In Silico Molecular Docking against C-KIT Tyrosine Kinase of 2-Isoxazoline Derivatives

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Abstract

2-Isoxazoline derivatives have been well known for their profound biological activities. A number of fluorinated derivatives with variable functional groups attached to the five-membered heterocyclic ring have been synthesized and subjected to molecular docking studies, against C-KIT Tyrosine kinase target protein (1T46) in order to understand their binding properties and variations due to presence of fluorine atom. Molecular docking is a computer software-based study that is used to understand how a drug binds to a protein in the body. The interactions, binding, and affinity variations due to differences in functional groups have been studied using ChemDraw Ultra 7.0, RCSB – Protein Data Bank, BIOVIA Discovery Studio Visualizer 2021, MGL AutoDock Tools, AutoDock Vina and Vina Split software. The docking studies showed good interaction of the designed molecules with the 1T46 target protein.

Keywords

2-Isoxazoline, Fluorine, Bioinformatics, Molecular Docking, C-KIT Tyrosine Kinase

Introduction

In the current era there is an increase in types of diseases, in addition to which new or existing viruses and bacteria are becoming resistant to drug molecules available in the market. For enhanced and effective protection against these diseases there is need for search of new moieties which can target these diseases and help to cure them. Molecular docking is an advantage since the structure of target provides a template for the discovery of novel ligands, which are different to those previously known.[1]

Nowadays structures of proteins and nucleic acids are easily accessible due to which the importance of molecular docking has been increased drastically. The molecular docking is a structure-based drug design method where the major focus is on protein-ligand interaction and provides prediction regarding the mode of binding and the affinity between receptors and ligands. In short, it is the study which displays how effectively the desired ligand fits into the pocket site of the target protein which is governed by the Lock-and-Key and Induced Fit theories for ligand binding. This type of computational technique is capable to improve the efficiency of drug and to reduce the research cost since by using the database the researchers are conveniently able to screen the potential pharmacophores by which they are able to purchase, synthesize and complete follow-up pharmacological tests. So molecular docking technique is widely used in drug design research. By performing docking studies researchers are being able to predict important properties such as, the drug-likeness, examine ligand-protein complex, binding energies, bond length, types of bond, as well as specificity of docking hits.[2-5] Molecular docking studies have gained immense support in the scientific world since it gives an idea regarding what types of molecules can act as potential drugs, without the use of animal models. These studies are used to save several animals such as mice, rats, rabbits and guinea pigs which have been used and sacrificed over the years in the name of scientific studies. These software studies may not be as accurate but do act as guiding forces towards the type of molecular structures the drugs need to possess to act to give the necessary healing actions.

Molecular docking analysis attempts to arrange molecules in favourable configurations via matching complementary features. This is a tricky task as there are numerous ways in which complex molecules can be linked.[3-6] Further, due to an exponential dependence on molecule size, numerous possible configurations are possible during docking involving biological macromolecules such as proteins or nucleic acid polymers, which adds to the difficulties.[7-9]

Currently the importance of small and highly electronegative fluorine atom in drug discovery and development is being realized. The selective addition of fluorine while synthesizing drug molecules can enhance several physicochemical and pharmacokinetic properties such as improved metabolic stability and enhanced membrane permeation. Increased binding affinity of fluorinated drug molecules to target protein has been recognized in various cases. After critical examination of the role of fluorine in medicinal chemistry it has been revealed that substitution of even a single fluorine atom or trifluoromethyl group in a key position of a biologically active organic compound imparts beneficial properties to that compound. At present, various fluorinated compounds are being synthesised regularly in pharmaceutical research which are widely used in the treatment of diseases such as anti-cancer agents, antidepressants, anti-inflammatory agents, anaesthetics and central nervous system drugs.[10,11]

Isoxazoline derivatives[12-16] are an important class of 5-membered oxygen and nitrogen containing heterocycles having profound biological properties. Also, from literature review it was found that this heterocycle could be a potent anticancer agent[17-20]. In addition to this, if these moieties contain fluorine or fluoroalkyl substituents it not only exhibits important pharmacological and agrochemical properties but also act as useful precursors for the synthesis of some biologically potent agents.[21-24] Due to this reason we have decided to perform structure-based ligand design on 2-Isoxazoline derivatives which target kinase protein. In this study we have performed molecular docking on designed moieties as ligand against C-kit tyrosine protein.

Experimental Section

Targeting and suppressing specific protein kinases is gaining prominence as a novel therapeutic approach in the quest to combat cancer. STI-571 (imatinib, sold under the trade names Gleevec and Glivec) functions as a blocker of protein-tyrosine kinases within the Abl group. Imatinib is primarily used in the treatment of certain types of cancer, particularly chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GISTs). So, in our present selected protein STI was pre attached ligand and in this study, we are trying to check cancer potential for our synthesized structures, due to which we have used this protein and replaced STI with our synthesized structures. This was the reason we have selected this protein. Docking studies of all the 2-isoxazoline derivatives **(Figure 1)** were done via Discovery studio docking suite on target protein (PDB ID 1T46) **(Figure 2)**.[10]

Ligand preparation

We have synthesized structures with varied fluorine atoms to investigate the correlation between the number and positioning of fluorine[25] atoms within the moiety and compared there docking scores. Additionally, we explored the impact on docking by altering the positions of the trifluoromethyl group (ortho, meta, para). The structures of the 2-isoxazoline derivatives i.e. ligands **(Figure 1)** were drawn using Chemdraw Ultra 7.0 and saved in PDB format using Biovia Discovery Studio and the ligand was prepared by applying charges using MGL tools.[10]

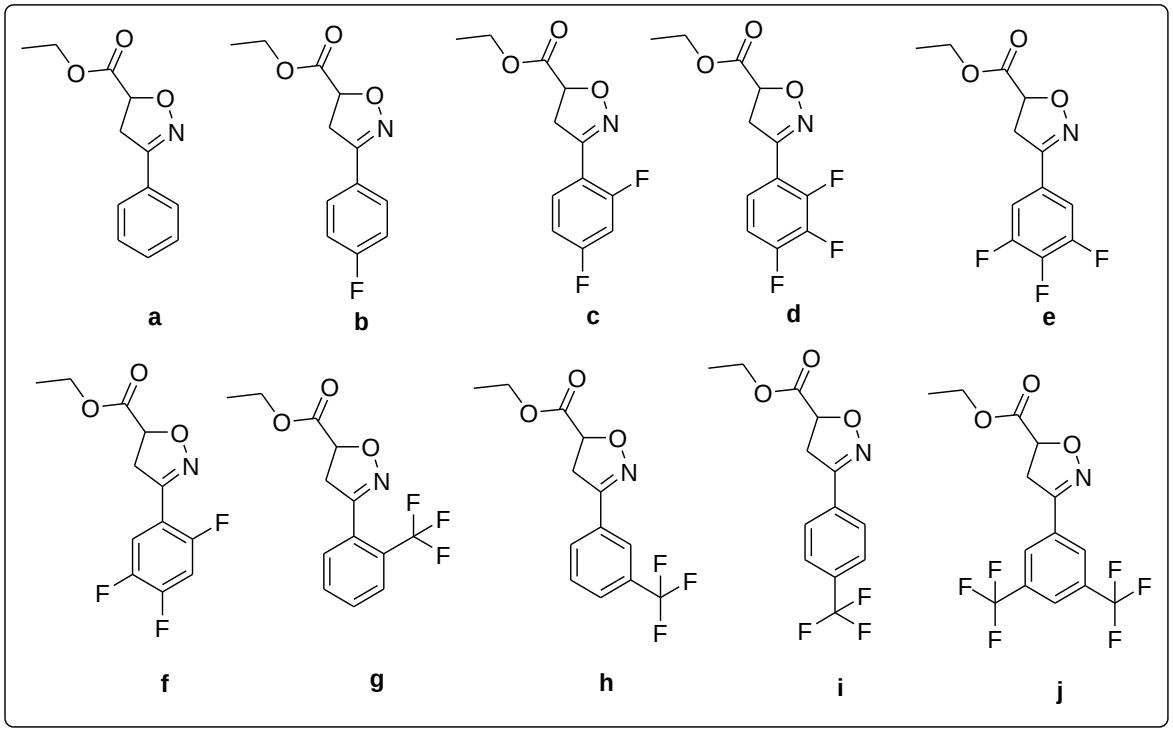

Figure 1: Synthesized novel 2-isoxazoline derivatives

Protein selection & Preparation

The X-ray crystal structure of kinase (PDB ID 1T46) **(Figure 2)** was obtained from pdb database and saved as pdb format for further studies. Selected protein macromolecule was prepared using Biovia Discovery Studio and charges were applied using MGL Tools.[10]

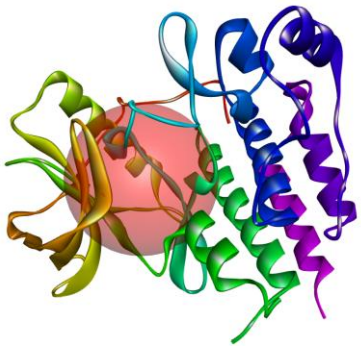

Figure 2: Structure of 1T46, C-KIT Tyrosine Kinase target protein used for Molecular Docking

The figure depicts the structure of the protein **1T46** on which molecular docking of all 10 2-Isoxazoline compounds have been performed.

Docking Studies[26]

In this study, the affinity, and binding modes of the examined molecules against the target protein were determined. Firstly, water molecules were removed from the crystal structures of target proteins, retaining only main-chain amino acids which are essential for binding. The ligands were used as reference ligands to predict the binding pockets. Then, the polar hydrogen atoms were added to protein structures to protonate them. The structures of the examined compounds in PDB formats were opened using MGL AutoDock Tools software for preparation of protein which was selected as macromolecule and then saved in PDBQT format. The configuration file containing receptor name, ligand name, output file name, and X, Y, Z coordinates of the grid box were created. The ligand was prepared and any rotatable bonds if available were added. Next, in Command Prompt with the help of AutoDock Vina software docking process for each target receptor by ligand was carried out by entering necessary codes or commands.

In each case, 9 docked structural poses, affinity and RMSD data were generated using the algorithm. The output from the Vina split software was further analyzed and visualized with the help of BIOVIA Discovery Studio Visualizer 2021.[10]

Results and Discussion

The synthesized 2-isoxazoline derivatives have been docked against kinase (PDB ID 1T46), the best compound was selected as per few criteria such as binding modes, good molecular interactions with the active site components of protein and docking energy at 0.00 RMSD

value. Docking scores and interacting amino acids were depicted in the **Table 1** below. Substituted 2-isoxazoline derivatives **(a-j)** have shown various Pi-Sigma, H-bonding, and halogen interactions towards 1T46 protein molecule. All the affinity values were observed to be within the range of -8.3 to -9.5 kcal/mol with favorable binding poses. One of the amino acids ASP810 in the 1T46 protein showed hydrogen bonding with almost all the compounds. Besides, two more amino acid CYS673 and ASP677 were also involved in H-bonding with various compounds. Among these compounds **h** and **i** exhibit strong interaction with active site amino acids with binding energy of -9.5 and -9.4 kcal/mol respectively towards 1T46 Kinase protein. This indicates that the presence of trifluoromethyl groups in in meta and para positions show the best affinity to the protein with maximum number of interactions. Fluorine atoms directly attached to the benzene rings show lesser interactions.

Compounds	Docking Energy	RMS	No of Interactions	Interaction Residues			No of H Bonds	Bond Length (Å)
				Pi-Sigma	Halogen	H-Bonding		
Doxorubicin	-7.7	0.00	9	VAL643	-	ASP810	2	2.62003
						GLY812		2.95235
A	-8.3	0.00	10	LEU595, LEU799, LEU799	-	ASP810	2	2.1546
						UNK0		3.28066
B	-8.5	0.00	9	LEU595, LEU799	-	ASP810	2	2.14156
						UNK0		3.25818
C	-8.8	0.00	9	LEU595, LEU799	-	ASP810	2	2.19469
						UNK0		3.1813
D	-9.1	0.00	8	THR670	GLU640, GLU640	-	0	-
E	-9.0	0.00	12	LEU595, LEU799	LEU595, CYS673	CYS673	3	2.11907
						ASP810		2.15353
						UNK0		3.29566

f	-9.1	0.00	11	LEU595, LEU799	CYS673	CYS673	3	2.16072
						ASP810		2.26825
						UNK0		3.24061
G	-8.8	0.00	14	LYS623, THR670	GLU640, ASP810, ASP810, UNK0	ASP810	1	2.3568
H	-9.5	0.00	15	LEU595, LEU799	LEU595	LYS623	3	2.60068
						ASP677		2.96075
						LYS623		3.39221
I	-9.4	0.00	14	LEU799, LEU799	LEU595, GLY676	ASP677	3	2.82292
						ASP810		2.25582
						UNK0		3.23812
J	-9.0	0.00	26	LEU799, LEU799	LEU595, LEU595, GLU671, TYR672, TYR672, CYS673	LYS623	5	2.93194
						CYS673		1.58375
						ASP677		2.89693
						ASP810		1.86061
						PHE811		3.59015

Table 1: Docking scores and interactions of the analyzed molecules.

To visualize the interactions the 2D diagram and 3D interactions of compounds **h** and **i** are displayed below which have lowest affinity value -9.5 and -9.4 kcal/mol. (The colours of bond interactions is depicted in the table)

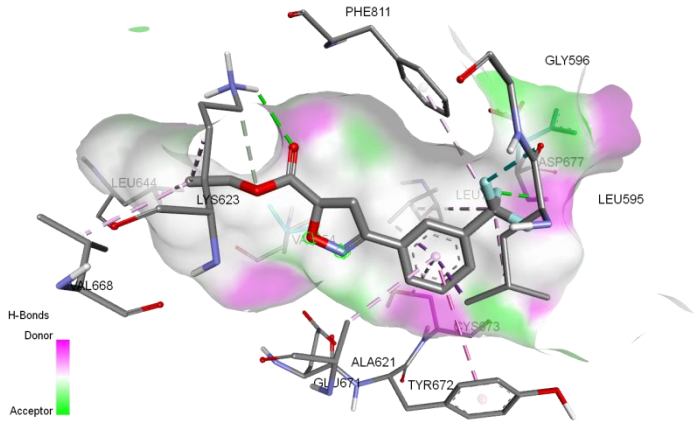

Figure 3: Molecular Docking 3D interaction output of 3-(3-Trifluoromethyl-phenyl)-4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester, h against 1T46 protein

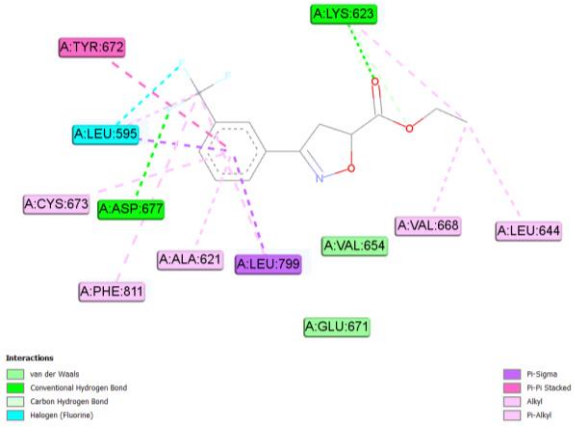

Figure 4: Molecular Docking 2D interaction output of 3-(3-Trifluoromethyl-phenyl)-4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester, 7 against 1T46 protein

Sr No.	NAME	COLOUR	DISTANCE	CATEGORY	TYPES OF BONDS	FROM	BONDS	TO	BONDS
1	A:LYS623:HZ1 - :UNK0:O13		2.60068	Hydrogen Bond	Conventional Hydrogen Bond	A:LYS623:HZ1	H-Donor	:UNK0:O13	H-Acceptor
2	A:ASP677:HN - :UNK0:F20		2.96075	Hydrogen Bond;Halogen	Conventional Hydrogen Bond;Halogen (Fluorine)	A:ASP677:HN	H-Donor;Halogen Acceptor	:UNK0:F20	H-Acceptor;Halogen

3	A:LYS623:CE - :UNK0:O14		3.3922	Hydrogen Bond	Carbon Hydrogen Bond	A:LYS623:CE	H-Donor	:UNK0:O14	H-Acceptor
4	A:LEU595:O - :UNK0:F19		2.9121	Halogen	Halogen (Fluorine)	A:LEU595:O	Halogen Acceptor	:UNK0:F19	Halogen
5	A:LEU595:CD1 - :UNK0		3.7260	Hydrophobic	Pi-Sigma	A:LEU595:CD1	C-H	:UNK0	Pi-Orbitals
6	A:LEU799:CD1 - :UNK0		3.8947	Hydrophobic	Pi-Sigma	A:LEU799:CD1	C-H	:UNK0	Pi-Orbitals
7	A:TYR672 - :UNK0		5.6804	Hydrophobic	Pi-Pi Stacked	A:TYR672	Pi-Orbitals	:UNK0	Pi-Orbitals
8	:UNK0:C16 - A:LYS623		4.201	Hydrophobic	Alkyl	:UNK0:C16	Alkyl	A:LYS623	Alkyl
9	:UNK0:C16 - A:LEU644		5.0632	Hydrophobic	Alkyl	:UNK0:C16	Alkyl	A:LEU644	Alkyl
10	:UNK0:C16 - A:VAL668		4.1545	Hydrophobic	Alkyl	:UNK0:C16	Alkyl	A:VAL668	Alkyl
11	:UNK0:C17 - A:LEU595		4.0081	Hydrophobic	Alkyl	:UNK0:C17	Alkyl	A:LEU595	Alkyl
12	:UNK0:C17 - A:LEU799		5.2630	Hydrophobic	Alkyl	:UNK0:C17	Alkyl	A:LEU799	Alkyl
13	A:PHE811 - :UNK0:C17		5.3043	Hydrophobic	Pi-Alkyl	A:PHE811	Pi-Orbitals	:UNK0:C17	Alkyl
14	:UNK0 - A:ALA621		4.4739	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	A:ALA621	Alkyl

15	:UNK0 - A:CYS673		5.1215	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	A:CYS673	Alkyl

Table 2: Total Number of Favorable Interactions: 15

The structure of 3-(3-Trifluoromethyl-phenyl)-4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester as a ligand has been subjected to molecular docking with a protein molecule that would act as a receptor. Docking results exhibited 9 poses out of which the pose having the lowest affinity (kcal/mol) was selected as the best docking pose and so considered for the ligand interaction. Here, 15 favorable interactions **Table 2** were observed where the ligand has bonded at the chosen pocket site in the selected pose. The given table shows the information about the bonds between the ligand and amino acids which contains bond distance, types of bonds, from where the bond is forming and their types. The bonding interactions observed in this moiety while docking were -: three hydrogen bonds [conventional hydrogen bond, conventional hydrogen bond with fluorine and carbon hydrogen bond], one halogen bond [with fluorine] and eleven hydrophobic interactions [i.e. two pi-sigma, one pi-pi stacked, five alkyl and three pi-alkyl].

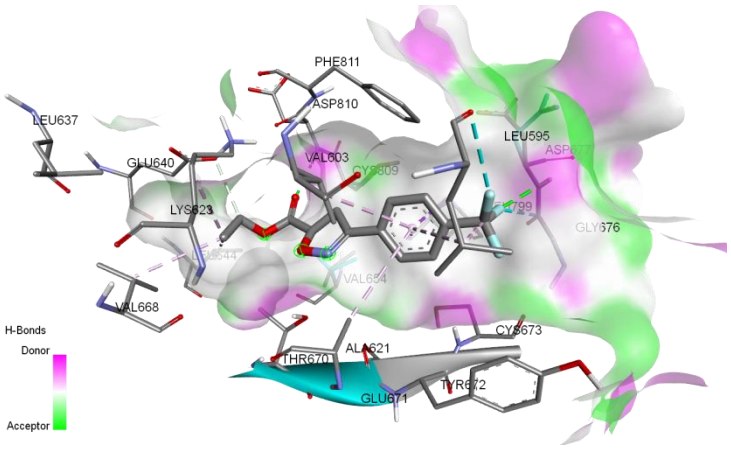

Figure 5: Molecular Docking 3D interaction output of 3-(4-Trifluoromethyl-phenyl)-4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester, i against 1T46 protein

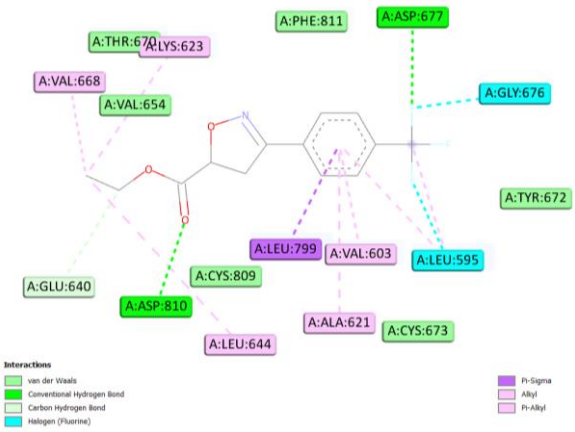

Figure 6: Molecular Docking 2D interaction output of 3-(4-Trifluoromethyl-phenyl)-4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester, i against 1T46 protein

Sr No.	NAME	COLOUR	DISTANCE	CATEGORY	TYPES OF BONDS	FROM	BONDS	TO	BONDS
1	A:ASP677:HN - :UNK0:F20		2.82292	Hydrogen Bond;Halogen	Conventional Hydrogen Bond;Halogen (Fluorine)	A:ASP677:HN	H-Donor;Halogen Acceptor	:UNK0:F20	H-Acceptor;Halogen
2	A:ASP810:HN - :UNK0:O14		2.25582	Hydrogen Bond	Conventional Hydrogen Bond	A:ASP810:HN	H-Donor	:UNK0:O14	H-Acceptor
3	:UNK0:C16 - A:GLU640:OE1		3.23812	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C16	H-Donor	A:GLU640:OE1	H-Acceptor
4	A:LEU595:O - :UNK0:F18		3.37294	Halogen	Halogen (Fluorine)	A:LEU595:O	Halogen Acceptor	:UNK0:F18	Halogen
5	A:GLY676:C - :UNK0:F20		3.29798	Halogen	Halogen (Fluorine)	A:GLY676:C	Halogen Acceptor	:UNK0:F20	Halogen
6	A:LEU799:CD1 - :UNK0		3.74699	Hydrophobic	Pi-Sigma	A:LEU7	C-H	:UNK0	Pi-Orbitals

						99:CD1			
7	A:LEU799:CD2 - :UNK0		3.9615	Hydrophobic	Pi-Sigma	A:LEU799:CD2	C-H	:UNK0	Pi-Orbitals
8	:UNK0:C17 - A:LYS623		4.0604 9	Hydrophobic	Alkyl	:UNK0:C17	Alkyl	A:LYS623	Alkyl
9	:UNK0:C17 - A:LEU644		5.4624 5	Hydrophobic	Alkyl	:UNK0:C17	Alkyl	A:LEU644	Alkyl
10	:UNK0:C17 - A:VAL668		4.1100 5	Hydrophobic	Alkyl	:UNK0:C17	Alkyl	A:VAL668	Alkyl
11	:UNK0:C7 - A:LEU595		3.6475 6	Hydrophobic	Alkyl	:UNK0:C7	Alkyl	A:LEU595	Alkyl
12	:UNK0 - A:LEU595		4.5938 9	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	A:LEU595	Alkyl
13	:UNK0 - A:VAL603		4.9171 9	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	A:VAL603	Alkyl
14	:UNK0 - A:ALA621		4.5465 2	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	A:ALA621	Alkyl

Table 3: Total Number of Favourable Interactions: 14

In order to perform molecular docking analysis, the structure of 3-(4-Trifluoromethyl-phenyl)-4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester has been selected as a ligand which is docked against protein molecule 1T46 acting as a receptor. During the analysis 9 different poses were observed, which was refined and the pose having lowest affinity (kcal/mol) was selected as the best docking pose and was considered for the ligand interaction. During this pose the ligand showed 14 favorable interactions in the chosen pocket site. **Table 3** provides information regarding bonding interaction between the ligand and amino acids in the protein chain which contains bond distance, types of bonds, from where the bond is forming and their types. The bonding interactions observed in this moiety while docking were three hydrogen

bonds [conventional hydrogen bond, conventional hydrogen bond with fluorine and carbon hydrogen bond], two halogen bonds [with fluorine] and nine hydrophobic interactions [i.e. two pi-sigma, four alkyl and three pi-alkyl].

Conclusion and Future directions

The synthesized 2-isoxazoline derivatives have shown good interactions with the C-KIT Tyrosine Kinase (1T46) target protein, which indicates good anti-viral potential of the molecules. The presence of trifluoromethyl groups on the benzene ring show better interaction to the protein as compared to simple fluorine atoms directly attached to the benzene ring. This could give a proper direction to the synthetic organic chemist for synthesizing bio-active derivatives of 2-isoxazoline. In addition to this the *in-silico* analysis performed in this study will act as a base in future for conducting *in-vitro* analysis in the lab by carefully examining the computational data generated in this research article.

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Glossary

2-Isoxazoline- 2-Isoxazoline are nitrogen and oxygen containing five-membered heterocyclic scaffolds with extensive biological activities.

Bioinformatics- It is defined as the application of tools of computation and analysis to the capture and interpretation of biological data.

Molecular Docking- It is a process under category of bioinformatic modelling which involves the interaction of two or more molecules to give the stable adduct. Depending upon binding properties of ligand and target (protein), it predicts the three-dimensional structure of any complex.

Protein ligand interactions- Protein–ligand interactions are fundamental to almost all processes occurring in living organisms. This interaction occurs through the molecular mechanics involving the conformational changes among low affinity and high affinity states. Ligand binding interactions changes the protein state and protein function.

Binding Site- The region within a macromolecule i.e. protein that binds to another molecule with specificity. The binding partner of the macromolecule is often referred to as a ligand.

Binding affinity- It quantifies the binding strength of a ligand to a macromolecule i.e. protein.