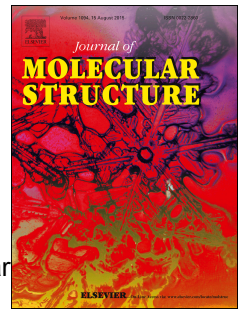

Design and synthesis of triazole conjugated novel 2,5-diaryl substituted 1,3,4-oxadiazoles as potential antimicrobial and anti-fungal agents

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Author contribution

KRA, SB, SSR conceived the idea and provided critical inputs to the concept. KRA planed the experiment, SB and RD. generated synthesis data. Molecular docking and Biological assays are carried out by SSR and AAG. All the authors KRA,SB,SSR, RD, PMR, SB2, AAG and BVK contributed to analyze, interpret data and wrote the manuscript. All authors contributed to the final reading and approved the submitted revised version.

Design and Synthesis of Triazole Conjugated Novel 2,5-Diaryl Substituted 1,3,4-Oxadiazoles as Potential Antimicrobial and Anti-fungal Agents

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Abstract: A series of triazole conjugated novel 2,5-diaryl 1,3,4-oxadiazole derivatives **8a-q** are efficiently synthesized starting from methyl salicylate. All the synthesized compounds were characterized based on their ^{1}H NMR, ^{13}C NMR, Mass and IR data. All the compounds have been investigated for antibacterial activity against *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* and antifungal activity against *Aspergillus niger* and *Saccharomyces cerevisiae*. It is interesting to note that all compounds **8a-q** were found to be effective, potent and active against microbial growth when compared with standard antibacterial agents. Our findings suggest that compounds **8d-f, 8l** and **8o** are the major ones shown anti-fungal activity among all the synthesized compounds. From our results, it is found that all the compounds **8a-q** have exhibited no appreciable potency towards clearing the free radicals in solution. Molecular docking studies of the synthesized compounds with penicillin-binding proteins (PDB ID: 3HUN and 3ITA) are in good agreement with their inhibition activity. The compounds **8p** and **8q** were found to be effective against microbial growth in gram-positive bacteria, whereas in gram-negative bacteria compound **8d** shown good synergy with biological assays and docking studies**.** These novel series of compounds have shown promising features in inhibiting the microorganisms by interacting with enzymes involved in peptidoglycan synthesis and bacterial cell wall biosynthesis.

Keywords: 1,3,4-Oxadiazole, 1,2,3-triazole, anti-bacterial activity, anti-fungal activity.

1. Introduction:

Microbial infection is one of the major areas of concern in healthcare and community environments as it remains a global threat. The United Nations adhoc Interagency Coordination Group (IACG) on Antimicrobial Resistance has recently reported an estimate of 10 million deaths each year by 2050 due to drug-resistant microbial diseases. It could force up to 24 million people into extreme poverty by 2030 [1]. Effective antimicrobial drugs are prerequisites for both preventive and curative measures, protecting patients from potentially fatal diseases and ensuring that complex procedures, such as surgery and chemotherapy, can be provided at low risk [2]. A particular concern was severe infections caused by Gram-positive bacteria *Staphylococcus*

aureus [3], *Bacillus subtilis* [4] and Gram negative bacteria *Escherichia coli* [5]. The mode of action of β-lactam antibiotics found to have similar in their sugar–amino acid backbone structures and involved in peptidoglycan formation. In due course of time, it has become eminent that β-lactam antibiotics became ineffective. Thus, raise in microbial antibiotic resistance is necessary to avoid or to treat the current infections. 1,3, 4- Oxadiazoles, a novel class of non-β lactam antibiotics might fight against bacteria in a way different to β-lactam penicillins.

Nitrogen and oxygen-containing heterocyclic ring compounds have gained significant importance globally not only due to their prevalence in natural products but also due to their pharmacological, photochemical optoelectronic properties and industrial value [6-12]. 1,3,4-Oxadiazoles and its derivatives became interesting over the past few years and found wide applications in pharmaceuticals, dyes, photographic materials, agrochemicals and corrosion inhibition [13-14]. Most importantly, the isomeric arrangement of heteroatoms and other five-membered rings like triazoles, pyrazoles, and isoxazoles were potent in reporting antibacterial activity [15]. The oxadiazoles are privileged scaffolds found in diverse areas such as medicinal, pesticidal, polymer and material science [16]. A number of compounds containing an oxadiazole moiety possess broad-spectrum bioactivities like antibiotic [17-19], anticancer [20-22], and anti-hypertensive [23-25]. Significant efficacy of 1,3,4-oxadiazoles have also been demonstrated for past few years in various pharmacological properties including anti-microbial [26-30], anti-inflammatory, analgesic [31-32], anti-HIV [33-35], anti-mycobacterial [36], inhibitors of Cathepsin K [37], inhibitors of tyrosinage [38-40], anti-convulsant [41-43], and muscle-relaxant [44]. On the other side, raltegravir, an antiretroviral drug for the treatment of HIV infection, has been launched into the market (**Fig-1**) [17,25,45-46]. In addition to their latent potential in medicinal chemistry, the oxadiazoles also find application in the field of material science where butyl-PBD or b-PBD is used in the liquid scintillator neutrino detector [47]. Hence diverse ranges of oxadiazoles applications have attracted the scientific community to develop novel oxadiazoles, which have an enormous impact on multiple drug discovery processes.

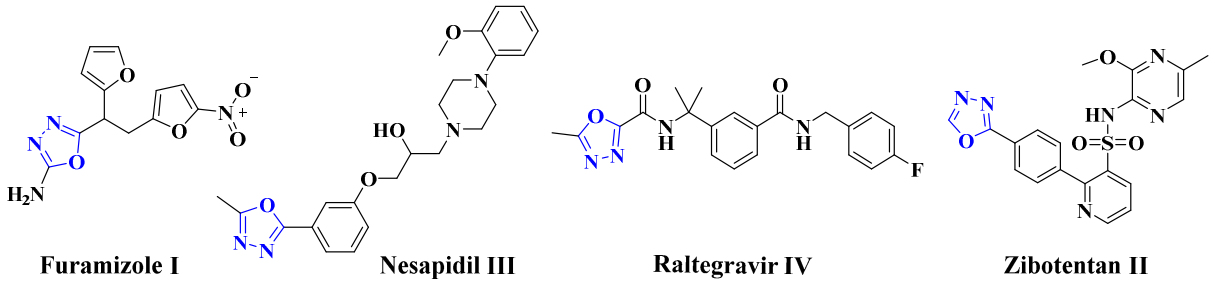


Fig -1: Oxadiazole containing biologically active drugs

On the other hand, 1,2,3-triazole's is a versatile moiety which is continuously attracting the attention of medicinal chemists due to its synthetic feasibility and numerous biological activities (**Fig-2**) when combined with other heterocyclic moieties [48-52].

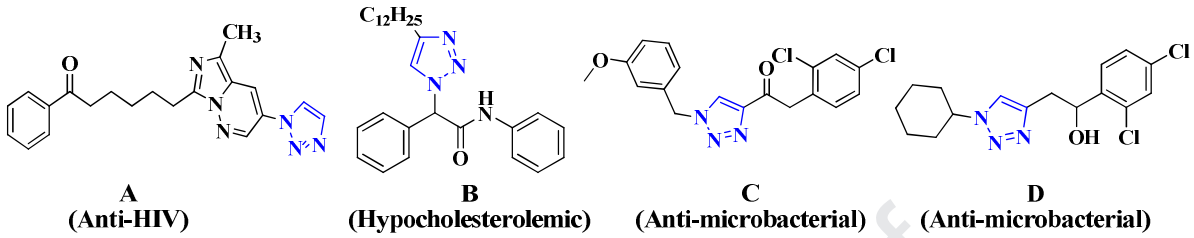


Fig -2: 1,2,3-Triazole based biologically active agents

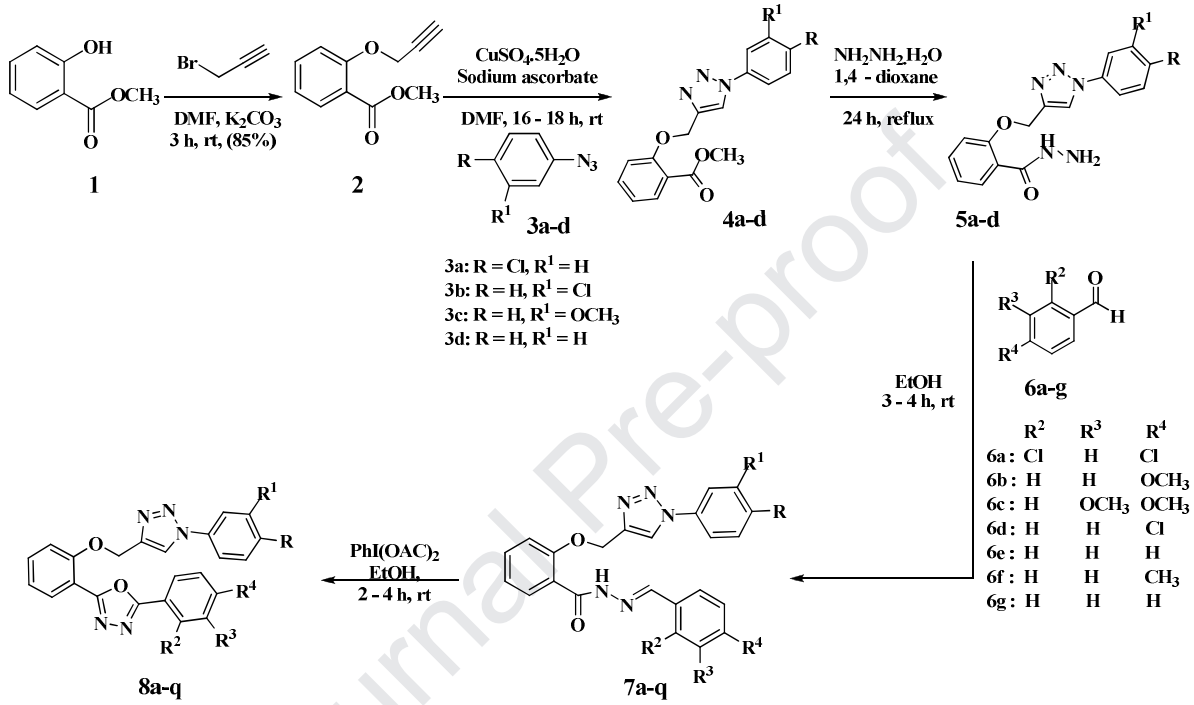
As part of on-going research in our lab, encouraged by the above finding towards the development of novel antimicrobial and anti-fungal agents with improved therapeutic efficacy and stimulated by the biological significance of triazole containing 1,3,4-oxadiazoles [53], herein, we have described the design and synthesis of a series of 1,2,3-triazole conjugated with 2,5-diaryl substituted 1,3,4-oxadiazoles. All the synthesized compounds were thoroughly characterized based on their Proton NMR, ^{13}C NMR, Mass and IR spectral data. All the synthesized **8a-q** compounds are investigated for their antimicrobial, anti-fungal and anti-oxidant activities.

2. Results and Discussion:

2.1. Chemistry

The synthetic approach followed in achieving the designed target oxadiazoles **8a-q** is summarized in **Scheme 1**. The synthesis was initiated with the propargylation on methyl salicylate **1** using propargyl bromide in the presence of potassium carbonate base and dry dimethyl formamide solvent to afford compound **2** in 85% yield. The spectral data of alkyne **2** is in agreement with the reported data [54]. The selective synthesis of required triazoles **4a-d** was efficiently achieved by using copper (I) catalyzed [CuAAC] Huisgene 1,3-dipolar cyclo addition of alkyne **2** with azides **3a-d** in good yields. The formation of triazole **4a** was confirmed by its spectral data where the ^{1}H NMR spectrum of **4a** showed characteristic triazole proton absorption at δ 8.02 as a singlet, O-CH_2- absorption appeared as a singlet at δ 5.40, ester -OCH_3 signal appeared at 3.89 as a singlet, it is further confirmed by its Mass spectral data which has given (M+1) peak atm/z 344. The obtained triazoles **4a-d** are then treated with hydrazine hydrate using 1,4-dioxane as a solvent to afford the benzohydrazides **5a-d** in quantitative yields. The disappearance of -OCH_3 singlet peak form δ 3.89 in the ^{1}H NMR and appearance of two new signals at δ 4.53 (a singlet for two protons) and δ 9.21 (a singlet for one proton) due to -NH_2 and

-NH respectively confirmed the structure of compound **5a**. Further, Mass spectral data is in agreement with its molecular formula. The condensation reaction between benzohydrazides **5a-d** and different substituted benzaldehydes **6a-g** was afforded the Schiff's base products **7a-q**, which without any further purification were subjected to intramolecular cyclization under $PhI(OAc)_2$ in ethanol solvent conditions to yield the final target 1,2,3-triazole conjugated 2,5-diaryl substituted 1,3,4-oxadiazoles **8a-q** in good yields.



	R	R^1	R^2	R^3	R^4	M.P.	Yield
8a :	Cl	H	Cl	H	Cl	184-186	84%
8b :	Cl	H	H	H	OCH_3	167-169	86%
8c :	Cl	H	H	OCH_3	OCH_3	170-172	89%
8d :	Cl	H	H	H	Cl	173-175	88%
8e :	Cl	H	H	H	H	161-163	84%
8f :	Cl	H	H	H	CH_3	168-170	86%
8g :	H	H	H	H	H	158-160	90%
8h :	H	H	H	H	OCH_3	163-165	84%
8i :	H	H	H	OCH_3	OCH_3	167-169	89%

	R	R^1	R^2	R^3	R^4	M.P.	Yield
8j :	H	H	Cl	H	Cl	181-183	88%
8k:	H	H	H	H	Cl	169-171	85%
8l :	H	H	H	H	CH_3	180-182	86%
8m:	H	H	F	H	H	176-178	89%
8n :	H	Cl	H	H	H	185-187	88%
8o :	H	Cl	Cl	H	Cl	177-179	85%
8p :	OCH_3	H	H	OCH_3	OCH_3	184-186	90%
8q :	OCH_3	H	Cl	H	Cl	171-173	86%

Scheme - 1: Synthesis of Triazole Conjugated Novel 2,5-Diaryl Substituted 1,3,5-Oxadiazoles **8a-q**

All the synthesized final compounds **8a-8q** were analyzed by their respective spectral data. The IR spectrum of compounds **8a** exhibited its characteristic **C=N** absorption band at 1595 cm^{-1}. The single triazole proton of compound **8a** appeared as a singlet at δ 8.36 in its ^1H NMR, and a singlet corresponding to two **$-OCH_2$** protons appeared at δ 5.49. Further, the ^{13}C NMR spectrum of compound **8a** given the absorption of **$-OCH_2$** carbon at δ 63.3. Mass spectral data of

compound **8a** has given the **M+1** peak at m/z 481 (M.F: $C_{23}H_{14}Cl_3N_5O_2$) which is in agreement with its calculated mass.

2.2. Biology

2.2.1. Antibacterial activity

The synthesized final compounds **8a-q** were evaluated for antibacterial activity *in vitro*. To assess the antimicrobial properties of synthesized compounds, two strains of Gram-positive bacteria (*Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 96) and one strain of Gram-negative bacteria (*Escherichia coli* MTCC 443*)* were chosen based on their capability of raising resistance to develop drugs [55]. A specific concentration of compound where the bacterial growth inhibition has started is termed as a minimal concentration of compound required for bacterial inhibition. Based on the study of tested antibacterial activities against different strains [56-57], it is noted that all compounds **8a-q** were found to be very effective, potent and active against microbial growth when compared with standard antibacterial agents (**Table -1**) & **(Fig -3**). These series of compounds have shown promising features in inhibiting the microorganism by interaction with enzymes involved in peptidoglycan synthesis and bacterial cell wall biosynthesis. The compounds **8p & 8q** have shown good inhibition in gram positive bacteria whereas **8p** not able to inhibit the gram-negative bacteria. Compound **8d** inhibited best against the gram negative bacteria, it might be having better mode of binding than the **8p**. In overall all the compounds shown good antimicrobial activity except few compounds in gram negative bacteria (**8f, 8h, 8o, and 8p**). The strain of *E. coli* might have bestowed with alternative pathway and not shown good inhibition activity.

Table-1:
Antibacterial activity: Minimum inhibitory concentration (MIC) of the synthesized compounds (μg/ml) against gram-positive and gram-negative bacterial strains

Compound No.	Gram positive bacteria		Gram negative bacteria
	S. aureus **MTCC 96**	***B.subtilis*** **MTCC 441**	***E. coli*** **MTCC 443**
8a	9.9	5	9
8b	8.8	6	10
8c	8.9	5.6	8.5
8d	8.4	5.5	4.8
8e	8.7	5.6	9.8

8f	8.7	5.4	>50
8g	9.8	6	10
8h	8.4	5.8	>50
8i	9	5.7	9.7
8j	8.9	5	8.2
8k	8.6	5.4	10
8l	8.5	5	25
8m	8.6	5.9	9.8
8n	9.7	5.8	10
8o	8.9	5.4	>50
8p	8.8	5.7	>50
8q	7.9	5.5	9
Ampicillin	10	10	4

Anti-bacterial activity of Triazole Conjugated Novel 2,5-Diaryl Substituted 1,3,4-Oxadiazoles in micro dilution method:

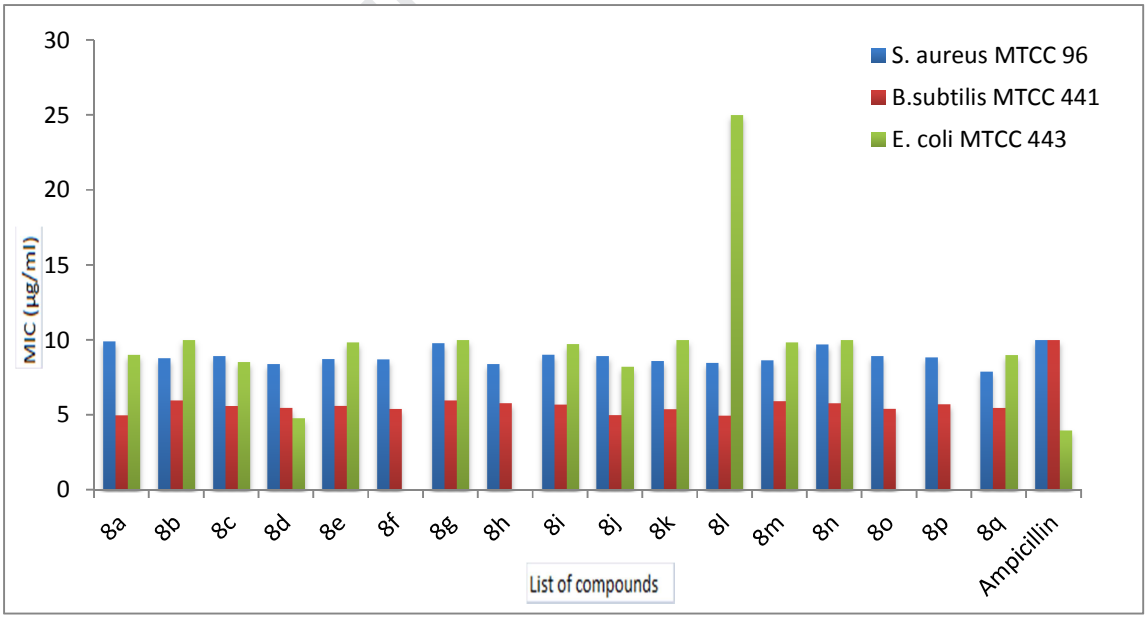


Fig -3. Graphical representation of antibacterial activity in bar diagram.

2.2.2. Anti-fungal activity

The anti-fungal activities of the synthesized **8a-q** compounds were tested against filamentous fungi *Aspergillus niger* MTCC 404 and *Saccharomyces cerevisiae* MTCC 1344 yeast cultures [58]. Our findings suggested that **8d, 8e, 8f, 8g, 8l** and **8o** are the compounds showed potent anti-fungal activity among all other synthesized triazole conjugated 1,3,4-oxadiazole compounds (**Table -2**).

Table – 2: Zone of fungal growth inhibition (mm) after incubating for 2 days at 37 ^{o}C.

Compound no.	*Aspergillus niger* MTCC 404			*Saccharomyces cerevisiae* MTCC 1344		
	Compound concentration (µM)					
8a	2 (10)	3 (20)	3 (30)	2 (10)	2 (20)	3.2 (30)
8b	2 (10.8)	23. (21.7)	2.5 (32.6)	2 (10.8)	2.5 (21.7)	2.6 (32.6)
8c	2 (10.2)	2 (20.4)	2.3 (30.6)	3 (10.2)	3 (20.4)	3.5 (30.6)
8d	**5.2 (10.7)**	**6 (21.5)**	**6 (32.3)**	**3 (10.7)**	**3.3 (21.5)**	**4 (32.3)**
8e	**5.2 (11.6)**	**6 (23.2)**	**6.2 (34.8)**	**4 (11.6)**	**5 (23.2)**	**5.3 (34.8)**
8f	**7.2 (12.6)**	**8 (25.2)**	**8.2 (37.8)**	**3 (12.6)**	**3 (25.2)**	**5 (37.8)**
8g	**6 (12.6)**	**7 (25.2)**	**7 (37.8)**	**5 (12.6)**	**5.4 (25.2)**	**6 (37.8)**
8h	2 (11.7)	2.4 (23.4)	2.7 (35.2)	1 (11.7)	1.5 (23.4)	2 (35.2)
8i	3 (10.9)	3.3 (21.9)	3.5 (32.8)	3.3 (10.9)	3.6 (21.9)	4 (32.8)
8j	3 (10.7)	3.2 (21.5)	3.3 (32.3)	4 (10.7)	4.2 (21.5)	4.6 (32.3)
8k	4 (11.60	4 (23.2)	4.2 (34.8)	2 (11.6)	2.2 (23.2)	2.4 (34.8)
8l	**4.6 (12.1)**	**5 (24.3)**	**5.3 (36.5)**	**5 (12.1)**	**6 (24.3)**	**6.2 (36.5)**
8m	3 (12)	3.2 (24.1)	3.5 (36.2)	2.4 (12)	2.8 (24.1)	3.5 (36.2)
8n	3.7 (11.6)	4 (23.2)	4 (34.8)	4 (11.6)	4.6 (23.2)	5 (34.8)
8o	**6 (10)**	**7.2 (20)**	**7.6 (30)**	**3.5 (10)**	**4.5 (20)**	**5.5 (30)**
8p	4 (10.2)	4.2 (20.5)	4.2 (30.8)	2 (10.2)	2.2 (20.5)	3 (30.8)
8q	3 (10.1)	3.5 (20.2)	3.7 (30.3)	3 (10.1)	4 (20.2)	5 (30.3)
Miconazole	**8 (12)**	**10 (24)**	**12 (36)**	**8 (12)**	**9 (24)**	**12 (36)**

Anti-fungal activity of Triazole Conjugated Novel 2,5-Diaryl Substituted 1,3,4-Oxadiazoles against *Aspergillus Niger* MTCC 404 and *Saccharomyces cerevisiae* MTCC 1344 by cross streak plate method.

2.2.3. Anti-oxidant activity

DPPH radical scavenging assay revealed that, all **8a-q** Oxadiazole derivatives exhibited minor potency towards clearing the free radicals in solution (**Table-3**) (**Fig- 4**).

Table – 3: % Antioxidant activity of Triazole Conjugated Novel 2,5-Diaryl Substituted 1,3,4-Oxadiazole compound at various concentrations**.**

Compound No.	**Compound concentration (µM)**			
	10	**50**	**100**	**200**
Ascorbic acid	44.1	44.5	45.8	79.3
8a	-2.1	4.3	20.9	30.3
8b	-20.6	-6.9	-1.8	7.5
8c	-89.3	-70.5	-46.5	8.4
8d	-3.7	1.5	23.2	39.4
8e	-1.2	14.1	26.4	37
8f	-38.5	-17.1	-13.2	-0.5
8g	-18	0.2	0.8	23.4
8h	-83.1	-38.1	-36.5	41.9
8i	-38	-10.1	-1.2	1.5
8j	-40.3	-18.5	-13.9	-0.2
8k	-62.5	-29.3	-26	1
8l	-13	-10.8	3.9	6.1
8m	-0.2	-0.9	3.6	15.6
8n	1.1	3.9	5.8	14.9
8o	-86.3	-15.6	-1.1	20.1
8p	-53.7	25.5	-21.7	-3.9
8q	-17.8	-4.8	16.5	20.3

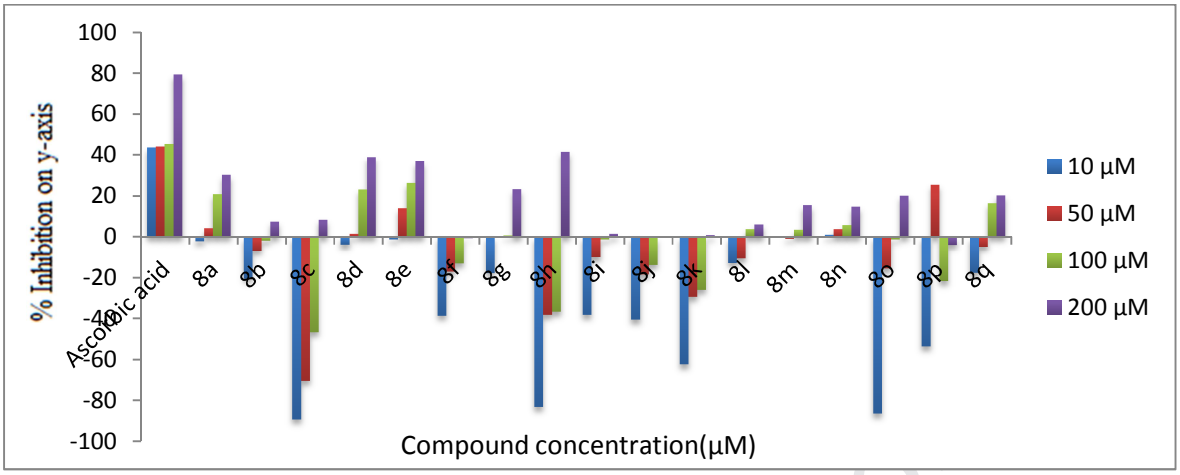


Fig-4. Graphical bar diagram representation of percent antioxidant activity of 8a-8q synthesized compounds**.**

2.2.4. *In silico* Molecular Docking Studies:

Synthesized ligands were screened *insilico* for potent antimicrobial agents using Molegro Virtual Docker (MVD). It is well known that the β-lactam antibiotics often target more than one (PBP) Penicillin binding protein during bacterial inhibition due to several closely related PBP's. As the β-lactam antibiotics found to be similar in their sugar–amino acid backbone structures, the following cell wall biosynthesis mechanism treats these antibiotics as own and forms the peptidoglycan layers. In a deal with this successful aspect the present study focused on synthesis of non-β-lactam derivatives called oxadiazoles, which would likely target multiple PBP's in various strains combating drug resistance. The principles from electronic properties, geometry suggest that the electrostatic interaction plays a pivotal role in attraction between compound aromatic chain and amino acid residues [59]. In addition to this, it is well known that the arrangement of heteroatoms and five-membered rings like triazoles and pyrazoles were potent in antibacterial activity with nitrogen and oxygen atoms in their aromatic rings. We have selected two co-crystal structure of two different PBPs (PDB ID: 3HUN; PBP4) and (3ITA; PBP6) from *S. aureus (*Gram positive) and *E. coli (*Gram negative), respectively. Both the structures were determined with ampicillin in their active site with high resolution of 2 Å [60] from *S. aureus* where as an *E. coli* structure with the 1.85 Å [61]. The oxadiazole derivatives were subjected to *in silico* docking against 3HUN and 3ITA to predict their ability and mode of binding. Best dock score depicts the interactions of protein-ligand complexes, thus, revealing the synthesized

compounds' ability to bind to spectrum of PBP4 and PBP6. The compounds **8p** and **8q** with high moldock score showed maximum number of pi-bond interactions at specific amino acid residues present in the binding pocket. Pi-alkyl, pi-sigma, pi-pi interactions along with other hydrogen interactions prevalent in protein crystal structures contribute to the microbial growth susceptibility. All the 17 ligands synthesized have shown good docking scores with strong binding affinities towards PBPs (**Table -4**). The active site of PBP4 (PDB id: 3HUN) & 3ITA identified from the earlier studies as well confirmed with the Protein ligand server: "https://projects.biotec.tu-dresden.de/plip-web/plip/index". The crystal structure of 3HUN is bound with ampicillin as inhibitor by having hydrogen bonding with Ser 75, Ser116 Ser 386 hydrophobic interaction with Phe 241 and water bridges with Lys 78, Ser 116 and Ser 262 [60]. Our docking also showed binding of **8p** and **8q** molecules in the same active site vicinity with Ser 75 (2.75 Å) and Tyr 291 (2.57 Å); Ser 75 (2.23 Å) and Ser 116 (1.98 Å) respectively via hydrogen bonding (**Fig-5**). Similarly, 3ITA docking results confirmed the same with higher docking scores for **8p** and **8q**. The co-crystal ampicillin also made few hydrogen bonding interactions with active site residues consisting of Ser 40 (2.92 Å), Thr 210 (3.68 Å) and Thr 212 (2.92 Å) [61]. Our docking results with synthesized compounds had shown similar interactions which were found in co-crystal structures. The compounds had shown hydrogen bonding Ser 40 (2.95 Å) Arg 244 (2.46 Å) in **8**, where as in **8q**, Thr 212 (1.91 Å) and Arg 194 (2.5 Å) as well both inhibitors were stabilized by hydrophobic and pi-pi interactions **(Fig-6).** The superposition of 3HUN and 3ITA co-crystal structures with the docked **8p-8q** structures confirmed the orientation and mode of binding **(Fig-7)** all of them were well aligned within the active site vicinity. The docking was executed with PBP6 (3ITA) protein as target whereas inhibition assay was carried out in living *E. coli*. In spite of good docking score, the compound 8p displayed turbid growth when treated with a concentration of >50 µg/ml in vitro revealing that *E. coli* might be bestowed with a complementing system or an alternative pathway to overcome the inhibition by 8p compound. On the other hand docking score of compound **8d** in the same series was in accord with *in vitro* inhibition values. Docking scores and biological assays of **8p and 8q** had shown synergy in gram positive bacteria.

Table - 4: *In silico* Molecular Docking of synthesized compounds against PBP4 (PDB ID: 3HUN) and PBP6 (PDB ID: 3ITA).

Ligand	Mol Dock Score (3HUN)	Rerank score (3HUN)	Mol Dock Score (3ITA)	Rerank Score (3ITA)
8a	-158.68	-101.37	-155.31	-109.27
8b	-155.23	-106.50	-151.87	-116.13
8c	-157.46	-111.63	-152.62	-115.67
8d	-149.78	-106.67	-149.76	-108.12
8e	-150.34	-85.071	-148.13	-103.93
8f	-154.27	-105.51	-154.78	-99.95
8g	-146.57	-90.25	-141.03	-102.11
8h	-146.62	-106.72	-146.15	-18.42
8i	-157.83	-107.13	-157.07	-108.47
8j	-164.57	-112.85	-141.03	-94.48
8k	-151.14	-101.03	-151.94	-99.95
8l	-148.20	-104.89	-147.69	-80.97
8m	-147.28	-90.73	-148.52	-119.01
8n	-151.04	-98.16	-143.56	-89.35
8o	-145.58	-82.91	-159.84	-118.59
8p	**-162.38**	-115.12	**-163.56**	-124.70
8q	**-165.92**	-113.62	**-165.83**	-111.42

3. Conclusion:

In this study, we have designed and synthesized a series of triazole conjugated novel 2, 5-diaryl substituted 1,3,4-oxadizole derivatives **8a-q.** These Oxadiazoles are valuable five-membered aromatic heterocycles bestowed with a wide range of biological inhibiting activities including antibacterial, anti-fungal and antiparasitic. All the compounds **8a-q** were investigated for their antimicrobial, anti-fungal and antioxidant activities. It is noted that all compounds **8a-q** were found to be effective, potent and active against microbial growth when compared with standard antibacterial agents. All the compounds were found in completely inhibiting the microbial growth by binding efficiently to proteins of bacterial cell wall biosynthesis. Our findings suggested that among all other synthesized triazole conjugated 1,3,4-oxadiazole compounds **8p** and **8q** are the best compounds showing anti-fungal as well as antimicrobial activity. Based on the DPPH radical scavenging assay it was observed that all derivatives (**8a-q**) displayed a minor potency towards clearing the free radicals in solution. The best dock scored compounds from our current *in silico* study were further confirmed with the minimal microbial growth. The compounds **8p** and **8q** were effective against gram positive bacteria, and **8d** had shown promising features against gram negative bacteria.

4. Experimental:

4.1. Chemistry

General Remarks:

All solvents, chemicals, reagents were purchased in LR grade from the commercial vendors Merck, Sigma-Aldrich and Avra chemicals and used as such in the reactions. NMR spectra was recorded on Bruker Avance-II 400 MHz instrument, TMS is used as an internal reference standard and $CDCl_3$/d^6-DMSO used as solvents.Aluminum sheets coated with 60F254 silica gel (Merck, 0.2 mm) are used for TLC. Uncorrected melting points are obtained from melting point instrument and reported in degrees centigrade.Chemical shifts (d) are reported in parts per million. *J* (Coupling constant) values are reported in Hz (Hertz). Proton spin multiplicities are denoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), ddd (doublet of doublet of doublets). IR and Mass spectra were obtained from Schimadzu FTIR-8400S and GCMS-QP-1000EX respectively.

4.1.1. Synthesis of methyl 2-(prop-2-ynyloxy) benzoate (2):

The compound **2** was prepared by the propargylation of compound **1** (1.0 eq) using the propargyl bromide (1.5 eq) and K_2CO_3 (1.5 eq) in dry dimethyl formamide solvent under nitrogen atmosphere at rt for 3 hrs, after completing the reaction, it was extracted using dichloromethane and ice cold water (3 x 50 mL), the combined organic layer was dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by silica gel column using 25% EtOAc in hexane as an eluent to afford compound **2** as light yellow solid in 85% yield. Spectral data of isolated compound **2** is in agreement with the reported data[54]. Yield:85%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (dd,*J* = 7.7, 1.8 Hz, 1H) 7.51-7.44 (m, 1H), 7.14 (d, *J*= 8.4, Hz, 1H), 7.04 (td,*J* = 7.6, 0.9 Hz, 1H), 4.79 (d, *J* = 2.4 Hz, 2H), 3.89 (s, 3H), 2.52 (t, *J* = 2.4 Hz, 1H); ^{13}C NMR (100 MHz,$CDCl_3$): δ 166.0, 156.0, 133.0, 130.0, 121.0 120.0, 114.0, 79.0 78.5 56.0, 51.9. M.F: $C_{11}H_{11}O_3$, ESI-MS; m/z: 191 (M+1).

4.1.2. General procedure for the synthesis of aromatic azides (3a-d):

The aromatic azides **3a-d** were prepared by addition of 5N HCl solution to a mixture of respective amine in CH_2Cl_2 at 0 ^oC followed by drop wise addition of $NaNO_2$ solution to above mixture and stirred at 0 ^oC for 30 minutes. To this was added NaN_3 at 0 ^oC and continued the stirring for 2 hrs at rt, then it was allowed to stand for separation of organic and aqueous layers. The organic layer was washed using $NaHCO_3$ followed by brine and the solvent was evaporated *in vacuo* to obtain the required aryl azides **3a-d**. The azides **3a-d** are used in next step without any additional purification.

4.1.3. General procedure for the synthesis of 1,2,3-triazoles (4a-d):

To a mixture of methyl salicylate derivative **2** (1.0 eq), compound **3a-d** (1.2 eq) in dry dimethyl formamide (30mL) was added to a solution of $CuSO_4.5H_2O$ (0.01 eq) and sodium ascorbate (0.01 eq) and the mixture was stirred for overnight at rt. After completion of the reaction as indicated by TLC, the reaction contents were extracted from ice cold water and ethyl acetate (3 x 50 mL). The organic layer was washed and dried using brine and dry Na_2SO_4 respectively. The solvent was removed *in vacuo* and the crude produced was purified by passing through silica gel column (eluent: 30% EtOAc in hexane) to get the desired pure product **4a-d** with 90-95% yield.

4.1.3.1. Methyl 2-((1-(4-chlorophenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)benzoate (4a):
Yield: 81%; White solid; M.p. 77 – 79. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 (s, 1H), 7.85 (dd, *J* = 17.8, 1.6, 1H), 7.72 – 7.69 (m, 2H), 7.53 – 7.47 (m, 3H), 7.22 – 7.15 (d, *J* = 8.1 Hz, 1H), 7.06 – 7.02 (m, 1H), 5.40 (s, 2H), 3.89 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ158.2, 145.5, 133.7, 131.8, 129.9, 121.7, 121.1, 121.0, 114.1, 63.51, 51.9. IR (neat): 3077, 2956, 2923, 2852, 1643, 1501, 1464, 1247, 1190, 1046, 932 cm^{-1}. Anal. Calcd. For $C_{17}H_{14}ClN_3O_3$: C, 59.40; H, 4.10; N, 12.22; Found: C, 59.38; H, 4.09; N, 12.19. ESI-MS: m/z: 344 (M+1).

4.1.3.2. Methyl 2-((1-(3-chlorophenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)benzoate (4b):
Yield: 80%; White solid; M.p 73 – 75. ^{1}H NMR (400 MHz, $CDCl_3$):δ 8.22 (s, 1H), 7.88 – 7.79 (m, 2H), 7.70 – 7.62 (m, 1H), 7.55 – 7.39 (m, 3H), 7.16 (d, *J* = 8.2 Hz, 1H), 7.09 – 6.95 (m, 1H), 5.41 (s, 2H), 3.91 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 157.7, 135.6, 133.7, 131.8, 130.8, 128.9, 121.2, 120.9, 120.6, 118.5, 114.1, 63.4, 52.0. IR (neat): 3394, 3117, 3027, 2923, 2852, 1727, 1596, 1530, 1493, 1449, 1293, 1255, 1166, 1048, 962 cm^{-1}.Anal. Calcd. For $C_{17}H_{14}ClN_3O_3$:C, 59.40; H, 4.10; N, 12.22; Found: C, 59.41; H, 4.06; N, 12.17. ESI-MS: m/z: 344 (M+1).

4.1.3.3. Methyl 2-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)benzoate (4c):
Yield: 87%; White solid; M.p 77 – 79. ^{1}H NMR (400 MHz, $CDCl_3$):δ 8.12 (s, 1H), 7.83 (dd, *J*= 7.7, 1.8 Hz, 1H), 7.72 – 7.58 (m, 2H), 7.54 – 7. 21 (m, 1H), 7.18 (d, *J* = 8.1 Hz, 1H), 7.08 – 6.96 (m, 3H), 5.40 (s, 2H), 3.89 (s, 3H), 3.87 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$):δ 166.3, 159.8, 157.84, 133.7, 131.7, 130.5, 122.2, 121.2, 121.0, 120.6, 114.7, 114.1, 63.5, 55.6, 52.0. IR (neat): 3136, 2924, 2853, 1723, 1599, 1517, 1490, 1451, 1303, 1250, 1160, 1034 cm^{-1}. Anal. Calcd. For $C_{18}H_{17}N_3O_4$:C, 63.71; H, 5.05; N, 12.38; Found: C, 63.68; H, 5.05; N, 12.35. ESI-MS: m/z: 340 (M+1).

4.1.3.4. Methyl 2-((1-phenyl-1*H*-1,2,3-triazol-5-yl)methoxy)benzoate (4d):
Yield: 82%; White solid; M.p 71-73.^{1}H NMR (400 MHz, $CDCl_3$), δ 8.21 (s, 1H), 7.84 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.80 – 7.70 (m, 2H), 7.58 – 7.42 (m, 4H), 7.18 (d, *J* = 7.9 Hz, 1H), 7.03 (tt, *J*= 7.8, 3.9 Hz, 1H), 5.42 (s, 2H), 3.90 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.3, 157.8, 137.0, 133.7, 131.8, 129.7, 128.8, 121.1, 120.6, 114.1, 63.5, 52.0. IR (neat): 3420, 3088, 2922, 2852, 1726, 1642, 1597, 1494, 1432, 1286, 1253, 1168, 1033, 998 cm^{-1}. Anal. Calcd. For $C_{17}H_{15}N_3O_3$:C, 66.01; H, 4.89; N, 13.58; Found: C, 65.98; H, 4.84; N, 13.54. ESI-MS: m/z: 310 (M+1).

4.1.4. General procedure for the synthesis of bezohydrazides (5a-d):

To a solution of compound **4a-d** (1.0 eq) in 1,4-dioxane solvent was added hydrazine hydrate (1.2 eq) and it was refluxed for 24 h. After completion of reaction as indicated by TLC, the solvent 1,4 dioxane was removed under reduced pressure and the crude reaction mixture was extracted by using ethyl acetate and water. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified using silica gel column to get compounds **5a-d** with 85-90% yield.

2-((1-(4-Chlorophenyl)-1H-1,2,3-triazol-5-yl)methoxy)benzohydrazides (5a):
Yield: 90%; White solid; M.p. 87 – 89. ^{1}H NMR (400 MHz, d^6-DMSO): δ 9.21 (s, 1H), 8.93 (s, 1H), 7.94 – 7.90 (m, 2H), 7.65 (ddd,*J* = 22.2, 10.4, 4.7 Hz, 3H), 7.54 – 7.46 (m, 2H), 7.37 (d, *J* = 8.1 Hz, 1H), 5.40 (s, 2H), 4.53 (s, 2H). ^{13}C NMR (100 MHz, d^6-DMSO): δ 155.3, 143.8, 135.2, 133.0, 131.8, 130.1, 129.9, 123.1, 122.7, 121.8, 121.0, 113.5, 61.8. IR (neat): 3394, 3097, 2956, 2923, 2852, 1783, 1711, 1643, 1501, 1464, 1247, 1046, 994 cm^{-1}. Anal. Calcd. For $C_{16}H_{14}ClN_5O_2$:C, 55.90; H, 4.10; N, 20.37; Found: C, 55.86; H, 4.11; N, 20.40. ESI-MS: m/z: 344 (M+1).

2-((1-(3-Chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzohydrazides (5b):
Yield: 88%; White solid; M.p. 88 – 90. ^{1}H NMR (400 MHz, d^6-DMSO): δ 10.34 (s, 1H), 9.08 (s, 1H), 8.05 (s, 1H), 7.93 (d, *J* = 7.8 Hz, 2H), 7.70 – 7.56 (m, 4H), 7.48 – 7.42 (m, 1H) 7.14 (dd, *J* = 12.9, 5.5 Hz, 1H), 5.46 (s, 2H), 3.33 (s, 1H). ^{13}C NMR(100 MHz, d^6-DMSO): δ 160.5, 155.6, 154.8, 142.9, 137.4, 134.2, 132.8, 131.2, 128.7, 123.9, 121.6, 121.4, 118.8, 113.3, 61.5.IR (neat): 3337, 3102, 2957, 2923, 1656, 1595, 1529, 1483, 1451, 1223, 1145, 1019, 992 cm^{-1}. Anal. Calcd. For $C_{16}H_{14}ClN_5O_2$:C, 55.90; H, 4.10; N, 20.37; Found: C, 55.92; H, 4.07; N, 20.31. ESI-MS: m/z: 344 (M+1).

2-((1-(4-Methoxyphenyl)-1H-1,2,3-triazol-5-yl)methoxy)benzohydrazides (5c):
Yield: 87%; White solid; M.p. 85 – 87. ^{1}H NMR (400 MHz, d^6-DMSO): δ 10.35 (s, 1H), 8.90 (s, 1H), 7.94 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.83 – 7.76 (m, 2H), 7.61 – 7.53 (m, 1H), 7.46 (d, *J* = 8.2 Hz, 1H), 7.20 – 7.10 (m, 3H), 5.43 (s, 2H), 3.84 (s, 3H), 1.90 (s, 2H). ^{13}C NMR (100 MHz, d^6-DMSO): δ 160.4, 159.4, 155.7, 154.7, 142.5, 132.9, 131.3, 129.8, 123.7, 121.9, 121.4, 114.9, 113.3, 61.9, 55.5. IR (neat): 3399, 3329, 2957, 2924, 2852, 1642, 1599, 1517, 1482, 1253, 1036, 835 cm^{-1}. Anal. Calcd. For $C_{17}H_{17}N_5O_3$:C, 60.17; H, 5.05; N, 20.64; Found: C, 60.20; H, 5.01; N, 20.60. ESI-MS: m/z: 340 (M+1).

2-((1-Phenyl-1H-1,2,3-triazol-5-yl)methoxy)benzohydrazides (5d):
Yield: 89%; White solid; M.p. 83 – 85. ^{1}H NMR (400 MHz, d^6-DMSO): δ 9.21 (s, 1H), 8.93 (s, 1H), 7.94 – 7.90 (m, 2H), 7.69 – 7.61 (m, 3H), 7.54 – 7.46 (m, 2H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.07 (t, *J* = 7.4 Hz, 1H), 5.40 (s, 2H), 4.53 (s, 2H). ^{13}C NMR (100 MHz, d^6-DMSO): δ 155.4, 143.7, 136.4, 131.8, 130.1, 129.9, 128.8, 123.1, 122.6, 121.0, 120.1, 113.5, 61.9. IR (neat): 3396, 3332, 3075, 2956, 2924, 2853, 1837, 1709, 1642, 1599, 1483, 1224, 1047, 994 cm^{-1}. Anal. Calcd. For$C_{16}H_{15}N_5O_2$:C, 62.13; H, 4.89; N, 22.64; Found: C, 62.10; H, 4.90; N, 22.61. ESI-MS: m/z: 310 (M+1).

4.1.5. General procedure for the synthesis of Schiff's base (7a-q):

To a solution of benzohydrazide **5a-d** (1.0 eq) in ethanol was added aldehyde 6 (1.0) and it was refluxed for 3- 4 hours. Once the reaction is completed as denoted by TLC, the ethanol was distilled under vacuum and the obtained compound was washed with hexane which yielded the desired pure products in about 90% yield.

4.1.6. General procedure for the synthesis of triazole conjugated novel 2,5-diaryl substituted 1,3,4, oxadiazoles (8a-q):

To a solution of Schiff's base compound **7** (1.0 eq) in ethanol was added $PhI(OAc)_2$ (5 mol%) and it was stirred for 2 to 4 hours at rt. When the reaction was completed, as denoted by TLC, ethanol solvent was removed *in vauco*. The obtained compound was subjected to silica gel column using 15% of EtOAc in hexane eluent to afford the final compound as a white solid with 80-90% yield.

2-(2-((1-(4-Chlorophenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)phenyl)-5-(3,4-dichlorophenyl) -1,3,4-oxadiazole (8a):

Yield: 84%; White solid; M.p. 184 – 186. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (s, 1H), 8.08 (dd, *J* = 7.8, 1.7 Hz, 1H), 8.01 (d, *J* = 8.5 Hz, 1H), 7.70 – 7.66 (m, 2H), 7.65 – 7.53 (m, 2H), 7.52 – 7.49 (m, 2H), 7.38 (dd, *J* = 8.5, 2.1 Hz, 1H) 7.29 (s, 1H), 7.18 (td, *J* = 7.7, 0.9 Hz, 1H), 5.49 (s, 2H).^{13}C NMR (100 MHz, $CDCl_3$): δ 162.3, 160.8, 156.0, 145.3, 138.1, 135.3, 134.6, 133.5, 131.9, 131.1, 130.6, 130.0, 127.7, 121.6, 121.3, 113.5, 63.3. IR (neat): 2929, 2856, 1595, 1501, 1464, 1263, 1097, 1038, 828 cm^{-1}. Anal. Calcd. For $C_{23}H_{14}Cl_3N_5O_2$:C, 55.39; H, 2.83; N, 14.04; Found: C, 55.41; H, 2.80; N, 14.00. ESI-MS: m/z:498(M+1).

2-(2-(2-(1-(3-Chlorophenyl)-1*H*-1,2,3-triazol-5-yl)ethyl)phenyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (8b):

Yield: 86%; White solid; M.p. 167 – 169. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (s, 1H), 8.06 – 8.00 (m, 3H), 7.64 – 7.62 (m, 2H), 7.56 – 7.51 (m, 1H), 7.47 – 7.45 (m, 2H), 7.27 (s, 1H), 7.17 (t, *J* = 8.2 Hz, 1H), 6.99 – 6.94 (m, 2H), 5.47 (s, 2H), 3.85 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$): δ 162.7, 162.3, 156.3, 145.3, 134.5, 133.0, 130.4, 129.9, 128.6, 121.7, 121.6, 121.1, 114.5, 113.5, 63.4, 55.4. IR (neat): 3063, 1612, 1500, 1464, 1258, 1177, 1032, 843 cm^{-1}. Anal. Calcd. For $C_{24}H_{18}ClN_5O_3$: C, 62.68; H, 3.95; N, 15.23; Found: C, 62.70; H, 3.90; N, 15.20. ESI-MS: m/z: 460 (M+1).

2-(2-(2-(1-(3-Chlorophenyl)-1*H*-1,2,3-triazol-5-yl)ethyl)phenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (8c):

Yield: 89%; White solid; M.p. 170 – 172. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (s, 1H), 8.06 (dd, *J* = 1.5 Hz, 1H), 7.64 –7.56 (m, 5H), 7.47 – 7.45 (m, 2H), 7.24 (s, 1H), 7.18 (t, *J*= 8.0 Hz, 1H), 6.86 – 6.84 (m, 1H), 5.47 (s, 2H), 3.96 (s, 3H), 3.91 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$):δ 162.8, 156.3, 151.9, 149.4, 145.3, 135.3, 134.6, 133.1, 130.4, 129.9, 121.7, 121.6, 121.1, 120.2, 116.5, 113.7, 113.4, 111.0, 109.4, 63.3, 56.2, 55.9. IR (neat): 2945, 1606, 1503, 1462, 1270,

1141, 1028, 832cm^{-1}. Anal. Calcd. For $C_{25}H_{20}ClN_5O_4$:C, 61.29; H, 4.11; N, 14.30; Found: C, 61.30; H, 4.09; N, 14.27. ESI-MS: m/z: 490 (M+1).

2-(4-Chlorophenyl)-5-(2-(2-(1-(4-chlorophenyl)-1*H*-1,2,3-triazol-5-yl)ethyl)phenyl)-1,3,4-oxadiazole (8d):

Yield: 88%; White solid; M.p. 173-175.^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H), (dd, *J* = 8.6, 5.1 Hz, 3H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.60 – 7.44 (m, 5H), 7.28 (s, 1H), 7.18 (t, *J* = 7.5, Hz, 1H), 5.49 (s, 2H). ^{13}C NMR(100 MHz, $CDCl_3$): δ 162.0, 156.7, 133.4, 131.6, 130.4, 130.0, 129.5, 128.8, 128.2, 127.3, 121.7, 121.2, 113.5, 63.2. IR (neat): 2928, 2860, 1597, 1499, 1268, 1093, 1015, 825 cm^{-1}. Anal. Calcd. For $C_{23}H_{15}Cl_2N_5O_2$:C, 59.50; H, 3.26; N, 15.08; Found: C, 59.46; H, 3.20; N, 15.10. ESI-MS: m/z: 464.

2-(2-((1-(4-Chlorophenyl)-1H-1,2,3-triazol-5-yl)methoxy)phenyl)-5-phenyl-1,3,4-oxa-diazole (8e):

Yield: 84%; White solid; M.p. 161 – 163. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H), 8.10 – 8.05 (m, 3H), 7.66 – 7.63 (m, 2H), 7.53 – 7.49 (m, 6H),7.29 (s, 1H), 7.18 (t, *J* = 7.5 Hz, 1H), 5.48 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.4, 135.4, 134.5, 133.2, 131.6, 130.4, 129.9, 129.1, 128.8, 126.9, 124.0, 121.7, 121.6, 121.1, 113.6, 113.5, 63.4. IR (neat): 2928, 2859, 1600, 1498, 1267, 1129, 1030, 831 cm^{-1}. Anal. Calcd. For $C_{23}H_{16}ClN_5O_2$:C, 64.26; H, 3.75; N, 16.29; Found: C, 64.22; H, 3.71; N, 16.30. ESI-MS: m/z: 430 (M+1).

2-(2-((1-(4-Chlorophenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)phenyl)-5-p-tolyl-1,3,4-oxa-diazole (8f):

Yield: 86%; White solid; M.p. 168 – 170. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H), 8.06 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.97 (d, *J* = 8.2 Hz, 2H), 7.65 – 7.62 (m, 2H), 7.56 (ddd, *J* = 11.8, 7.2, 2.6 Hz, 2H), 7.48 – 7.44 (m, 2H), 7.26 – 7.24 (m, 2H), 7.18 (t, *J* = 7.6, Hz, 1H), 5.49 (s, 2H), 2.41 (s, 3H). ^{13}CNMR(100 MHz, $CDCl_3$): δ 156.5, 145.3, 142.1, 134.6, 133.1, 130.4, 129.9, 129.8, 126.8, 121.7, 121.6, 113.4, 63.3, 22.0. IR (neat): 2937, 1628, 1504, 1464, 1269, 1101, 1028, 840 cm^{-1}. Anal. Calcd. For $C_{23}H_{18}ClN_5O_2$:C, 63.96; H, 4.20; N, 16.22; Found: C, 64.00; H, 4.18; N, 16.19. ESI-MS: m/z: 396 (M+1).

2-Phenyl-5-(2-((1-phenyl-1H-1,2,3-triazol-5-yl)methoxy)phenyl)-1,3,4-oxadiazole (8g):

Yield: 90%; White solid; M.p. 158 – 160. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (s, 1H), 8.08 (dd, *J* = 8.1, 1.3 Hz, 3H), 7.76 – 7.66 (m, 2H), 7.61 – 7.54 (m, 1H), 7.53 – 7.41 (m, 6H), 7.28 (d, *J* = 7.6 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 5.50 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.3, 163.2, 156.4, 144.9, 136.9, 133.2, 131.6, 130.5, 129.7, 129.1, 128.8, 126.8, 124.0, 121.6, 121.1, 120.5, 113.6, 113.5, 63.4. IR (neat): 3149, 2921, 1598, 1472, 1265, 1023, 755 cm^{-1}. Anal. Calcd. For $C_{23}H_{17}N_5O_2$:C, 69.86; H, 4.33; N, 17.71; Found: C, 69.85; H, 4.31; N, 17.69. ESI-MS: m/z: 396 (M+1).

2-(4-Methoxyphenyl)-5-(2-(2-(1-phenyl-1*H*-1,2,3-triazol-5-yl)ethyl)phenyl)-1,3,4-oxa-diazole (8h):

Yield: 87%; White solid; M.p. 163 – 165. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (s, 1H), 8.07 (dd, *J* = 8.5 Hz, 1H) 7.70 – 7.67 (m, 2H), 7.61 – 7.57 (m, 2H), 7.51 – 7.43 (m, 4H), 7.22 (s, 1H), 7.16 (t, *J* = 8.1 Hz, 1H), 6.94 – 6.92 (m, 2H), 5.58 (s, 2H), 3.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.6, 156.6, 144.8, 137.2, 133.0, 130.4, 129.7, 128.8, 128.6, 121.6, 121.1, 120.5, 116.6, 114.5,

113.5, 63.4, 55.4. IR (neat): 2927, 2859, 1608, 1499, 1465, 1258, 1036, 807 cm^{-1}. Anal. Calcd. For $C_{24}H_{19}N_5O_3$:C, 67.76; H, 4.50; N, 16.46; Found: C, 67.74; H, 4.48; N, 16.43. ESI-MS: m/z:426 (M+1).

2-(2-(2-(1-Phenyl-1*H*-1,2,3-triazol-5-yl)ethyl)phenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (8i):

Yield: 89%; White solid; M.p. 167 – 169. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27 (s, 1H), 8.07 (dd, *J* = 1.5 Hz, 1H), 7.68 – 7.65 (m, 3H), 7.56 – 7.55 (m, 2H), 7.54 – 7.42(m, 3H), 7.27 (s, 1H), 7.19 (t, *J* = 7.6 Hz, 1H), 6.82 – 6.80 (m, 1H), 5.48 (s, 2H), 3.96 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ164.3, 162.9, 156.3, 151.8, 149.3, 144.8, 136.8, 133.1, 130.4, 129.7, 128.8, 121.6, 121.1, 120.4, 120.2, 116.5, 113.6, 113.4, 111.0, 109.3, 63.3, 56.1, 55.9. IR (neat): 3010, 2947, 1602, 1543, 1501, 1463, 1267, 1140, 1030, 758 cm^{-1}. Anal. Calcd. For $C_{25}H_{21}N_5O_4$:C, 65.93; H, 4.65; N, 15.38; Found: C, 65.90; H, 4.63; N, 15.37. ESI-MS: m/z: 456 (M+1).

2-(3,4-Dichlorophenyl)-5-(2-((1-phenyl-1*H*-1,2,3-triazol-5-yl)methoxy)phenyl)-1,3,4-oxa-diazole (8j):

Yield: 88%; White solid; M.p. 181 – 183. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (s, 1H), 8.09 (dd, *J*= 7.8, 1.7 Hz, 1H), 7.99 (d, *J* = 8.5 Hz, 1H), 7.73 – 7.69 (m, 2H), 7.58 – 7.47 (m, 5H), 7.36 (dd, *J* = 8.5, 2.0 Hz. 1H), 7.29 (d, *J* = 8.3 Hz, 1H), 7.17 (t, *J* = 7.6 Hz, 1H), 5.50 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.7, 161.6,156.5, 144.9, 133.7, 133.5, 131.9, 131.1, 130.6, 129.8, 128.9, 127.6, 121.8, 121.7, 121.4, 120.5, 113.5, 63.2.IR (neat): 3076, 2938, 1598, 1502, 1469, 1295, 1262, 1076, 1042, 820 cm^{-1}. Anal. Calcd. For $C_{23}H_{15}Cl_2N_5O_2$:C, 59.50; H, 3.26; N, 15.08; Found: C, 59.47; H, 3.25; N, 15.10. ESI-MS: m/z: 464 (M+1).

2-(2-((1-Phenyl-1H-1,2,3-triazol-5-yl)methoxy)phenyl)-5-p-tolyl-1,3,4-oxadiazole (8k):

Yield: 85%; White solid; M.p. 169 – 171.^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (s, 1H), 8.08 (d, *J* = 6.2 Hz, 1H), 8.01 (d, *J* = 8.5 Hz, 2H), 7.70 (d, *J*= 7.6, Hz, 2H), 7.53 (dd, *J* = 14.5, 7.3 Hz, 4H), 7.42 (d, *J* = 8.5 Hz, 2H), 7.29 (s, 1H), 7.18 (t, *J* = 7.4 Hz, 1H), 5.49 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.4, 135.3, 134.5, 132.9, 131.5, 130.4, 129.9, 128.9, 126.7, 123.9, 121.7, 121.1, 113.5, 63.3. IR (neat): 2930, 2863, 1599, 1469, 1265, 1090, 1029, 806 cm^{-1}. Anal. Calcd. For $C_{23}H_{16}ClN_5O_2$:C, 64.27; H, 3.75; N, 16.29; Found: C, 64.25; H, 3.73; N, 16.30. ESI-MS: m/z: 430 (M+1).

2-(2-(2-(1-Phenyl-1H-1,2,3-triazol-5-yl)ethyl)phenyl)-5-p-tolyl-1,3,4-oxadiazole (8l):

Yield: 86%; White solid; M.p. 180 – 182. ^{1}H NMR (400 MHz $CDCl_3$): δ 8.30 (s, 1H), 8.08 (dd, *J* = 1.2 Hz, 1H), 7.96 – 7.95 (m, 2H), 7.69 – 7.67 (m, 2H), 7.56 – 7.43 (m, 5H), 7.27 – 7.24 (m, 2H), 7.17 – 7.15 (m, 1H), 5.49 (s, 2H), 2.39 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ164.8, 163.0, 156.4, 144.9, 142.1, 133.1, 130.5, 129.8, 129.7, 128.8, 126.8, 121.6, 121.2, 120.5, 113.5, 63.4, 21.6. IR (neat): 2928, 2867, 1602, 1502, 1464, 1271, 1124, 1079, 1032, 824 cm^{-1}. Anal. Calcd. For$C_{24}H_{19}N_5O_2$:C, 70.40; H, 4.68; N, 17.10; Found: C, 70.38; H, 4.65; N, 17.09. ESI-MS:m/z:410 (M+1).

2-(2-Fluorophenyl)-5-(2-((1-phenyl-1H-1,2,3-triazol-5-yl)methoxy)phenyl)-1,3,4-oxadi-azole (8m):

Yield: 89%; White solid; M.p. 176 – 178. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (s, 1H), 8.08 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.80 – 7.69 (m, 2H), 7.40 (m, 6H), 7.27 (d, *J* = 5.8 Hz, 1H), 7.24 – 7.12 (m,

2H), 6.57 (dd, *J* = 3.5, 1.8 Hz, 1H), 5.50 (s, 2H), ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.6, 157.2, 156.4, 145.5, 145.0, 139.6, 137.0, 133.4, 130.5, 129.8, 128.8, 121.7, 121.3, 120.7, 114.0, 113.4, 113.0, 112.2, 63.6. IR (neat): 3142, 2926, 1602, 1505, 1466, 1267, 1240, 1046, 902 cm^{-1}. Anal. Calcd. For $C_{23}H_{16}FN_5O_2$:C, 66.82; H, 3.90; N, 16.94; Found: C, 66.79; H, 3.89; N, 16.89. ESI-MS: m/z: 414 (M+1).

2-(2-((1-(3-Chlorophenyl)-1H-1,2,3-triazol-5-yl)methoxy)phenyl)-5-phenyl-1,3,4-oxadi-azole (8n):

Yield: 88%; White solid; M.p. 185 – 187.^{1}H NMR (400 MHz, $CDCl_3$): δ 8. 36 (s, 1H), 8.12 – 8.05 (m, 3H), 7.79 (dd, *J* = 2.5, 1.4 Hz, 1H), 7.60 – 7.43 (m, 7H), 7.27 (d, *J* = 5.7 Hz, 1H), 7.22 – 7.14 (m, 1H), 5.50 (s, 2H).^{13}C NMR (100 MHz, $CDCl_3$): δ 156.3, 145.2, 135.6, 133.2, 131.7, 130.8, 130.4, 129.1, 128.9, 126.8, 124.0, 121.7, 121.2, 120.8, 118.4, 113.6, 113.5, 63.3. IR (neat): 2955, 2922, 2853, 1593, 1461, 1264, 1262, 1044, 780 cm^{-1}. Anal. Calcd. For $C_{23}H_{16}ClN_5O_2$:C, 64.26; H, 3.75; N, 16.29; Found: C, 64.24; H, 3.71; N, 16.26. ESI-MS: m/z: 430 (M+1).

2-(2-((1-(3-Chlorophenyl)-1H-1,2,3-triazol-5-yl)methoxy)phenyl)-5-(2,4-dichlorophenyl) -1,3,4-oxadiazole (8o):

Yield: 85%; White solid; M.p. 177 – 179. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (s, 1H), 8.08 (dd, *J* = 7.8, 1.7 Hz, 1H), 8.01 (d, *J* = 8.5 Hz, 1H), 7.70 – 7.66 (m, 2H), 7.61 – 7.48 (m, 4H), 7.38 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.28 (d, *J* = 7.8 Hz, 1H), 7.18 (td, *J* = 7.7, 0.9 Hz, 1H), 5.49 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.7, 156.4, 145.0, 138.0, 137.7, 135.6, 133.7, 133.5, 131.9, 131.1, 130.8, 130.6, 129.0, 127.7, 121.7, 121.4, 120.8, 118.4, 113.5, 113.2, 63.2. IR (neat): 2956, 2923, 2853, 1582, 1460, 1285, 1253, 1045, 987 cm^{-1}. Anal. Calcd. For $C_{23}H_{14}Cl_2N_5O_2$:C, 59.63; H, 3.05; N, 15.12; Found: C, 59.59; H, 3.03; N, 15.09. ESI-MS:m/z:498(M+1).

2-(3,4-Dimethoxyphenyl)-5-(2-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-5yl)methoxy)phenyl-1,3,4-oxadiazole (8p):

Yield: 90%; White solid; M.p. 184 – 186.^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (s, 1H), 8.07 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.65 (d, *J* = 1.9 Hz, 1H), 7.59 – 7.53 (m, 4H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.00 – 6.95 (m, 2H), 6.82 (d, *J* = 8.4 Hz, 1H), 5.47 (s, 2H), 3.96 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H). ^{13}C NMR(100 MHz, $CDCl_3$): δ164.4, 159.8, 156.3, 151.1, 149.3, 144.7, 133.1, 130.5, 122.3, 122.1, 121.6, 120.2, 116.6, 114.8, 113.7, 111.0, 109.4, 63.3, 56.1, 55.9, 55.6. IR (neat): 2955, 2923, 2853, 1605, 1517, 1498, 1255, 1230, 1142, 1028, 749 cm^{-1}. Anal. Calcd. For $C_{26}H_{23}N_5O_5$:C, 64.32; H, 4.78; N, 14.43; Found: C, 64.29; H, 4.77; N, 14.40. ESI-MS:m/z: 486 (M+1).

2-(2,4-Dichlorophenyl)-5-(2-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)phe-nyl)-1,3,4oxadiazole (8q):

Yield: 86%; White solid; M.p. 171 – 173.^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (s, 1H), 8.09(dd, *J* = 7.8, 1.7 Hz, 1H), 7.99 (d, *J* = 8.5 Hz, 1H), 7.62 – 7.51 (m, 4H), 7.35 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 1H), 7.17 (t, *J* = 7.2 Hz, 1H), 7.05 – 6.97 (m, 2H), 5.48 (s, 2H), 3.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ162.5, 156.6, 145.1, 132.9, 130.5, 129.8, 129.0, 121.8, 121.3, 120.5, 114.5, 113.4, 63.4. IR (neat): 2955, 2922, 2852, 1594, 1494, 1461, 1377, 1266, 1044, 817

cm^{-1}. Anal. Calcd. For $C_{24}H_{17}Cl_2N_5O_3$:C, 58.31; H, 3.47; N, 14.17; Found: C, 58.28; H, 3.44; N, 14.15.ESI-MS:m/z:494 (M+1).

4.2. Biological Screening

4.2.1. Antimicrobial activity

Materials and methods:

Strains and media:

Two strains of Gram-positive bacteria (*Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 96*)* and Gram-negative bacteria (*Escherichia coli* MTCC 443*)* were used for screening the antibacterial activity. Strains were obtained from the Department of Microbiology, Osmania University. Dimethyl sulfoxide (DMSO) was used as compound dissolving agent, Ampicillin (Standard drug) as test control, Muller-Hinton Agar, Luria-Bertani Broth, Miller (LB broth) for culturing microbes.

Preparation of microbial cultures:

All types of microbial stock cultures received were streaked separately onto Muller-Hinton Agar slants (20 ml/tube) and incubated at 37°C for 24 hrs under sterile conditions. The grown cultures were inoculated into 10 mL Luria-Bertani Broth, Miller (LB) broth medium (containing Tryptone 10 g/L, Yeast extract 5 g/L, Sodium chloride 10 g/L, pH 7.5) by transferring a loop full of culture and incubated for 12 hr at 37°C with continuous shaking at 220 rpm.. 1% of grown culture was re-inoculated into fresh LB broth and incubated for 3-4 hours for Log phase culture. An absorbance of 0.4-0.6 OD was measuredat 600 nm suggesting that cultures are now ready to use for testing antibacterial activity.

Antibacterial Activity

To test the antimicrobial efficacy of the compounds, Luria-Bertani Broth, Miller (LB broth) a potent microbiological growth medium which was commonly used for antibiotic susceptibility testing was used. LB broth was prepared and sterilized in autoclave under aseptic conditions, 121 °C /15 Lbs pressure for 30 minutes and cooled to room temperature. In the present study, Minimum Inhibitory Concentration (MIC) of the compound against several bacterial strains was determined by following the broth dilution method. Microbial strains to be tested were grown at 37°C for 12 h with continuous shaking at 220 rpm [55-57] and arrested at log phase by storing at 4°C. The synthesized compounds to be tested for antimicrobial activities were dissolved in DMSO consequently to produce a concentration of 1 mg/mL and are treated as a stock solution. Subsequently different concentration of compound ranging from minimum to maximum was added to bacterial cultures to determine its MIC value. Antibacterial activity of the compound

was determined by treating various concentrations of compound with log phase culture (1 µl/ml) and incubating for 12 hrs at 37℃ with continuous shaking at 220 rpm [56]. Ampicillin a standard drug of 1% is used as a positive control for antibacterial activity and blank was prepared by adding only 1µl/ml culture to the broth and it served as a negative control. MIC values of different compounds were noted by comparing the growth turbidity in tubes. A specific concentration of compound where the bacterial growth inhibition has started is termed as a minimal concentration of compound required for bacterial inhibition.

4.2.2. Anti-fungal activity

The anti-fungal activities of the synthesized compounds were tested against filamentous fungi *Aspergillus niger* MTCC 404 and *Saccharomyces cerevisiae* MTCC 1344 yeast cultures [58]. They were obtained from the Culture Collection Division at Department of Microbiology, Osmania University. The fungal cultures were grown on yeast extract agar (YEP) media containing chloramphenicol (50 µg/mL) for 2-4 days at 28 °C. After 2 days, the black spores and lawn of cultures were collected and stored in an aqueous solution of 40% (v/v) glycerol at -80 °C. Anti-fungal assay can be done by cylinder plate, disc method, cross streak plate method, and broth dilution method. Here in the current study we used cross steak plate method to identify the minimum concentration of compound required to inhibit the fungal growth.YEP agar media (commonly used to assess susceptibility of diverse mutants) was prepared by mixing the bactopeptone, sodium chloride and maintained final pH 7 at 25°C. Sterilization of media is carried out at 121^0C/15 lbs pressure for 30 minutes, and cooled to room temperature and poured into sterile petriplates. Aqueous cultures of fungal strains were spreaded evenly on plates and different concentrations of diluted compounds were streaked on plate. The measurement of clear zonal area length (mm) on YEP agar plate determined the compound efficacy for fungal growth inhibition.

4.2.3. Antioxidant activity

DPPH assay is one of the most widely used method for testing free radical scavenging activity [62-63]. The UV spectrophotometric assay proposed by Yen and Chen in 1995 [64], determines the antioxidant ability of synthesized compounds. It uses stable light sensitive freshly prepared 0.5 mM DPPH dissolved in methanol as a reagent solution. Standard and other synthesized compounds at various concentrations were successively prepared from 1 mM stock by dissolving in DMSO. Antioxidant activity of diluted test compounds was checked by transferring various concentrations from 10 µM to 200 µM.100 µl of DPPH• reagent was transferred constantly to tube and

1. Sterile distilled water was added to tube to make up to 1 ml. The mixture was shook gently and permitted to stand in dark at room temperature for 30 minutes. Blank solution was prepared by adding various concentrations of compound diluents and constant amount of reagent solution to make up to 1000 ml.

2. Ascorbic acid a well known stable antioxidant of 1 mM stock diluted in DMSO was used as a positive control for maintenance.
3. All determinations for each concentration were carried in triplicates to avoid mean variations.
4. Absorbance of solutions was measured by using UV spectrophotometer at 517 nm. The plotted by taking concentrations of the compound in x-axis and absorbance at 517 nM in y-axis showed the percent antioxidant activity of samples.

The free radical scavenging activity was calculated using the following equation:

% Antiradical activity= [(Control abs - Sample abs) / Control abs] x100

The standard curve was linear between 10 and 200 μM concentrations and the results of **Table – 3** listed compounds at 517 nM were expressed as % antioxidant activity at various molecular concentrations.

4.3. Molecular docking

The structures of all the synthesized compounds were generated using MarvinSketch 5.6.0.2. (1998-2011, Copyright © ChemAxon Ltd), cleaned in 3D and saved in .pdb format for docking studies. The PDB file of the target protein downloaded from RCSB PDB (www.rcsb.org), PBP co-crystal structures (PDB ID: 3HUN & 3ITA). Docking was performed with Molegro Virtual Docker (MVD 2012.5.5) [65]. While docking first the protein and ligands were prepared by assigning bonds, bond orders, charges, explicit hydrogens, flexible torsions in ligands if they were missing as per manual instructions. Possible binding sites were detected for both PBP's, first cavity in 3HUN and third cavity in 3ITA were used respectively. The search algorithm is taken as Mol Dock SE and number of runs are taken as 10 and max iterations were 1500 with population size 50 and with an energy threshold of 100. Further investigations of the binding interactions of the most active docked compounds were performed using Biovia discovery studio 2020 Client and PyMol.

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6. Supplementary:

^{1}H NMR, ^{13}C NMR, MASS and IR data for all compounds provided with supplementary.

7. Conflict Interest:

The authors hereby declare no conflict of interest.

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9. Figures and legends:

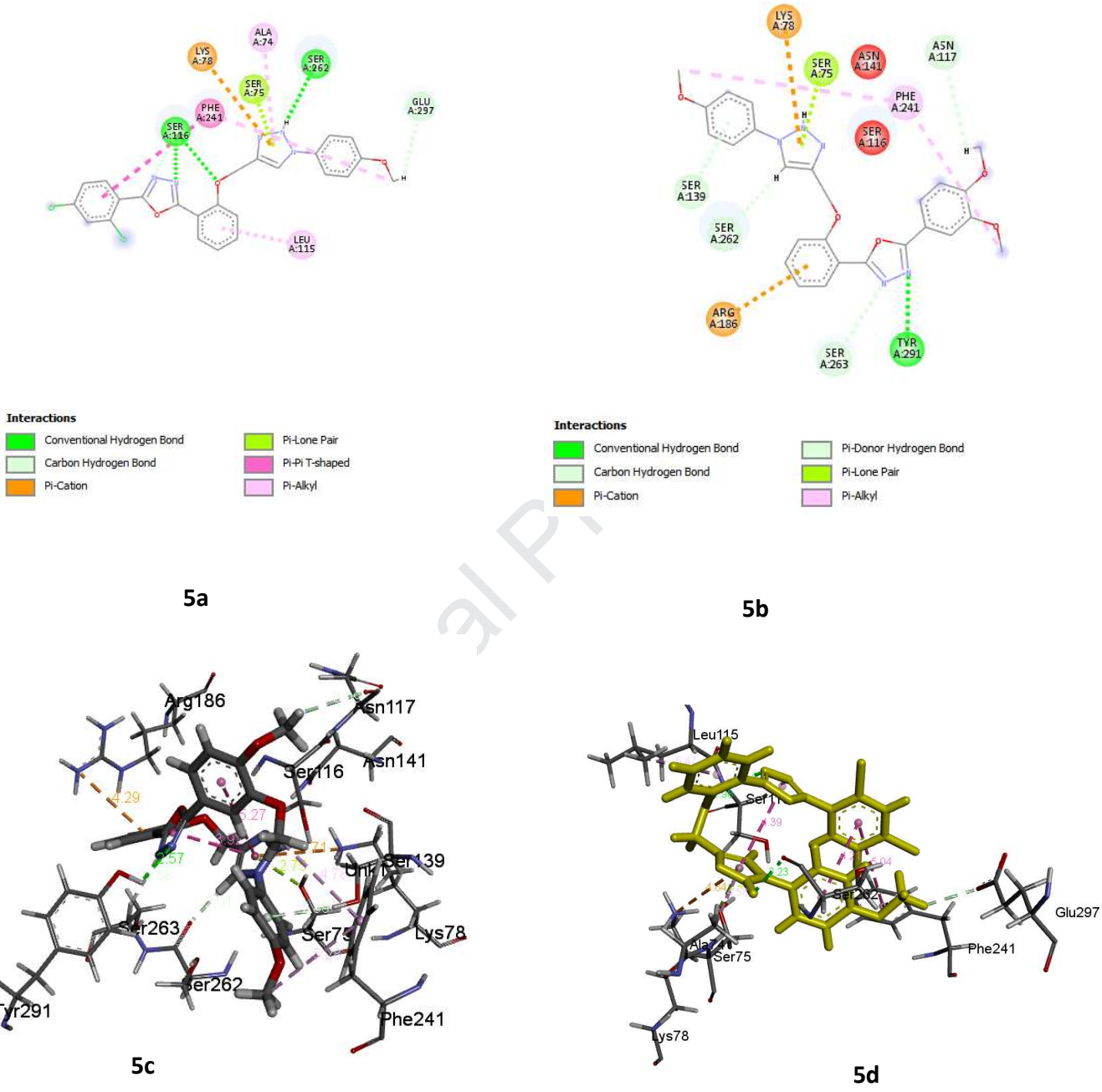
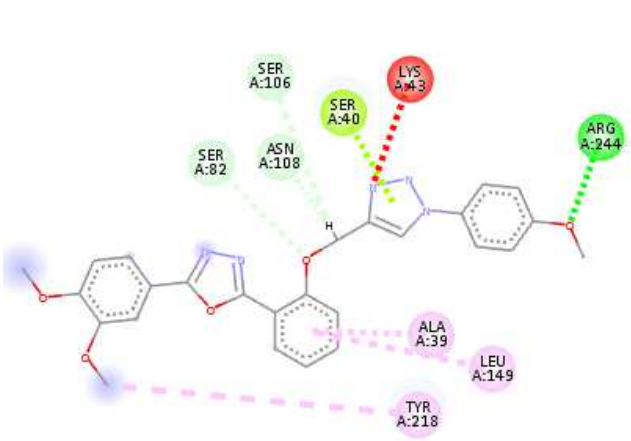
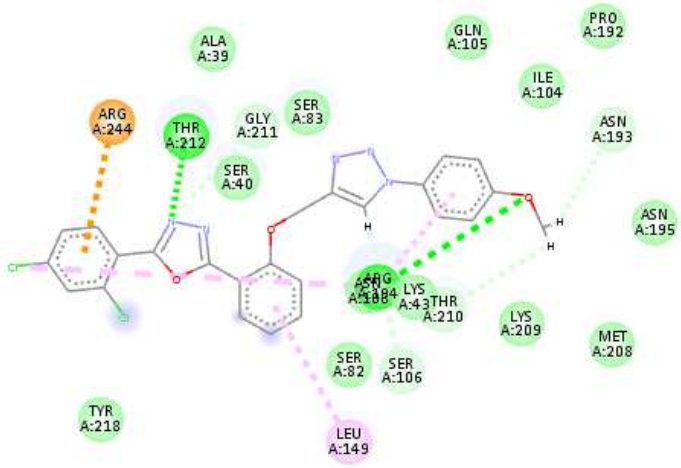


Figure -5: 5a & b represents Docked 2D image of compound 8p & 8q with Penicillin binding protein 4 (PBP4) (PDB ID: **3HUN); 5c & d** represents 3D-stick enlarged view of compound **8p** and **8q** with **3HUN** (Ribbons). The green dotted lines in the figure represent the hydrogen bonding between the ligand and protein. Different protein ligands are stabilized by different interactions and color codes are given in the bottom of figure 1a and 1b.



6a



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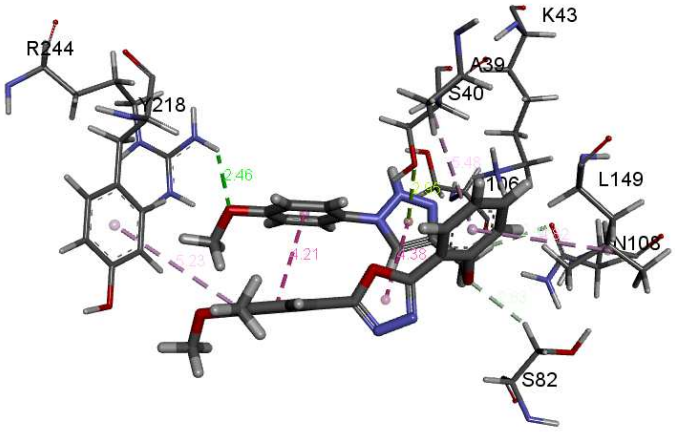

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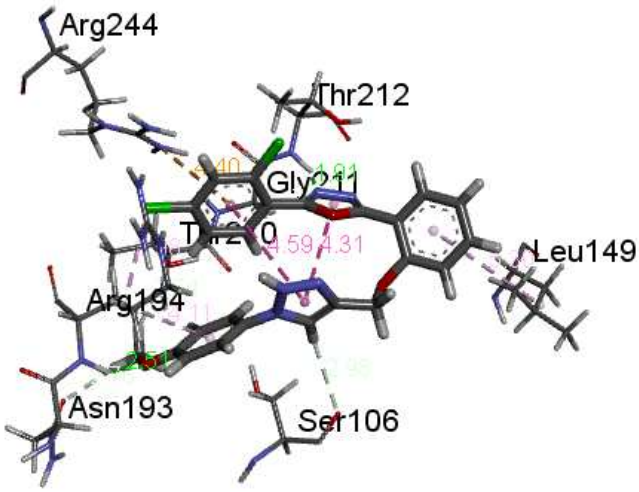
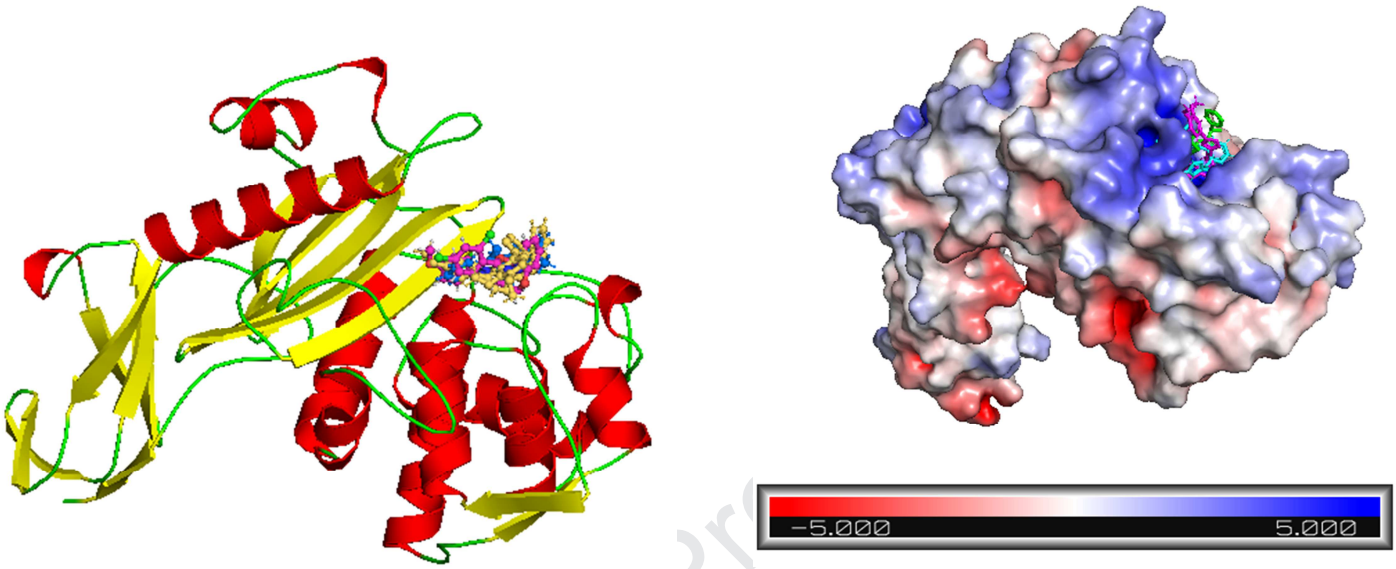


6d

Figure -6: Docked 2D image of compound **8p & 8q (6a & 6b**) with Penicillin binding protein 6 (PBP6) (PDB ID: **3ITA); 6c & d** represents 3D-stick enlarged view of compound **8p** and **8q** with **3ITA** (Ribbons). The green dotted lines in the figure represent the hydrogen bonding between the ligand and protein.



7a

7b

Figure -7: Superposition of docked compounds and co-crystal structure with Pymol: 8a) Cartoon diagram representing secondary structural elements of **3HUN** helices in red, barrels in yellow and loops in green color, respectively. The **8p** (magenta) & **8q** (dark yellow) are aligned with ampicillin shown in marine color. **8b)** Surface representation of **3ITA** with electrostatic potential, red, grey and blue showing negative, neutral and positive charges, respectively. In the active site **8p** (magenta) & **8q** (cyan) inhibitors are aligned on ampicillin (green) in the active site.

Highlights of Manuscript:

- The synthesized novel 2,5-diaryl substituted 1,3,5-oxadiazole derivatives **8a-q** exhibited promising activity for anti-microbial.
- Molecular docking studies of the synthesized compounds are in good agreement with their activity, compounds like **8d**,**8p** and **8q** were found to be effective against bacterial growth.
- Among all the compounds, **8d-f, 8l** and **8o** are the major compounds shown significant antifungal activity.

*Credit Author Statement

Author contribution:

KRA, SB, SSR conceived the idea and provided critical inputs to the concept. KRA planed the experiment, SB and RD. generated synthesis data. Molecular docking and Biological assays are carried out by SSR and AAG. All the authors KRA, SB, SSR, RD, PMR, SB2, AAG and BVK contributed to analyze, interpret data and wrote the manuscript. All authors contributed to the final reading and approved the submitted revised version.

Declaration of Interest:

The authors declare that they have no know competing financial interests (or) personal relationships that could have appeared to influence the work reported in this manuscript.

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