Synthesis, characterisation and Docking studies of 6-(4-fluorophenyl)-8-(5-(4-nitrophenyl)furan-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazepines

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Abstract:

A series of 6-(4-fluorophenyl)-8-(5-(4-nitrophenyl)furan-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazepines were synthesised and screened these molecules for antimictobial activity and it was tested for cytotoxicity test and Docking scores for all the compounds are quite good against all the proteins. Among them, compounds 1 and 2 show best activities, having either the highest or second highest docking scores among the molecules. Compounds 1 and 2 can be potential leads as anti bacterials.

Introduction :

Traditional interest in pyrazoles stemmed from their use as drugs, dyes and anaesthetics. Pyrazoles themselves can be used as antioxidants in fuels, but their major practical applications have been in medical and agriculture fields. Pyrazole derivatives have been widely used as bacteriostats,bacteriocides,insecticides, fungicides,amoebicides, hypoglycemic, sedative, anticarcinogens, hypnotic, trichomonacidal and psychopharmacological agents.

Several books and reviews on pyrazoles and their derivatives have appeared. A recent one is that of Grimmett [1] in 'Comprehensive organic chemistry'. Jacobs' review [2] was the leading reference for many years and a permanent source of research ideas. Applications of pyrazoles derivatives as pharmaceuticals, agrochemicals, dyestuffs, analytical reagents and as plastics was reviewed by Elguero ins [3] 1984. Some of the most important pyrazoles with biological activity are shown in the table. Pyrazole itself and several N- substituted pyrazoles are inhibitors and deactivators of liver alcohol dehydrogenase [4-7] .

1. Materials and methods

Here we synthesised chalcones by using aromatic ketones and substituted aldehyde in the presence of Acid as a catalyst ,we obtained propenones,again on debromination in the presence of drybenzene and triethylamine we obtained Acetylinic ketone ,and it is made to react with triazole in the presence of alcohol medium to obtain thiazadapines .

Experimental section :

Thin layer chromatography was used to analyse the reaction progress and purity of the compounds synthesized. Melting points were determined in open glass capillary methods and were uncorrected. IR spectra were obtained in KBr discs on a Shimadzu- 8400 IR spectrophotometer, 1H NMR spectra were recorded on Brucker spectrometer (400 MHz) in DMSO-d6/CDCl3 using TMS as an internal standard, Mass spectra were recorded on Agilent 6320 Ion Trap method

Procedure for synthesis of propenones (1)

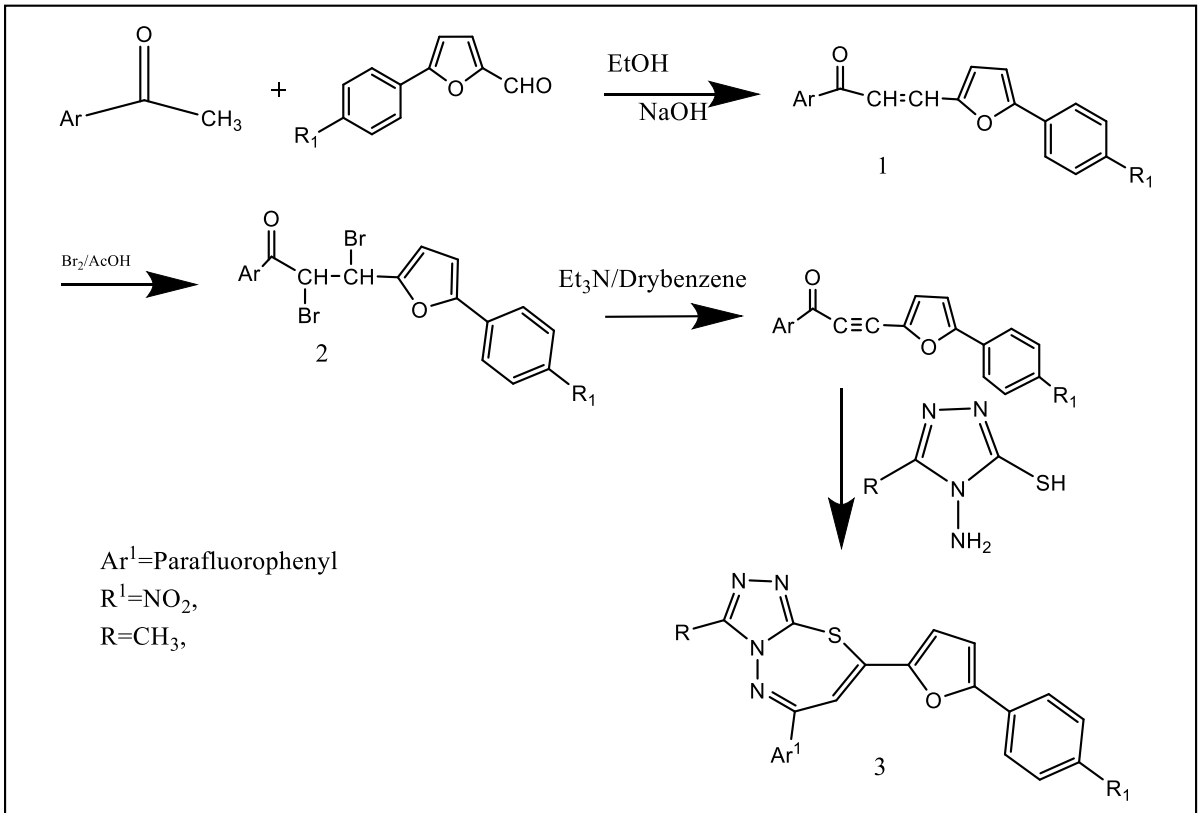
A solution of 5-(p-halo-phenyl-2-furfuraldehyde (0.01mol) and an appropriate acetophenone (0.01 mol) in ethanol (20ml) was cooled to 5-100C in ice bath. The cooled solution was treated with dropwise addition of aqueous sodium hydroxide (5ml, 50%) the reaction mixture was magnetically stirred for 30 minutes and allowed to stand at room temperature for 24 hours. The precipitated crystals of propenones were collected by filtration, washed with ethanol dried and recrystallized from alcohol.

2. Synthesis of dibromopropenones (2)

propen-1-ones (0.01mol) was dissolved in glacial acetic acid (10ml) by gentle warming. A solution of bromine in glacial acetic acid was added to it with constant stirring until the yellow colour of bromine persisted. The reaction mixture was kept aside at room temperature for overnight. Crystals of dibromo propanone separated were collected by filtration and washed with ethanol. It was dried and recrystallised from Alcohol.

2. Synthesis of thiazadapines by using acetylenic ketone (3)

Triazole (0.01mol), acetylenic ketone (0.01mol) and concentrated sulphuric acid in the presence of ethanol(25ml) were refluxed on a water bath for 1-2 hours. Yellow crystals separated pit pm cooling were collected by filtration and recrystallised from dioxane.



Scheme of 8-(5-(4'-fluoro-[1,1'-biphenyl]-4-yl)furan-2-yl)-6-(4-fluorophenyl)-3-methyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepine

Figure1: scheme of Synthesis of thiazadapines by using acetylenic ketone

Table 1: Perentage yield and molecular weight of compounds

compound No,	**R**	**Ar[1]**	**R[1]**	**Yield % m.pt in[0] C**	**Colour of crystals**	**Mol wt/ mol formula**
1	methyl	4-fluoro-phenyl	4-fluoro l	60 146^0C	Yellow powder	420 $C_{22}H_{14}N_4SOF_2$
2	methyl	4-fluoro-phenyl	4-chloro	73 132^0C	Yellow crystals	436.5 $C_{22}H_{14}N_4SOClF$
3	methyl	4-fluoro-phenyl	4-bromo -	75 110^0C	Yellow crystals	481.0 $C_{22}H_{14}N_4SOBrF$
4	methyl	4-fluoro-phenyl	4-nitro-	69 200^0C	Light orange crystals	447 $C_{22}H_{14}N_5SO_3F$
5	ethyl	4-fluoro-phenyl	4-fluoro	81 202^0C	Yellow crystals	434 $C_{23}H_{16}N_4SOF_2$
6	ethyl	4-fluoro-phenyl	4-chloro-	55 102^0C	Yellow flakes	450.5 $C_{23}H_{16}N_4SOClF$

Spectral values of synthesised compounds

Compound 1

H^1 NMR (400 MHz,) :δ 8.39 – 7.66 (m), 7.66 – 7.55 (m),6.7-6.45 (m) 4.44 – 4.37 (m), 2.72-2.53 (m), 1.13 – 0.18 (m).
m/z=420(M^++1)

Compound 2:

H^1NMR: δ 8.37 – 7.64 (m), 7.62 – 7.50 (m), 6.75-6.44 (m) 4.46 – 4.374(m), 2.73-2.51 (m), 1.10 – 0.14 (m).
m/z=436.5(M^++1)

Compound 3:

H^1NMR: δ 8.28 – 7.52 (m), 7.69 – 7.48 (m), 6.66-6.42 (m) 4.48 – 4.34(m), 2.69-2.49 (m), 1.08 – 0.09 (m).
m/z=481(M^++1)

Compound:4

H^1NMR: δ 8.21 – 7.48 (m), 7.64 – 7.43 (m), 6.64-6.38 (m) 4.43 – 4.31(m), 2.65-2.45 (m), 1.06 – 0.08 (m).

m/z=447(M^++1)

Compound 5:

H^1NMR: δ 8.28 – 7.52 (m), 7.69 – 7.48 (m), 6.66-6.42 (m) 4.48 – 4.34(m), 2.69-2.49 (m), 1.08 – 0.09 (m).

m/z=434(M^++1)

Compound 6:

H^1NMR: δ 8.06 – 7.46 (m), 7.62 – 7.41 (m), 6.62-6.39 (m) 4.42 – 4.31(m), 2.62-2.43 (m), 1.06 – 0.07 (m).

m/z=450.5(M^++1)

Biological Activity

Evaluation of antibacterial activity.

Determination of minimum inhibitory concentration by tube dilution method [8]

Antibacterial activity as MIC is evaluated by tube dilution method. Double strength Nutrient broth was prepared and autoclaved. Prepared various concentration of stock solution using serial dilution method (100- 1.56 mcg/ml).

Each 2.5ml of this solution double strength nutrient broth 2.5ml is added.

Microorganism was inoculated (E. coli) in all test tubes were incubated at 37ºC for 48h

Table 2: Minimum Inhibitory Concentration of compounds

Compounds	**Minimum Inhibitory Concentration (mcg/ml)**
1	**12.5**
2	**12.4**
3	24
4	45
5	48
6	25

The given compounds showed promising antimicrobial activity. The minimum

inhibitory concentration achieved by the compounds was in the range of 12.5 to

50 mcg/ml. Compound 1and 2 exhibited maximum activity with minimum inhibitory concentration (mic) of 12.5 mcg/ml.

Determination of zone of inhibition by cup-plate method. [9]

In this technique, melted agar inoculated with microorganisms is poured into Petri dishes. Wells were made in the agar plate and a specific volume of the antimicrobial substances were placed in them; plates were incubated at 37°C for the specified time. The antimicrobial substance diffuses through agar around its well and produces a clear zone of inhibition. The diameter of this zone gives an estimation of the degree of activity of the antimicrobial substance.

Table 3: Zone of inhibition.

Std:Ciprofloxacin

Compounds	Std (ciprofloxacin 5mcg)	C1	C2	C3	C4	C5	C6
Zone of inhibition (mm)	32mm	31.0±0.033	29.07±0.106	19.26±0.12	12.00±0.135	10.74±0.031	22.06±0.65

All the six samples at MIC concentration exhibited antimicrobial activity in the zone of inhibition studies carried out. Compound 1and 2 showed maximum activity which was comparable to the standard drug ciprofloxacin (5mcg).

Docking Studies:

Introduction

Molecular docking is used as a useful in silico tool to identify possible leads as useful drugs. [8] The proteins selected for molecular docking are common anti-bacterial targets[9], found across many bacterial families. Penicillin Binding Proteins are a set protein which are active targets of common penicillins. They are involved in the cross linking of peptidoglycans during cell division [10]. All penicillins like piperacilin, benzylpenicillin etc., and ceftazidine bind to these proteins. Dihydrofolate reductase (DHFR) catalyses the hydrogenation of dihydrofolate to tetrahydrofolate [11]. It is an important enzyme in the biosynthetic pathway of folate synthesis. Trimethoprim is a useful DHFR inhibitor [12]. Tyrosine tRNA ligase is an aminoacyl tRNA ligase, which is important in protein synthesis [13]. A number of new inhibitors for this protein have been developed, as the bacterial and human tyrRS enzymes are quite different [14]. Glutamine Synthatase is involved in the synthesis of glutamine from glutamic acid and ammonia, and is involved in many important cellular processes [15]. Never antibacterials developed are important GS targets. [16,17]

Materials and Methods

Ligand Preparation

The ligands Comp1-6 was drawn using Discovery Studio Visualiser 202111 and saved as SDF files. The structures were minimised using the MMFF94 forcefield using OpenBabel, for 10000 steps using steepest descent algorithm12. The ligands were saved as SDF files and converted to Autodock PDBQT files using OpenBabel. Polar Hydrogens and charges were added while converting to PDBQT. The ligand files were visualised and checked for errors using Discovery Studio 2021.

ADME and Toxicology Calculations

To predict the Absorption, Distribution, Metabolism and Excretion properties of the ligands, SwissADME13 web tool was used. The Toxicology studies were taken from pkCSM webserver14.

Protein Preparation

The proteins were downloaded from the RCSB PDB website15. The PDB files were visualised using Discovery Studio, and then loaded into the PyRx software, which automatically converted the PDB files to PDBQT files, with polar Hydrogens and Kollmann Charges added. The substrates/known inhibitors of the proteins were downloaded from PubChem database16.

Molecular Docking

The Autodock Vina17 module in PyRx was used for molecular docking of all proteins, one by one, against its own substrate/inhibitor and the nine ligands. The docking was performed at the active sites of each of the proteins, with an exhaustiveness of 16. The Inhibitory constant Ki was calculated as Ki=exp(ΔG/RT), where ΔG is the Binding Affinity obtained as Vina Score, R is the universal gas constant (=1.987×10-3 kcal mol-1 K-1) and T is the absolute temperature (298.15K). All the computational studies and visualisations were done using Discovery Studio Visualiser 2021.

Results and Discussion

Table 4: Protein Structures

PDB ID	Protein Name	Function	Organism	Inhibitor/Substrate
3ocn	Penicillin Binding Protein 3	Cell wall Synthesis	P. aeruginosa	Piperacilin(PIP), Ceftazidine(CTJ)
3srw	Dihydrofolate Reductase	Folic acid metabolism	S. aureus	Trimethoprim
1vbm	Tyrosine tRNA Ligase	Aminoacyl tRNA synthesis	E. coli	Ligand-SB219383
4s0r	Glutamine Synthatase	Amino acid metabolism	B. subtilis	Glutamine

Table 5: Description of the proteins used for molecular docking

ADMI

Molecule	Comp1	Comp2	Comp3	Comp4	Comp5	Comp6
#Heavy atoms	30	30	30	32	31	31
#Aromatic heavy atoms	22	22	22	22	22	22
#Rotatable bonds	3	3	3	4	4	4
#H-bond acceptors	6	5	5	7	6	5
#H-bond donors	0	0	0	0	0	0

Molecular Refractivity	114.58	119.64	122.33	123.45	119.39	124.44
Topological Polar Surface Area	81.51	81.51	81.51	127.33	81.51	81.51
MLOGP	4.55	4.66	4.76	3.26	4.76	4.87
ESOL Log S	-6.05	-6.48	-6.8	-5.95	-6.32	-6.76
ESOL Solubility (mg/ml)	0.000376	0.000143	7.67E-05	0.000507	0.000207	7.86E-05
ESOL Solubility (mol/l)	8.94E-07	3.28E-07	1.59E-07	1.13E-06	4.76E-07	1.74E-07
ESOL Class	Poorly soluble	Poorly soluble	Poorly soluble	Moderately soluble	Poorly soluble	Poorly soluble
GI absorption	High	High	High	Low	High	High
BBB permeant	No	No	No	No	No	No
Pgp substrate	No	No	No	No	No	No
CYP1A2 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes
CYP2C19 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes
CYP2C9 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes
CYP2D6 inhibitor	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	No
Skin Permeability(log Kp (cm/s))	-5.19	-4.92	-5.15	-5.55	-4.97	-4.7
Lipinski #violations	1	1	1	0	1	1
Bioavailability Score	0.55	0.55	0.55	0.55	0.55	0.55
PAINS #alerts	0	0	0	0	0	0

Table 6: ADME Prediction of the compounds 1-6.

Toxicity Prediction

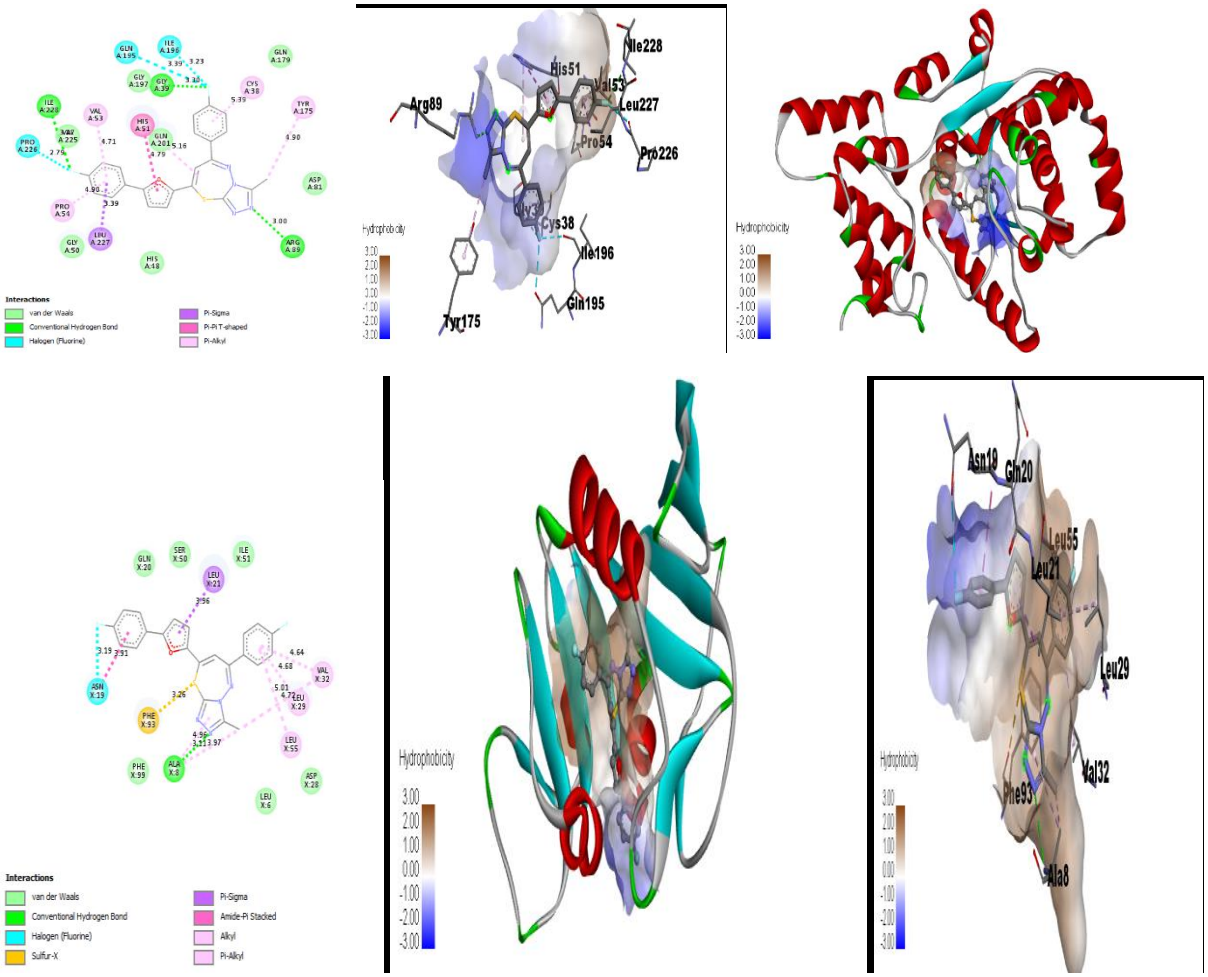
Compound	**Unit**	**Comp 1**	**Comp 2**	**Comp 3**	**Comp 4**	**Comp 5**	**Comp 6**
AMES toxicity	Categorical (Yes/No)	No	No	No	Yes	No	No
Max. tolerated dose (human)	Numeric (log mg/kg/day)	0.248	0.144	0.149	0.466	0.427	0.33
hERG I inhibitor	Categorical (Yes/No)	No	No	No	No	No	No
hERG II inhibitor	Categorical (Yes/No)	Yes	Yes	Yes	Yes	Yes	Yes

Oral Rat Acute Toxicity (LD50)	Numeric (mol/kg)	2.64	2.578	2.584	2.968	2.797	2.72
Oral Rat Chronic Toxicity (LOAEL)	Numeric (log mg/kg_bw/day)	0.926	0.811	0.801	0.767	0.774	0.66
Hepatotoxicity	Categorical (Yes/No)	No	No	No	No	No	No
Skin Sensitisation	Categorical (Yes/No)	No	No	No	No	No	No
T.Pyriformis toxicity	Numeric (log ug/L)	0.29	0.292	0.292	0.286	0.29	0.292
Minnow toxicity	Numeric (log mM)	-2.102	-2.65	-2.796	-3.689	-2.053	-2.601

Table 7: Docking Results

	Proteins							
	1vbm		**3ocn**		**3srw**		**4s0r**	
Ligand	**Binding Energy (kcal/mol)**	**Inhibitory Constant Ki(nM)**	**Binding Energy (kcal/mol)**	**Inhibitory Constant Ki(nM)**	**Binding Energy (kcal/mol)**	**Inhibitory Constant Ki(nM)**	**Binding Energy (kcal/mol)**	**Inhibitory Constant Ki(nM)**
Comp1	**-10.6**	**16.96**	**-9.7**	**77.47**	**-11.4**	**4.39**	**-8.4**	**695.22**
Comp2	**-10.4**	**23.77**	**-9.8**	**65.44**	**-11.2**	**6.16**	**-8.4**	**695.22**
Comp3	-10.4	23.77	-9.8	65.44	-11.0	8.63	-7.7	2266.11
Comp4	-10.3	28.14	-9.6	91.71	-11.3	5.20	-8.5	587.24
Comp5	-10.0	46.69	-9.7	77.47	-11.4	4.39	-8.2	974.41
Comp6	-9.9	55.27	-9.7	77.47	-11.2	6.16	-8.2	974.41
LigSB	-8.6	496.03	-	-	-	-	-	-
CTJ	-	-	-8.2	974.41	-	-	-	-
PIP	-	-	-9.5	108.58	-	-	-	-
TRI	-	-	-	-	-7.5	3176.12	-	-
GLN	-	-	-	-	-	-	-5.7	66289.01

Compound:1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Pi-Sigma
Pi-Pi T-shaped
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Gly37
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Interactions
van der Waals
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Halogen (Fluorine)
Sulfur-X
Pi-Sigma
Amide-Pi Stacked
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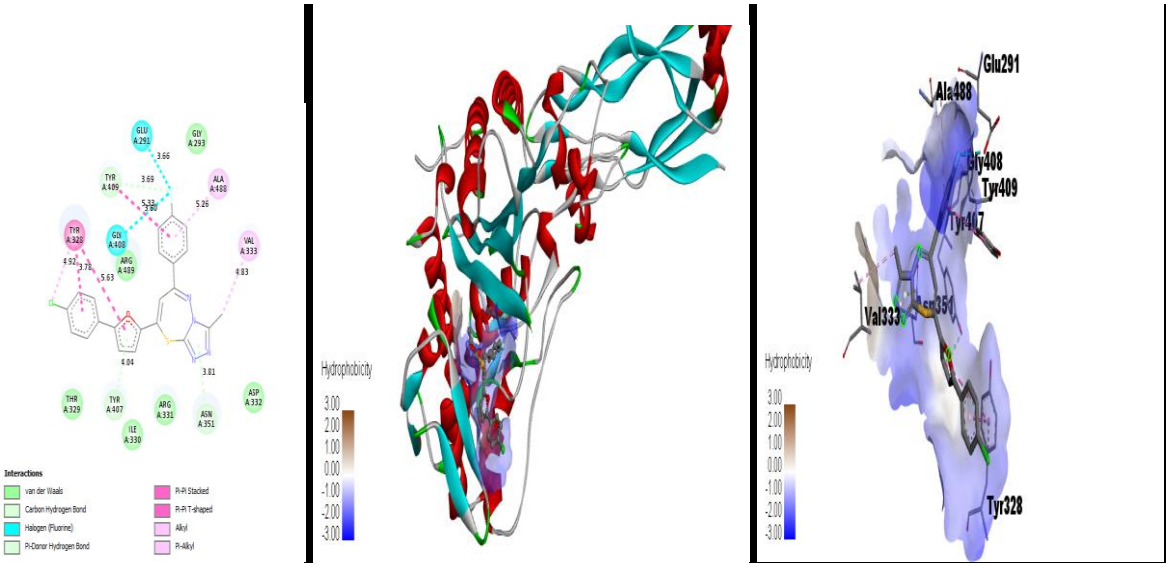
Compound:2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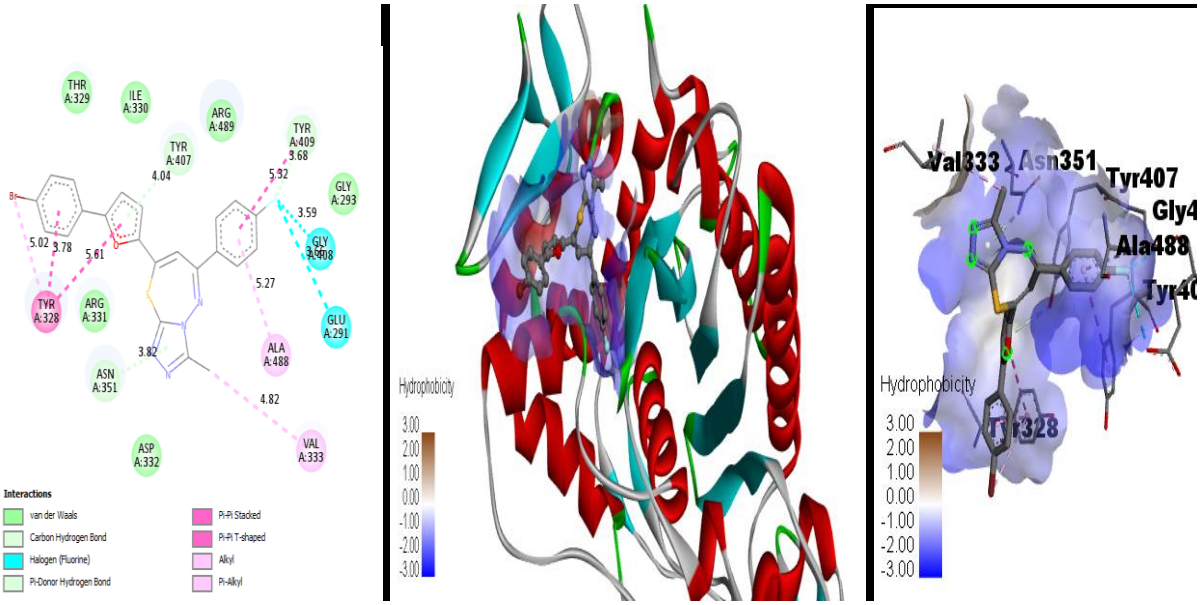
Compound:3



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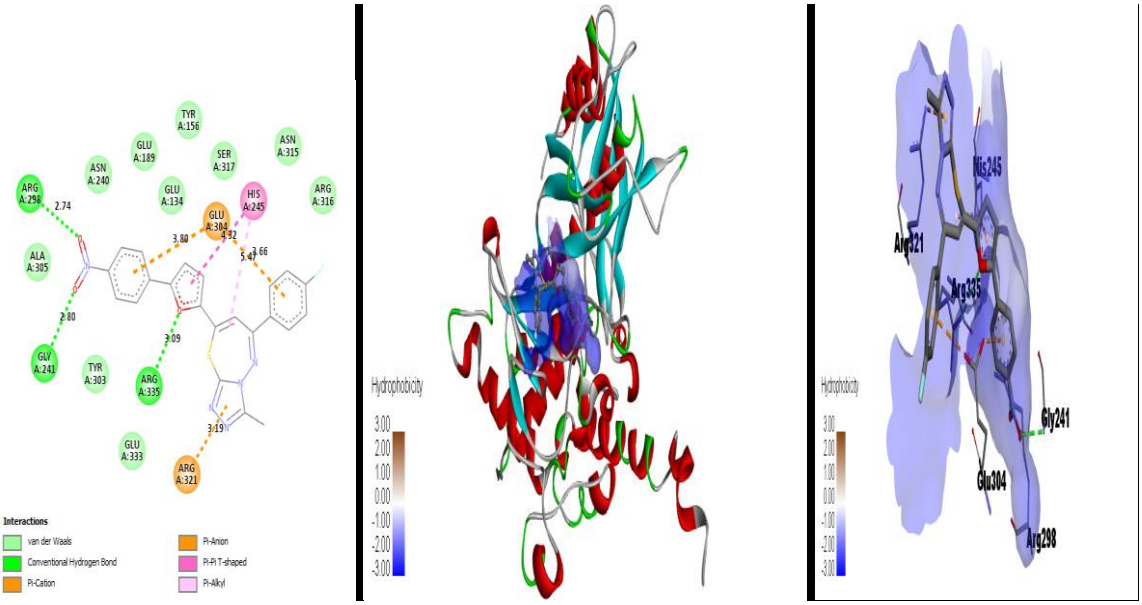

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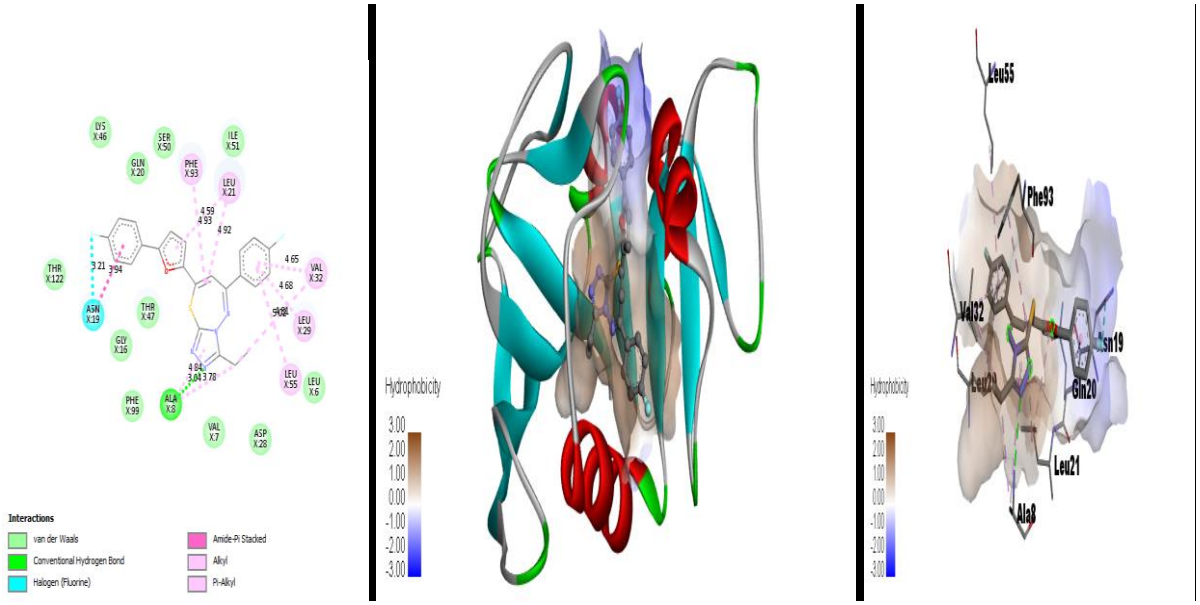

Result and discussion:

A series of 6-(4-fluorophenyl)-8-(5-(4-nitrophenyl) furan-2-yl)-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazepines were synthesised and screened these molecules for antimictobial activity and it was tested for cytotoxicity test and Docking scores for all the compounds are quite good against all the proteins. Among them, compounds 1 and 2 show best activities, having either the highest or second highest docking scores among the molecules. Compounds 1 and 2 can be potential leads as anti bacterials. From spectral studies we confirmed the structures by Mass as well as from NMR values all showed nearly same values in aromatic region.

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Conflict of interest:

No conflict of interests

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