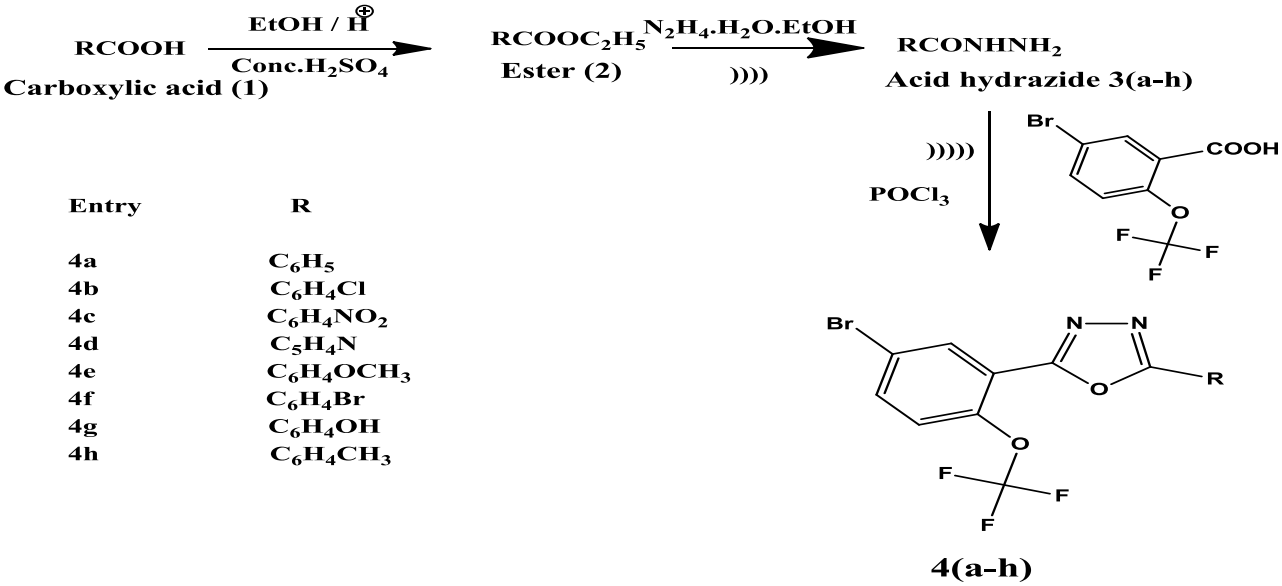
that were co-crystallized with various pathogenic FabH proteins and subsequently optimized using structure-guided drug design methods (MeenakshiPradhanet al.,2015; PriyaSwaminathan and Lilly Saleena.,2017). In view of this, an attempt has been made to study the antimicrobial activities of 1,3,4-oxadiazole. Here, we report our effort to study and develop more potent analogues of 1,3,4-oxadiazole derivatives. Hence, we prepared (2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-aryl-1,3,4-oxadiazoles(4a-h) and briefly present in-vitro antibacterialresults and screening experiments.

2. Experimental

2.1 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 aSHIMADZUFourier 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

2.2 Docking Studies

Docking study was performed on anAutodock 4.2 and Molecular docking server with an Intel Pentium D process or (3.0 GHz) and 4 GB of R A M was run on windows 7(Muhammad Arba et al.,2018,Muruganet al.,2018). The crystal structure of β-ketoacyl-ACP-synthase III (FabH) (PDB id: 1HNJ) has been obtained from the RCSB protein data bank (http://www.pdb.org). The 3D image β-ketoacyl-ACP-synthase III is shown in **Fig. 1.**

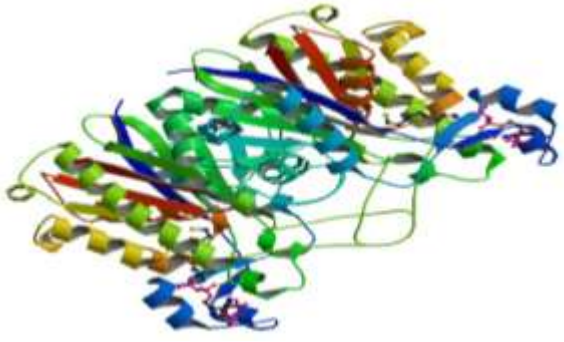


Fig. 1 3D image of **1HNJ** of E.Coli

For AutoDock 4.2, ligand molecules were drawn in ChemBioDraw 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 AutoDock in the next step. Lamarckian genetic algorithm method was employed for docking simulations. 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 was 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.

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2.3 Antibacterial Activity

The newly prepared compounds were screened for their antibacterial activity against Escherichia coli, Staphylococcus aureus,PseudomonasaeruginosaStreptococcus pyogenes and Bacillus Subtilis bacterial strains by disc-diffusion method (Andurmila Joshi et al.,2013; Collins,1976). 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. The discs measuring 6.25 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. 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 was measured after 24h. The plates were inverted and incubated for 24 h at 37 °C. Ciprofloxacin was used as a standard drug. Inhibition zones were measured and compared with the Ciprofloxacin.

3.Synthesis

3.1 Synthesis of ester:

The compounds **2a-h** was prepared according to the procedure given in literature with a little modification (Jha et al.,2010) 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 hour on an ultrasonic cleaning bath. The reaction mixture was concentrated on a rotatory evaporator. It was filtered and collected.

3.2 Synthesis of acid hydrazide 3a-h

The compounds **3a-h** was prepared according to the procedure given in literature with a little modification (Jha et al.,2010).Esterand 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.

3.3 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. The reaction mixture was cooled and poured into 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 Arstr); 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 Arstr); 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-Clstr). ^{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 Arstr); 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 Arstr); 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 Arstr); 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).

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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 Arstr); 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 Arstr); 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 Arstr); 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).

4.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 esterproduced 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

4.1 Antibacterial Activity

The compounds described were evaluated by measuring *in vitro* antibacterial activity against gram-positive organisms (*Staphylococcus aureus,Bacillussubtilis*and *Streptococcus pyogenes)* and gram-negative organisms *(Escherichia coli* and *Pseudomonas aeruginosa).* Results are summarized in **Table 1** along with the standard drug. These results have been validated by studying the inhibition efficiency if standard drug *Ciprofloxacin*.Ciprofloxacin possesses strong activity against gram-negative bacteria. Ciprofloxacin is commonly used for the treatment of a number of infections such as acute uncomplicated cystitis, urinary tract infections, acute sinusitis, and chronic bacterial prostatitis. The mechanism of antibacterial action of including ciprofloxacin, involves interfering with replication and transcription and leading to the production of cellular poisons and cell death.**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 the methoxy group at phenyl (compound **4e**) exhibited excellent activity against E.Coli strain, whereas unsubstituted compound (compound **4a**) shows good activity. Compounds **4a,4b** and **4e**, fails to inhibit the

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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 show good activity.

Table 2 Different types of interaction in compounds 4a-h against β-ketoacyl-ACP

Compounds	**Best ligand pose energy (kcal/mol)**	**Hydrogen bonding**	**Other interactions**
4a	-5.16	ARG A:36; ARG A:249; ASN A:247	GLY A:152; ILE A:156; ILE A:250; PHE A:213; ALA A:246; VAL A:212; TRP A:32
4b	-6.72	ASN A:247; ARG A:36	PHE A:213; ALA A:246; ILE A:250; ALA A:216; VAL A:212; TRP A:32
4c	-6.11	ASN A:210	MET A:207; VAL A:212; ASN A:247; ARG A:249; PHE A:213
4d	-5.86	ARG A:249	GLY A:152; GLY A:209; ARG A:36; ALA A:246; ILE A:156; MET A:207
4e	-7.34	ARG A:36	ILE A:156; ALA A:246; MET A:207; TRP A:32; ARG A:151; GLY A:152
4f	-6.58	ASN A:247; ARG A:36	ALA A:216; VAL A:212; ALA A:246; ILE A:250; TRP A:32; ILE A:156; MET A:207; PHE A:213
4g	-6.04	ARG A:24; ASN A:247	ASN A:210; PHE A:213; ARG A:36; MET A:207; ILE A:250; VAL A:212
4h	-5.69	ASN A:247; ARG A:249	ALA A:246; ALA A:216; ILE A:250; VAL A:212; ARG A:36; TRP A:32
Ciprofloxacin	-7.8	ASP 161 and ASP 51	ILE A:156; ALA A:246; MET A:207; TRP A:32; ARG A:151; GLY A:152

4.2 Molecular docking analysis

To explain the antibacterial effects of oxadiazole analogs, docking studies were carried out using the Auto-dock 4.2 program (Roshana Devi et al.,2018). The scoring functions and hydrogen bonds formed with the surrounding amino acids were used to explore the binding modes, binding affinities, and orientations of the docked compounds. The Binding pose of compound **4e** and enzyme is shown in **Fig.2.** The compound **4a** has the binding score of -5.16 Kcal/mol. The chloro substituted compound **4b** has high binding energy than parent oxadiazole. It forms the hydrogen bond with nearby amino acids such as ASN A:247 and ARG A:36.Insertion of electron withdrawing 1,3,4-oxadiazole offers an upgradedπ-electron delocalization across the donor-acceptor links and affords significant enhancement in the binding energy(SumanBala et al,2018). But the nitro-substituted compound decreases binding energy than compound **4b** and higher thancompound**4a.** As seen from **Table 2**, all the substituted compounds have more binding score compound **4a.**

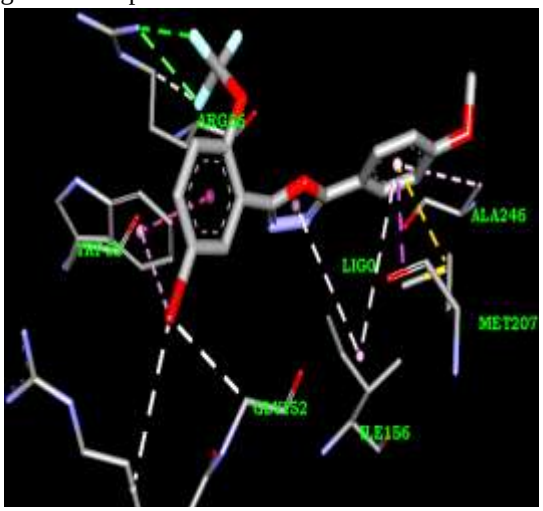

Fig. 2 Binding pose at the compound **4e** and enzyme.

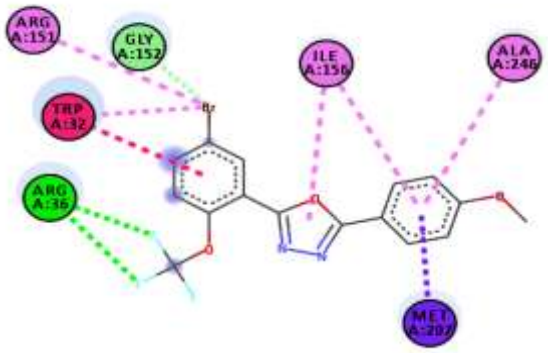

Fig. 3 Two-dimensional (2D) interactions of most active compound **4e**

Compound **4e** has been the most active analogs showing the highest binding recognition at the β-ketoacyl-ACP binding site (**Fig. 3**). Compound **4e** had a common methoxypharmacophore scaffold; the **4e** performed proper H-bonding with ARG A:36. Apart from this, hydrophobic interactions are observed between the compound **4e** with ILE A:156, ALA A:246, MET A:207, TRP A:32, ARG A:151 and GLY A:152. We also analyzed the interaction and energy profile of β-ketoacyl-ACP with the already available drug *Ciprofloxacin.*The standard drug Ciprofloxacin showed binding interactions with ASP 161 and ASP 57 with binding energy -7.82 (Table 2) which is close to docking score of compound 4e. It reveals that our designed one has more potent than the standard inhibitor.

5. Conclusion

Our study performed for the series of 2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-aryl-1,3,4-oxadiazole **4a-h.** The Oxadiazole structures have been characterized by basis of FT-IR, ^{1}H and ^{13}C NMR spectra data. The title compounds were evaluated for antibacterial activity by disc diffusion method against various bacterial strains. All the synthesized compounds were found to moderate activity against bacteria strains. It provided new information in the field of structural properties responsible for affinity and inhibitory activity toward the bacterial strains. Molecular docking showed that all new compounds bound to selected receptors. It is very important that the compound **4e** derivative from Oxadiazole was potential β-ketoacyl-ACP inhibitors. Finally, the resultssuggest that the compound **4e**is important lead compound for the continuing battle against antibacterial disease.

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