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Research Article

In Silico and *In Vitro* Study of Synthesized Derivatives of 2-aryl/3, 4-difluro aryl substituted 1, 3, 4-thidiazole-2-amine.

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ABSTRACT
In this research article synthesized derivatives of 2-aryl/3,4-difluro aryl substituted 1,3,4-thidiazole-2-amine were investigated *in silico* way by docked with selected drug target (Dihydrofolate reductase (DHFR); DNA Gyrase; Topoisomerase IV; S-adenosyl homocysteine nucleosidase) for anti-bacterial infections and Pencillin Binding Protein (PDB ID: 1VQQ) & Lanosterol 14 α-demethylase (PDB ID: 4LXJ) for anti-fungal activity. IC_{50} value and in micro molar levels binding energy is determined which showed potential activity when binded with ligand .NCIM provided culture is used to investigate *in vitro* activity Gram positive bacterial strains *Staphallococcus aureus* (Sa- 2178) *Bacillus subtilis* (Bs-2239) Gram negative bacterial strains *Eschereschia coli* (Ec-25744) Klibesiella aerogenus (Ka-2249) Antifungal strains are *Aspergillus niger* (An-504) and *Penicillium Chrysogenum*(Pc-709).The comparative study of these two investigated methods are explained. QSAR studies of synthesized derivatives were also highlight and support these investigations.

KEYWORDS
In silico, in vitro, NCIM, 2-aryl/3,4-difluro aryl substituted 1,3,4-thidiazole-2-amine

1. INTRODUCTION

Chemotherapy treatment is communicated in the world in the 1960s in the group of antibiotics such as tetracycline, cephalosporin, amino glycosides and macrolides. Current scenario is that efficacy of these antibiotics get reduce due to multidrug-resistant bacteria and chances of treatment failure get increase in rate of morbidity and mortality [1].

Synthetic organic chemist has always interested to synthesize new molecules; it may analogues of trade drugs available in the market. They modified the molecule by substituting replacing the substituent of known molecule and design their analogues *in silico* screening find out their biological potential. It is the way to saves money and time of researchers in this computer age. *In vitro* antimicrobial studies of synthesized molecules are the major part of our research. Now a day's resistance to antibiotic drugs causes severe problems to community and hospitalised patients as well as healthy persons. Major causes of AMR problem is due to population, industrialisation, pollution and deforestation, new microorganisms are continuously producing and cause diseases such as swine flu, antiviral infection, and asthma in short infectious diseases increased day by day. To fight against these harmful microorganisms there was continuous and consistent effort of scientist to develop new molecules which could be used for the treatment of infections. For example in 1976 due to over use of penicillin bacterial strain such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* acquire resistance to overcome this clavulanic acid used as antibiotics, to fight against these microorganisms. AMR problems started from 1960s and new term introduced i.e. MRSA - methicilline resistant *S.aureus* but this term is wide applied to penicillinase resistant penicillins not just methicilline antibiotic used in hospital to treat bacterial infections [2-4]. As a result the third generation cephalosporin, cefotoxins, cefuroxime, cefazoline synthesized antibiotics as analogues of penicillins are used in antimicrobial therapy. These antibiotics are used as reserve troops for troublesome infections which do not respond to the more commonly prescribed β-lactums [5] One of these list cefazolin as a antibiotic containing 1,3,4-thidiazole ring and it has promoted us to synthesized the derivatives of 1,3,4-thidiazole, 1,3,4-oxadiazole and 1,2,4-triazole moieties. Antimicrobial activity evaluation of synthesized derivatives are applied part of our synthetic research.

2. MATERIALS AND METHODS

Sample of synthesized derivatives were completely dried and purified labelled as per requirement of process. Homogeneous solution about 500µg/mL of each sample made separately in DMSO which acts as negative control. Total 08 samples are investigated under this treatment by *Kirbey Bauer* Method. NCIM provides pure, non-pathogenic, viable and authentic cultures with their standard code number and testing symbols are mentioned below;

Gram positive bacterial strains *Staphallococcus aureus* (Sa- 2178) *Bacillus subtilis* (Bs-2239)
Gram negative bacterial strains *Eschereschia coli* (Ec-25744) Klibesiella aerogenus (Ka-2249)
Antifungal strains are *Aspergillus niger* (An-504) and *Penicillium Chrysogenum*(Pc-709)

The ratio between the diameter of the inhibition zones (mm) produced by sample solution and the inhibition zones around the disc with standard ciprofloxacin (mm) was used to express antibacterial activity and fluconazole inhibition zone used to measure antifungal activity.

Activity index is equal to the ratio of Inhibition zone of the compound to the inhibition zone of standard into 100. It is formulated as

$$\text{Activity index} = \frac{\text{Inhibition zone of the compound}}{\text{Inhibition zone of the standard}} \text{ X } 100$$

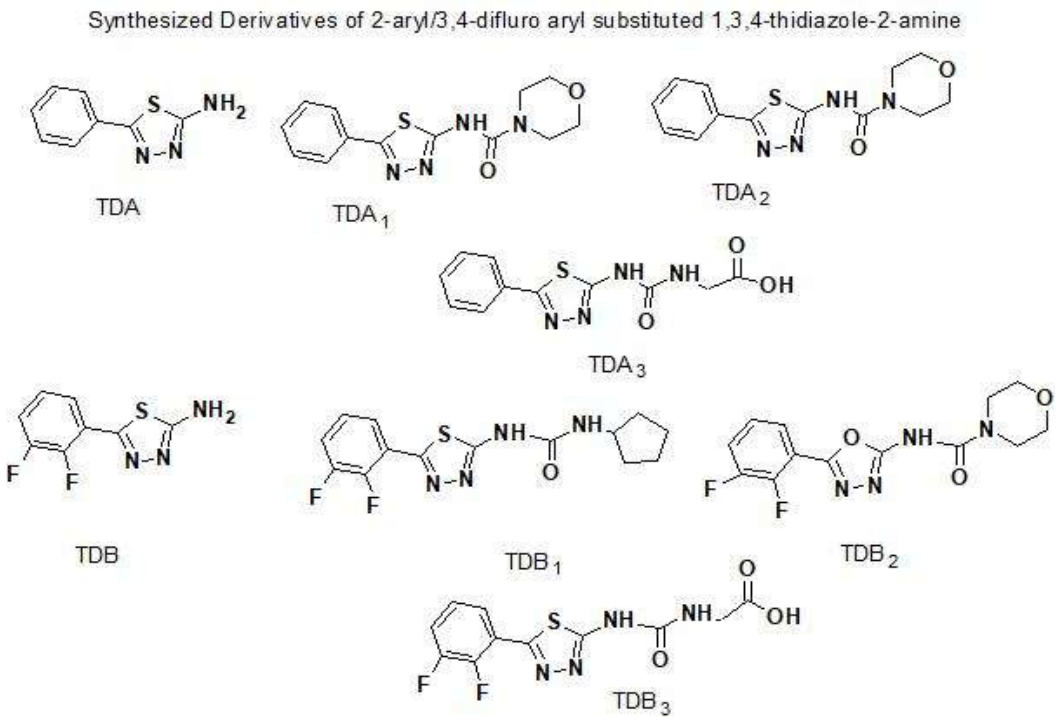
A set up of dilutions (sample solutions) ranging from 25 µg/mL to 200 µg/mL and for another series it was from 250 µg/mL to 750 µg/mL. Ciprofloxacin and fluconazole were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulfoxide with dilution of 1:10 was used as solvent control [6-7].

2.1. Software and program

Chemsketch was used to draw the ligand compounds. Accelry's Discovery studio v4.0 and Schrodinger's maestro visualization program v9.6 [8-10] were utilized to visualize the protein-ligand structures, H-bonds, measurement of bond lengths and to render images. Manual Pharmacophore hypothesis generation module of Schrodinger's maestro v9.6 was used for pharmacophore features mapping of the compounds along with location and calculation of distance between the pharmacophore features. MGL Tools version 1.5.6 was used for the preparation of the ligands and protein receptors in pdbqt format and to visualize and estimate the grid box size for docking calculations. Autodock 4.0 **[**11-13**]** is the software used for the docking calculation.

3. RESULTS AND DISCUSSION

Synthetic methods and characterization of these derivatives were reported in published research papers [14-18].

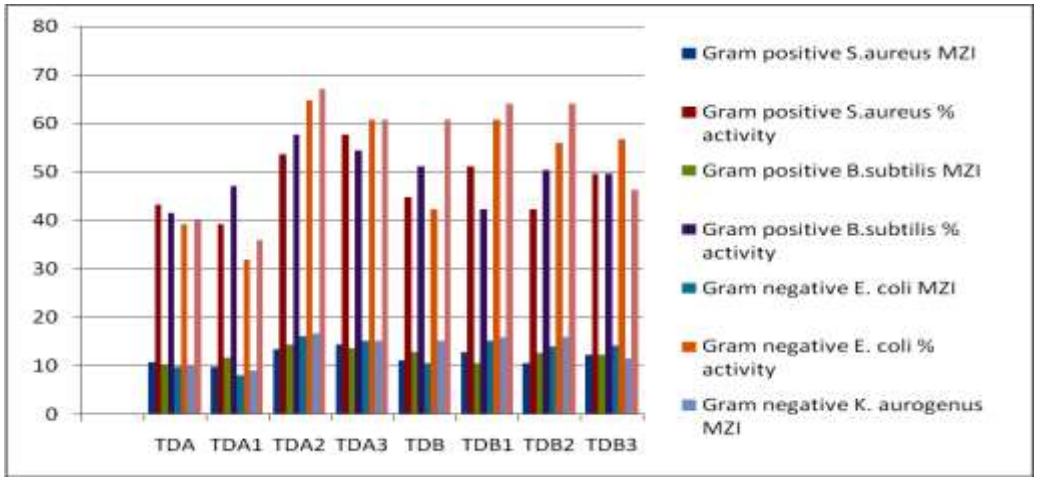


Scheme 1. Synthesis of 2-aryl/3,4-difluro aryl substituted 1,3,4-thidiazole-2-amine

Table 1. Antibacterial screening of the synthesized derivatives.

Sr. No.	Comp code	Gram positive				Gram negative			
		S.aureus		***B.subtilis***		***E. coli***		***K. aurogenus***	
		MZI (mm)	**% activity**	**MZI (mm)**	**% activity**	**MZI (mm)**	**% activity**	**MZI (mm)**	**% activity**
1	**TDA**	**10.8**	**43.2**	**10.4**	**41.6**	**9.8**	**39.2**	**10**	**40**
2	**TDA_1**	**9.8**	**39.2**	**11.8**	**47.2**	**8**	**32**	**9**	**36**
3	**TDA_2**	**13.4**	**53.6**	**14.4**	**57.6**	**16.2**	**64.8**	**16.8**	**67.2**
4	**TDA_3**	**14.4**	**57.6**	**13.6**	**54.4**	**15.2**	**60.8**	**15.2**	**60.8**
5	**TDB**	**11.2**	**44.8**	**12.8**	**51.2**	**10.6**	**42.4**	**15.2**	**60.8**
6	**TDB_1**	**12.8**	**51.2**	**10.6**	**42.4**	**15.2**	**60.8**	**16**	**64**
7	**TDB_2**	**10.6**	**42.4**	**12.6**	**50.4**	**14**	**56**	**16**	**64**
8	**TDB_3**	**12.4**	**49.6**	**12.4**	**49.6**	**14.2**	**56.8**	**11.6**	**46.4**

Bar graphs are plotted to explain % zone of inhibition and minimun zone of inhinbition to discuss the activity of compound against selected bacteria shown as below:



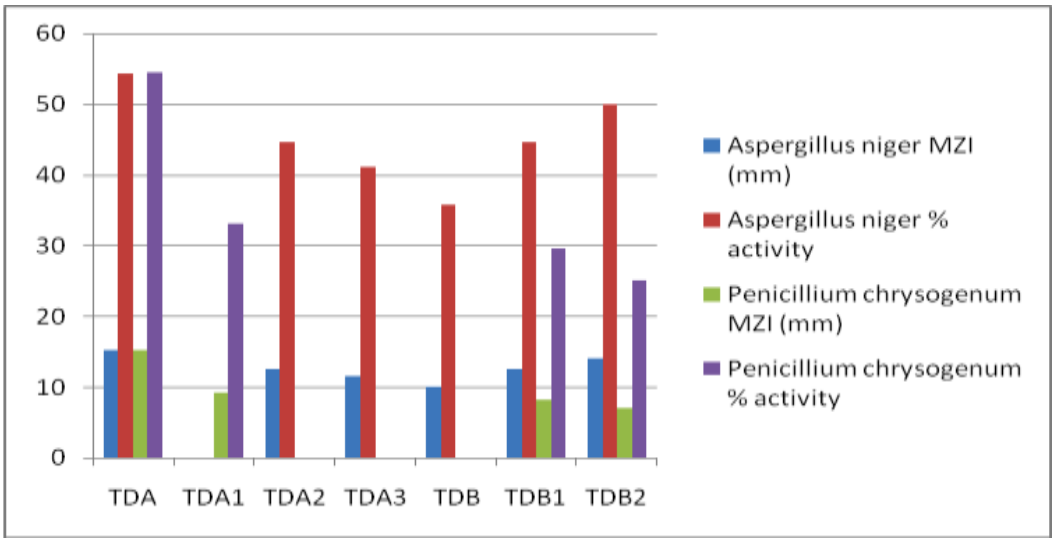
Graph 1. % zone of inhibition and minimun zone of inhinbition

Table 2: Antifungal screening of the synthesized derivatives.

Sr. No.	comp. code	*Aspergillus niger*		*Penicillium chrysogenum*	
		MZI (mm)	**% activity**	**MZI (mm)**	**% activity**
1	**TDA**	**15.2**	**54.28**	**15.25**	**54.46**
2	**TDA_1**	**…**	**…**	**9.25**	**33.10**
3	**TDA_2**	**12.5**	**44.64**	**…**	**…**
4	**TDA_3**	**11.5**	**41.10**	**…**	**…**

5	**TDB**	**10**	**35.7**	**..**	**..**
6	**TDB_1**	**12.5**	**44.64**	**8.25**	**29.50**
7	**TDB_2**	**14**	**50**	**7**	**25**
8	**TDB_3**	**12.5**	**44.64**	**9.75**	**34.82**

Bar graphs are plotted to explain % zone of inhibition and minimun zone of inhinbition to discuss the activity of compound against selected fungi shown as below:



Graph 2. % zone of inhibition and minimun zone.

Table 3: Docking results of Compounds targeting DHFR (PDB ID: 3Q1H), DNA Gyrase (PDB ID: 2XCT), Topoisomerase IV (PDB ID: 3FV5), S-adenosyl homocysteine nucleosidase (PDB ID: 4YML) for anti-bacterial activity:

	DHFR (PDB ID: 3Q1H)		DNA Gyrase (PDB ID: 2XCT)		Topoisomerase IV (PDB ID: 3FV5)		S-adenosyl homocysteine nucleosidase (PDB ID: 4YML)	
Compound Name	**Binding Energy in Kcal/mol**	**Predicted IC50 valueuM**	**Binding Energy in Kcal/mol**	**Predicted IC50 value nM**	**Binding Energy in Kcal/mol**	**Predicted IC50 valueuM**	**Binding Energy in Kcal/mol**	**Predicted IC50 value**
TDA	-4	5.24	-4.5	432.76	-4.4	3.34	-4.7	9.59
TDA_1	-6.4	3.56	-6.8	124.57	-6.4	1.97	-6.6	6.98

TDA_2	-6.9	2.29	-6.8	124.31	-5.6	2.79	-5.8	8.13
TDA_3	-5.5	4.73	-5.6	283.18	-5.4	2.85	-5.1	8.86
TDB	-4.6	4.98	-4.7	362.94	-4.4	3.32	-5.3	8.6
TDB_1	-6.3	3.72	-6.7	129.71	-5.8	2.54	-5.5	8.48
TDB_2	-6.6	2.79	-6.8	124.28	-6	2.34	-5.8	8.13
TDB_3	-5.7	4.55	-5.9	213.68	-5.7	2.67	-5	9.13

Table 4: Docking results of Compounds targeting Penicillin Binding Protein (PDB ID: 1VQQ), Lanosterol 14 α-demethylase (PDB ID: 4LXJ) for anti-fungal activity.

S.No	Compound Name	Penicillin Binding Protein (PDB ID: 1VQQ)		Lanosterol 14 α-demethylase (PDB ID: 4LXJ)	
		Binding Energy in Kcal/mol	**Predicted IC50 value**	**Binding Energy in Kcal/mol**	**Predicted IC50 value**
1	**TDA**	-4.3	6.68	-4	1.28
2	**TDA_1**	**-6.2**	4.56	-8.4	422.3
3	**TDA_2**	-6.4	4.32	-8.5	391.8
4	**TDA_3**	-5.3	5.48	-6.4	812.6
5	**TDB**	-4.6	6.35	-4.8	1.12
6	**TDB_1**	-6.1	4.68	-8.4	422.3
7	**TDB_2**	-6.4	4.32	-8.6	383.4
8	**TDB_3**	-5.4	5.36	-6.5	782.5

The antibacterial activity of synthesized derivatives and parent molecule (08) has been screened against selected above mentioned microorganism. These compounds showed strong activity against standard drug ciprofloxacin. TDB_3 has 50-60 % zone of inhibition against *Staphylococcus aureus* while TDA series exhibited more antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*. The % of activity and zone of inhibition against selected microorganism which is tabulated in table 1 at the same plotted bar graph against each microorganism has explained the antibacterial activity. *In vitro* study we can conclude that derivatives acts as potential active at MIC 50 µg/ mL to 200 µg/ mL against selected strain showed better results. The evaluation report resulted that the 08 compound tested against bacterial species are better but less active compare to std.drug ciprofloxacin. The antibacterial activity was found to be increased for particular substituent as morpholine, piperazine, amino acid glycine.

The antifungal activities has been screened against antifungal strains are *Aspergillus niger* and *P. Chrysogenum* against standard antibiotic fluconazole. The compounds of TDA series are active against *A.niger* except TDA_2 which has phenyl and morpholine substituent. TDA_1, TDA_3 and

TDB inactive against *P. Chrysogenum* .TDA$_3$and TDB is inactive against *P. Chrysogenum*. Target molecule TZA and TZB are inactive against fungal strain. It may be due to the presence of free NH_2 exert which may cause solvation effect and p^H.

Synthesized compounds targeting selected anti bacterial drug and anti fungal drug targets:

In order to evaluate the anti-bacterial activity of the synthesized compounds has been screened in to four different drug targets (Dihydrofolate reductase (DHFR); DNA Gyrase; Topoisomerase IV; S-adenosyl homocysteine nucleosidase) for anti-bacterial infections and Pencillin Binding Protein (PDB ID: 1VQQ) & Lanosterol 14 α-demethylase (PDB ID: 4LXJ) for anti-fungal activity[19-21]. Results from the docking studies predicted IC_{50} value range of 5.24 micro molar to 1.41 micro molar levels with binding energy in a range of -4.00 to -8.00 Kcal/mol for DHFR drug target respectively. IC_{50} value range of 432.76 nano molar to 20.02 nano molar levels with binding energy in a range of -4.50 to -8.50 Kcal/mol for DNA Gyrase drug target respectively (Table 8); IC_{50} value range of 3.32 micro molar to 1.38 micro molar levels with binding energy in a range of -4.4 to -7.0 Kcal/mol for Topoisomerase IV drug target respectively.IC_{50} value range of 9.59 micro molar to 5.66 micro molar levels with binding energy in a range of -4.7 to -7.8 Kcal/mol for S-adenosyl homocysteine nucleosidase drug target respectively .

The docking studies predicted IC_{50} value range of 2.55 micro molar to 6.68 micro molar levels with binding energy in a range of -4.30 to -8.10 Kcal/mol for Pencillin Binding Protein drug target respectively.IC_{50} value range of 1.28 micro molar to 137.50 nano molar levels with binding energy in a range of -4.00 to -10.00 Kcal/mol for Lanosterol **14** α-**demethylase** drug target respectively

4. CONCLUSION

In the present work we report the complete synthesis along with structural characterization of 08 compounds for 1, 3, 4-thiadiazole core nucleus. These compounds were undertaken for *in silico* investigations. It is interesting to note that the compounds from 1,3,4-thiadiazole series exhibited better binding interactions and gave rise to more stable systems with the target molecules.

The *in vitro* screening was also carried out for all the 08 compounds to validate the predictions made by *in silico* investigation. It is worth mentioning that in vitro screening also led to similar pattern of activity as that of *in silico* studies. They are more potent against all micro-organisms considered for the said experiments. However, it is also notable observation that gram positive species of *S. aureus* was affected to a larger extent than the other micro-organisms. These findings warrant further investigations to explore mechanistic details to under the actual mode of action of these compounds.

5. ACKNOWLEDGEMNT

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