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IN SILICO DESIGN, DOCKING, SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 2,5-DISUBSTITUTED 1,3,4-OXADIAZOLE DERIVATIVES

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Key words:

2,5-disubstituted 1,3,4-oxadiazole, Schrodinger, Glide XP, FabH inhibitors, antibacterial activity, antifungal activity

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ABSTRACT: Emergence of resistant bacterial and fungal strains towards existing antimicrobial agents is one of the major motives for research and development of new molecules to defend them. Oxadiazole is a major compound of heterocyclic nucleus for the development of new drugs. Oxadiazole ring system could be incorporated into many more ring systems which itself have their own activity and could lead to potent and highly active compounds. *In silico* is an expression used to mean "performed on computer or via computer Simulation". Docking is a method used to identify the fit between a receptor and a potential ligand. In this research work *In silico* design of 2,5-disubstituted 1,3,4-oxadiazole derivatives were carried out by Schrodinger software for antimicrobial activity. The antibacterial and antifungal proteins were selected from Protein Data Bank and docked with hundred derivatives of 2,5-disubstituted 1,3,4-oxadiazole by using glide XP. Then five derivatives with highest glide Score were selected and synthesized. Synthesis was a microwave cyclisation reaction between hydrazide and various aromatic acids. The newly synthesized derivatives were characterized and confirmed by physical and spectral studies. These compounds were screened for antibacterial activity against *Escherichia coli* and *Staphylococcus aures*, antifungal activity against *Aspergillus niger* and *Candida albicans*. Synthesized compounds exhibit significant biological activity and can certainly hold greater promise in discovering safer biologically active molecules.

INTRODUCTION: Infections triggered by some pathogenic microorganisms can bring illnesses even a fatal one. Although many kinds of antibacterial agents were discovered and used for clinical treatment, the incidences of drug resistance of microorganisms to antibacterial agents were constantly reported. Therefore, the development of new types of antibacterial agents is a very important task and much of the research effort is oriented to the design of new antibacterial agents with high efficiency.

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In recent years, different kinds of targets in key areas of the bacterial cell cycle have been studied that would be a new approach against the problem of drug resistance. One of the most attractive biochemical pathways to be used as the target for new antibacterial agents is the fatty acid biosynthesis (FAB).

This pathway has been demonstrated to be essential for bacteria cell survival. A key enzyme in this pathway is β-ketoacyl-acyl carrier protein synthase III (FabH), which is the enzyme responsible for the first reaction in the pathway and plays an important regulatory role. These facts suggest the idea that FabH can be used as an effective molecular target for the development of new antibacterial agents [1]. The incidence of fungal infections continues to increase rapidly because of the increased number of

immunocompromised patients (AIDS, cancer and transplants). *Candida* species is one of the most well-known fungal pathogens which accounts for majority of fungal infections occurring worldwide. Resistance to wide spectrum antifungal agents has initiated the search for new therapeutic agents, including those produced by the modification of existing antifungal drugs.

For antifungal agents, the mechanism involved is the inhibition of ergosterol synthesis and a key enzyme involved is sterol 14-α demethylase, so it is used as an effective molecular target for the development of new antifungal agents [2].

Li et al., (2012) designed and synthesized nitroimidazole derivatives containing 1,3,4-oxadiazole scaffold as FabH inhibitors. The derivatives were tested for antibacterial activities against *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus*. This new nitroimidazole derivatives class demonstrated strong antibacterial activities. *Escherichia coli* β-ketoacyl-acyl carrier protein synthase III (FabH) inhibitory assay and docking simulation by CDOCKER Dock protocol of Discovery Studio 3.1 indicates that synthesized derivatives of 1,3,4-oxadiazole compounds with MIC of 1.56-3.13 g/ml were most potent inhibitors of *Escherichia coli* FabH[1]. Ilango et al., (2009) synthesized 2,5-disubstituted 1,3,4-oxadiazole derivatives from 8-hydroxy quinoline. The synthesized compounds were evaluated for their *in vitro* antibacterial activity [3].

1,3,4-oxadiazole belongs to an important class of heterocyclic compounds and serves both as biomimetic and reactive pharmacophores and many are key elements with potential biological activities such as antibacterial, antifungal, anticancer etc[1]. Based on these findings, with the aim of developing new antibacterial and antifungal agents, we planned to synthesize 2,5disubstituted 1,3,4-oxadiazole derivatives, together with the hope of potent activity and lower toxicity. These compounds were studied for antibacterial activity against *Escherichia coli* and *staphylococcus aures,* antifungal activity against *Aspergillius niger* and *Candida albicans.*

MATERIALS AND METHODS:

Methodology of Docking: Schrodinger [Maestro 9.2]

The antibacterial (1HNJ pdb) and antifungal (1EA1 pdb) proteins (**Fig. 1 & Fig. 2)** were docked with hundred derivatives of 2,5-disubstituted 1,3,4-oxadiazole by using GLIDE XP. (**Fig. 3**)

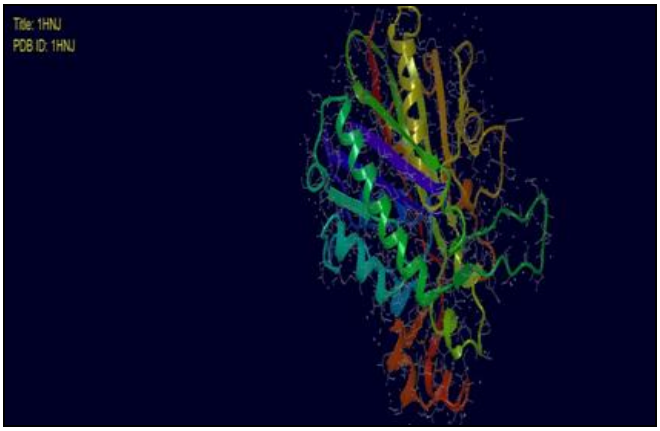


FIG.1: ANTIBACTERIAL PROTEIN: 1HNJ

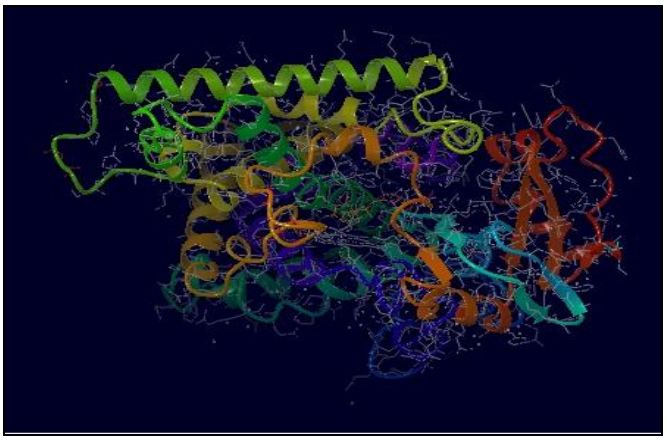


FIG.2: ANTIFUNGAL PROTEIN: 1EA1

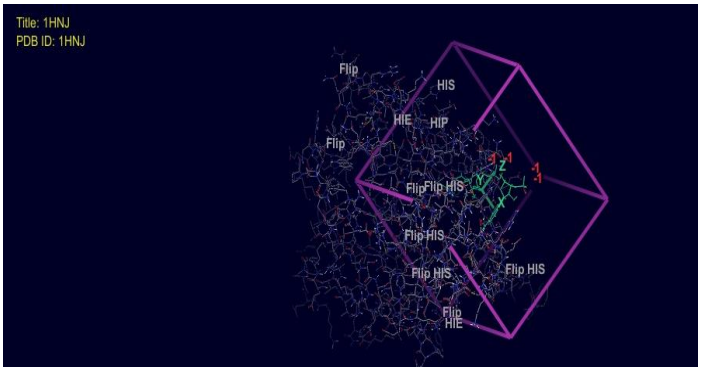


FIG.3: RECEPTOR GRID GENERATION

General method of synthesis of 2,5-disubstituted 1,3,4-oxadiazole derivatives:

1. **Synthesis of ethyl-2-(quinoline-8yloxy) acetate:** A mixture of 8-hydroxy quinoline (0.01mol), ethyl chloro acetate (0.01mol) and

anhydrous potassium carbonate (0.01mol) in dry acetone were refluxed for 6 hours and poured into ice-cold water. Solid product obtained was filtered and recrystallized from ethanol.

2. **Synthesis of 8-hydroxy quinoline acetyl hydrazide:** A mixture of ethyl-2-(quinolin-8yloxy) acetate (0.01 mol), hydrazine hydrate (80%, 0.07 mol) in methanol was refluxed for 6 hours. From the resultant mixture excess of ethanol was removed by distillation. On cooling needle crystals separates out and recrystallized from ethnol.

3. **Synthesis of 2-(quinoline-8yloxymethyl)-5-aryl-1,3,4-oxadiazole:** A mixture of 8-hydroxy quinoline acetyl hydrazide (0.01 mol) and various aromatic acids (0.01 mol) in phosphorous oxychloride (**Table 2**) was exposed to microwave irradiation at 420 W (i.e, 30% microwave power) intermittently at 2 minutes intervals for 8-15 minutes (**Table 1**). Completion of the reaction process was monitored by TLC using chloroform**:** acetone (9**:**1) as mobile phase. The contents were cooled and poured into the crushed ice. It was neutralized by 10% sodium hydroxide and solid product obtained was filtered, dried and recrystallized from ethanol. [3]

4. Synthetic Methodology: [3]

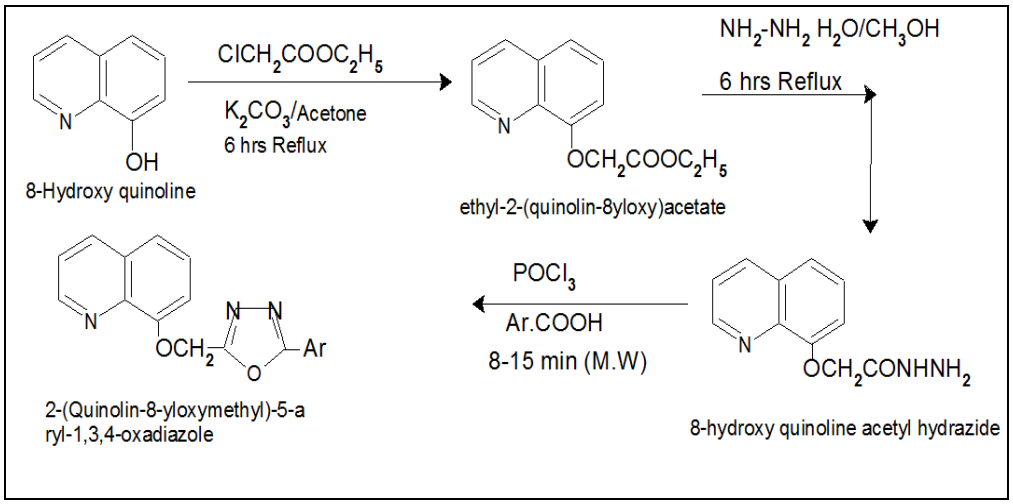
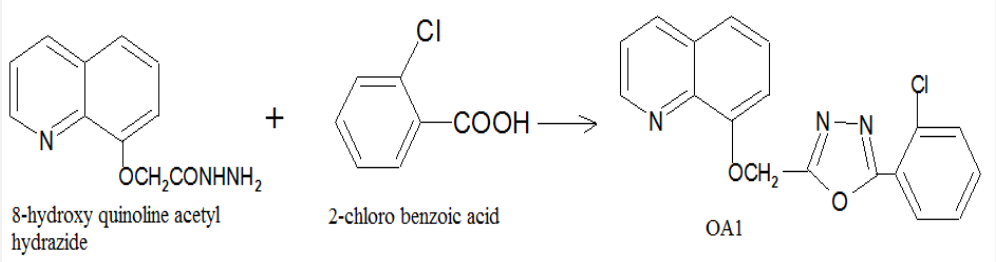


TABLE 1: MICROWAVE IRRADIATION TIME FOR EACH COMPOUNDS

Compound	Microwave irradiation Time (min)
OA-1	12
OA-2	10
OA-3	13
OA-4	14
OA-5	11

TABLE 2: LIST OF 2,5-DISUBSTITUTED 1,3,4-OXADIAZOLE DERIVATIVES.

SL No.	Derivatives
1	

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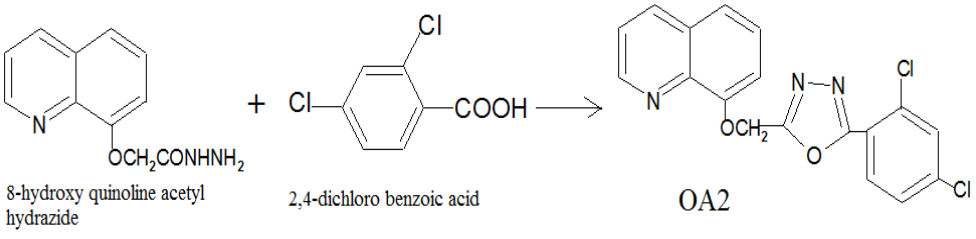
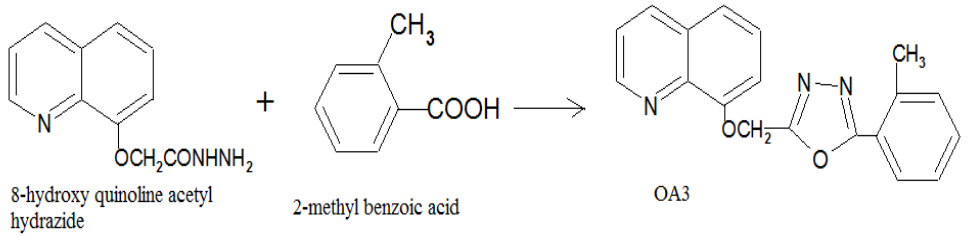


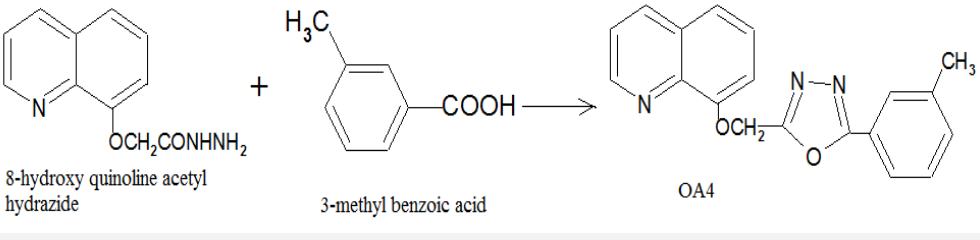
8-hydroxy quinoline acetyl hydrazide + 2,4-dichloro benzoic acid → OA2

3



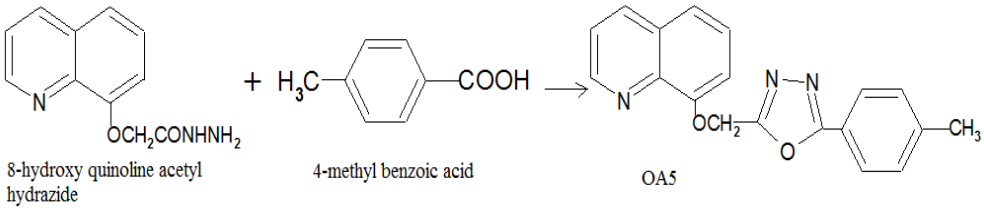
8-hydroxy quinoline acetyl hydrazide + 2-methyl benzoic acid → OA3

4



8-hydroxy quinoline acetyl hydrazide + 3-methyl benzoic acid → OA4

5



8-hydroxy quinoline acetyl hydrazide + 4-methyl benzoic acid → OA5

Antibacterial Activity:

Determination of Minimum Inhibitory Concentration:

Agar well diffusion method: [4]

Maintenance of Bacterial Strains:

Bacterial strains - *Staphylococcus aureus, Escherichia coli***.** The bacteria were maintained on nutrient agar slants by sub culturing them at monthly intervals.

Preparation of Inoculum:

Nutrient broth culture of organisms obtained after incubation for 24 hrs at 37^0C was used as inoculums

Preparation of Agar wells:

The well was made using a 5mm cork borer sterilized using flame and alcohol.

Procedure:

A sterile swab was dipped into nutrient broth culture and was rotated firmly against upper inside wall of the tube to expel the excess fluid. The entire surface of Mueller Hinton agar medium (MHA) was lawned with the swab soaked with inoculum. As soon as the MHA was partly solidified, the plates were inverted and left for 2hrs. When cooled, wells were made on the soldified medium in the plate. 100µl of various samples dissolved in DMSO was introduced into the wells. The plates were incubated overnight at 37^0C. Microbial growth was determined by measuring the diameter of zone of inhibition and compared with that of standard antibiotics. For each bacterial strain, controls were maintained where DMSO was used instead of sample. The results were obtained by measuring the zone diameter.

Antifungal Activity: [5]

Fungal strains - *Candida albicans, Aspergilius nige.*

Procedure:

Drops of fungus culture was mixed with the warm, melted, autoclaved Potato Dextrose Agar medium and poured into separate plates under aseptic conditions. The plates were covered and allowed to cool. As soon as the agar was partly solidified, the plates were inverted and left for 2h. When cooled wells were made on the solidified medium in the plate. The well was made by using a 5 mm cork borer that was sterilized with alcohol and flame. Samples were dissolved in DMSO and pipetted into the different wells in a sterilized environment in separate plates, using a micro pipette. DMSO was used as control. The plates were labelled, covered, inverted and placed in room temperature for about one week. After one week plates are examined for fungal growth and for zone of inhibition around the wells and compared with standard antifungal agents.

RESULTS AND DISCUSSION:

In Silico Molecular Modeling:

TABLE 3: CALCULATION OF SMILES AND LOG P VALUES OF PROPOSED DRUGS USING MOLINSPIRATION SOFTWARE

Sample	Smiles notation	Log P
OA-1	Clc1ccccc1c4nnc(COc2cccc3cccnc23)o4	3.599
OA-2	Clc4ccc(c3nnc(COc1cccc2cccnc12)o3)c(Cl)c4	4.253
OA-3	Cc1ccccc1c4nnc(COc2cccc3cccnc23)o4	3.369
OA-4	Cc4cccc(c3nnc(COc1cccc2cccnc12)o3)c4	3.394
OA-5	Cc4ccc(c3nnc(COc1cccc2cccnc12)o3)cc4	3.417

TABLE 4: LIPINSKI RULE OF FIVE ANALYSIS BY MOLINSPIRATION SOFTWARE

Sample	Log P	Molecular weight	No: of Hydrogen Bond acceptors	No: of Hydrogen Bond donors	n rotatable bonds	Lipinski Rule Alert Index
OA-1	3.599	337.766	5	0	4	0
OA-2	4.253	372.211	5	0	4	0
OA-3	3.369	317.348	5	0	4	0
OA-4	3.394	317.348	5	0	4	0
OA-5	3.417	317.348	5	0	4	0

TABLE 5: DRUG LIKENESS ANALYSIS OF PROPOSED DRUGS USING MOLINSPIRATION SOFTWARE

Sample	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
OA-1	-0.12	-0.36	-0.04	-0.32	-0.21	-0.02
OA-2	-0.10	-0.34	-0.05	-0.32	-0.20	-0.03
OA-3	-0.10	-0.35	-0.02	-0.20	-0.26	-0.07
OA-4	-0.10	-0.37	-0.05	-0.18	-0.23	-0.03
OA-5	-0.12	-0.38	-0.08	-0.22	0.25	-0.06

In silico molecular modeling studies were carried out on analogues of 2,5-disubstituted 1,3,4-oxadiazole derivatives using various softwares like ChemSketch, Molinspiration, and Schrodinger (version 9.2). Smiles notation and molecular descriptors were calculated using Molinspiration software. Analysis of Lipinski Rule of Five were carried out by Molinspiration software and found out that all the analogues obeyed Lipinski Rule of Five. Drug likeness profile of analogues was predicted by the methodology developed by Molinspiration.

Molecular docking:

Molecular docking studies were carried out on hundred analogues of 2,5disubstituted 1,3,4-oxadiazole by flexible docking using XP GLIDE (Extra Precision). According to the highest docking score and availability of chemicals, out of these hundred analogues, five analogues were chosen for wet lab synthesis.

TABLE 6: DOCKING SCORES OF PROPOSED COMPOUNDS FOR ANTIBACTERIAL ACTIVITY

Target	PDB ID	Compound code	Docking scores
β-ketoacyl-acyl carrier protein synthaseIII (FabH)	1HNJ	OA-1	-7.318
		OA-2	-7.178
		OA-3	-6.978
		OA-4	-7.939
		OA-5	-7.400

TABLE7: DOCKING SCORES OF PROPOSED COMPOUNDS FOR ANTIFUNGAL ACTIVITY

Target	PDB ID	Compound code	Docking scores
Cytochrome P450 14α-sterol demethylase (CYP51)	1EA1	OA-1	-6.497
		OA-2	-6.584
		OA-3	-6.776
		OA-4	-7.828
		OA-5	-7.621

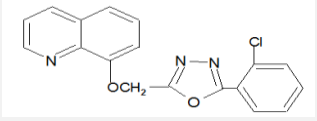
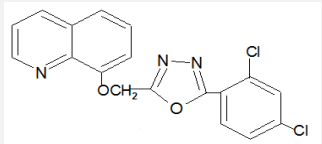
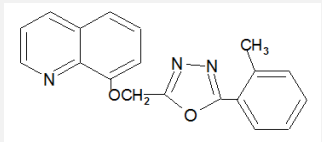
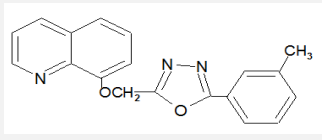
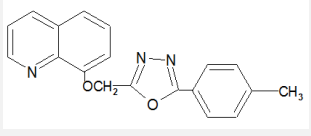
Docking was carried out for antibacterial activity, where the proposed compounds showed good binding interactions with β-ketoacyl-acyl carrier protein synthase III (FabH) from Escherichia coli (1HNJ).

The compound OA4 exhibited highest docking score. OA4 has a m-methyl substituted benzene ring at 5th position of 1,3,4-oxadiazole nucleus, so substitution with the same improves the binding interactions between protein and ligand.

Docking was carried out for antifungal activity, where the proposed compounds showed significant binding interactions with Cytochrome P450 14α-sterol demethylase (CYP51) from Mycobacterium Tuberculosis (1EA1). The compound OA4 exhibited highest docking score.

Characterization:

TABLE 8: PHYSICAL CHARACTERIZATION DATA OF SYNTHESIZED COMPOUNDS

Sample	Melting point	R_f value	Percentage yield	Molecular weight	Molecular formula
 OA1	147^{0}C	0.75	64.53%w/w	337.766	$C_{18}H_{12}N_3O_2Cl$
 OA2	154^{0}C	0.67	66.35%w/w	372.211	$C_{18}H_{12}N_3O_2Cl_2$
 OA3	166^{0}C	0.79	63.59%w/w	317.348	$C_{19}H_{15}N_3O_2$
 OA4	160^{0}C	0.82	60.62%w/w	317.348	$C_{19}H_{15}N_3O_2$
 OA5	167^{0}C	0.81	61.89%w/w	317.348	$C_{19}H_{15}N_3O_2$

Solvent system – Chloroform:acetone (9:1)

Antibacterial activity:

The synthesized analogues were taken for antibacterial screening. Streptomycin was used as standard for both gram positive *(Staphylococcus aureus)* and gram negative organism (*Escherichia coli*).

TABLE 9: ZONE OF INHIBITION OF SAMPLES

Sample	**Zone of inhibtion** (mm)									
	Gram –ve (*Escherichia coli*)					**Gram + ve (*Staphylococcus aureus)***				
	5 µg	10 µg	20 µg	40 µg	80 µg	5 µg	10 µg	20 µg	40 µg	80 µg
OA1	-	-	-	8±0.89	10±0.51	-	-	6±0.51	7±0.89	10±0.51
OA2	-	-	-	7±0.89	9±0.51	-	-	9±0.89	13±0.51	15±0.51
OA3	-	-	7±0.89	7.6±0.51	9±0.89	-	7±0.89	8±0.51	9±0.89	10±0.89
OA4	-	-	9±0.51	10±0.51	11±0.51	-	10±0.51	12±0.89	14±0.51	16±0.51
OA5	-	-	6±0.51	7±0.89	8±0.51	-	9±0.89	10±0.51	12±0.51	14±0.89
Streptomycin	6±0.5	7±0.5	8±0.51	9±0.51	10±0.51	6±0.5	7±0.89	8±0.51	9±0.51	10±0.51

TABLE 10: MINIMUM INHIBITORY CONCENTRATION OF SAMPLES

Sample	**Minimum Inhibitory Concentration [MIC] (µg/ml)**	
	Gram –ve (*Escherichia coli*)	**Gram + ve (*Staphylococcus aureus*)**
OA1	31.62	19.95
OA2	25.70	12.88
OA3	14.12	8.91
OA4	10.44	6.76
OA5	12.88	6.87
Streptomycin	4.67	5.0

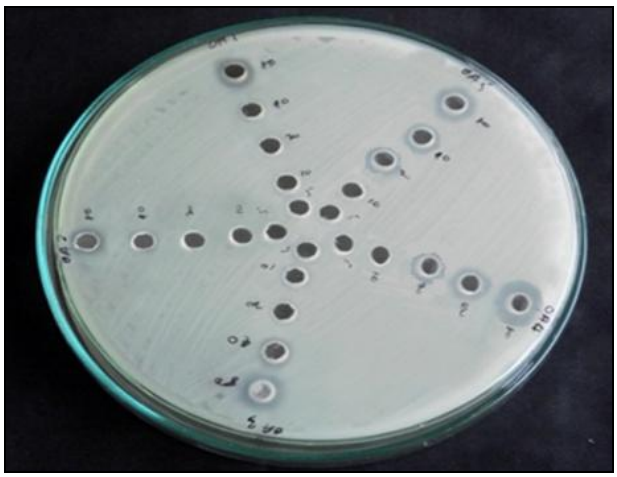


FIG.4: ANTIBACTERIAL ACTIVITY OF SAMPLES ON *E.COLI*

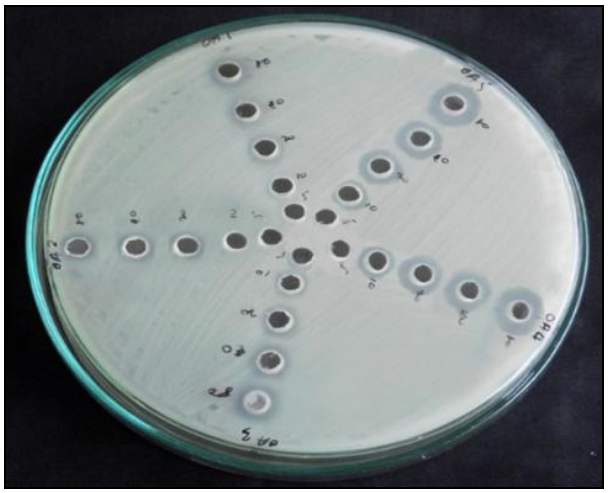


FIG.5: ANTIBACTERIAL ACTIVITY OF SAMPLES ON *S.AUREUS*

Antifungal activity:

The synthesized analogues were taken for antifungal screening. Clotrimazole was used as standard for both fungi *Candida albicans* and *Aspergillius niger*.

TABLE 11: ZONE OF INHIBITION OF SAMPLES

Sample	Zone of inhibtion (mm)					
	Candida albicans			*Aspergillius niger*		
	25	50	100	25	50	100
OA1	-	6±0.51	14±0.89	-	7±0.51	15±0.89
OA2	-	7±0.51	17±0.89	-	6±0.51	11±0.51
OA3	-	8±0.89	15±0.89	-	10±0.89	20±0.51
OA4	-	11±0.89	20±0.51	7±0.89	10±0.89	11±0.89
OA5	-	12±0.89	24±0.51	10±0.89	18±0.89	26±0.51
Clotrimazole	7±0.51	9±0.51	10±0.51	6±0.51	7±0.51	8±0.51

TABLE 12: MINIMUM INHIBITORY CONCENTRATION OF SAMPLES

Sample	Minimum Inhibitory Concentration [MIC] (µg/ml)	
	Candida albicans	*Aspergillius niger*
OA1	48.26	46.77
OA2	46.77	45.70
OA3	45.70	28.18
OA4	42.65	18.62
OA5	43.65	23.98
Clotimazole	22.38	15.84

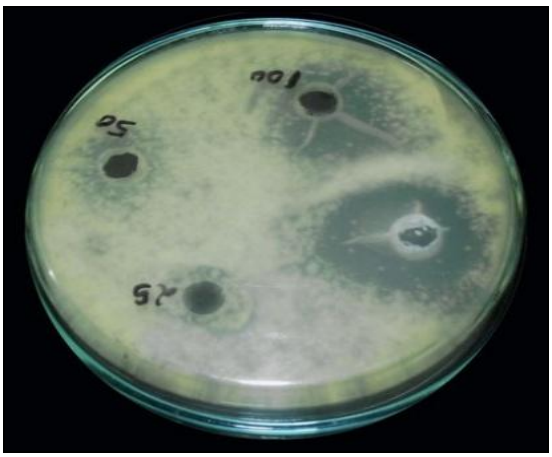


FIG. 6: ANTIFUNGAL ACTIVITY OF SAMPLES ON *C.ALBICANS*

All analogues were screened for antibacterial activity and showed good antibacterial activity. Streptomycin was used as standard for both gram positive (*Staphylococcus aureus*) and gram negative organism (*Escherichia coli*). Synthesized analogues were most active against gram positive organism (*Staphylococcus aureus*). OA4 analogue was found to be highly active against both gram positive and gram negative organism than other analogues in considerable amounts with respect to the standard drug Streptomycin.

All the analogues were screened for antifungal activity and showed good antifungal activity. Clotrimazole was used as standard for both *Candida albicans* and *Aspergillius niger*. Synthesized analogues were most active against *Aspergillius niger*. OA4 analogue was found to be highly active against both *Candida albicans* and *Aspergillius niger* than other analogues in considerable amounts with respect to the standard drug Clotrimazole.

CONCLUSION: The present research work involved in the preliminary *in silico* molecular modeling studies of 2,5-disubstituted 1,3,4-oxadiazole derivatives using Chem Sketch, molnspirstion, and PASS Online softwares and docking using schrodinger (Mastero 9.2). Among the proposed analogues, those obeying Lipinski

rule of five were selected for the wet lab synthesis. Synthesis of five analogues was performed and the purity of the same was ascertained by consistency in melting point and R_f value. The compounds were characterized by IR and MASS spectral studies. The synthesized analogues were screened for various biological activity studies, which includes, antibacterial and antifungal studies as per docking score. The activities of the screened analogues were promising.

ACKNOWLEDGEMENT: I take this opportunity to convey my sincere thanks to Mr. Raghu and Mr.Vinod Daivaraji, Application Scientist, Schrödinger Inc, for giving Schrödinger software which was very helpful in carrying out my docking studies.

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