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Design, in Silico Screening and Synthesis of Novel Pyrimidine Derivatives as Tyrosine Kinase Inhibitors

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Abstract

In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. The present aim of the research is to design, in silico screening and synthesis of novel pyrimidine derivatives as tyrosine kinase inhibitors. The designing of all the scheme and molecules done in Chemdraw Ultra 12.0. The NTK acid, derivatives (3a-d) were studied for their molecular properties by various software's and revealed that designed molecules are safer, non-toxic and drug like. Results revealed that all the ligands (3a-d) exhibited greater affinity than NTK acid for 3CS9. Out of these, compounds 3b and 3a has shown significant binding affinities -10.74 and -10.25 respectively. Based on the present research results, the designed molecules can be further studied for its biological activity.

Keywords: Tyrosine Kinase Inhibitors, Molecular Docking, In Silico Screening, NTK Acid, Pyrimidine

Introduction

Cancer is a leading cause of death worldwide. It accounts for about 7 million deaths/year (12.5% of deaths worldwide). It has been estimated that there will be 16 million new cancer cases every year. Cancer progresses from the uncontrolled growth of cells to the formation of a primary tumour mass, vascularisation and subsequent spread (metastasis) of cancer cells to other parts of the body where secondary tumours may form.

Lipinski's rule of 5 filtration, it is also known as the Pfizer's rule of five or simply rule of 5 (RO5) or rule of thumb. This rule helps us to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A Lipinski in 1997, based on the observation that most orally administered drugs are relatively. The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion (ADME). However, the rule does not predict if a compound is pharmacologically active.

OSIRIS molecular property explorer

The OSIRIS Property Explorer allows us to draw chemical structures and calculate their various drug-relevant properties (CLogP, solubility, molecular weight, toxicity risk assessment, overall drug-score, etc.) whenever a structure is valid. Prediction results are valued and colour coded. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red, whereas a green colour indicates drug-confirm behaviour (Fig 1).

The aim of the present research work is to design, in silico screening and synthesis of novel pyrimidine derivatives as tyrosine kinase inhibitors.

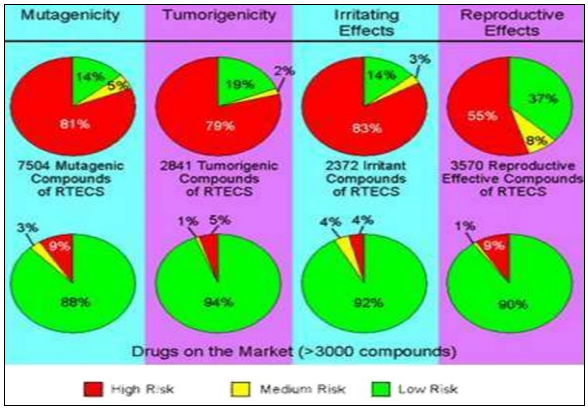


Fig 1: Toxicity prediction

Materials and methods

Chemicals used

Chemicals used for synthetic work were HATU (Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium), TEA (Triethylamine), ethanol, methanol, 4-chloroaniline, 2,6-dichloroaniline, anisidine, 4-(thiophene-2-yl) aniline, chloroform, dichloromethane, ethyl acetate are AR grade and LR grade chemicals.

Designing of molecules

All the molecules are designed in Chemdraw Ultra 12.0.

In silico screening

Lipinski's rule of 5 filtration

The files were inserted in *.pdb, *.mol,*.mol2,*.xyz,*.sdf,* or .smiles formats. Care was taken to avoid whitespace(s) in the input file name. The window opened and the files were uploaded in the above-mentioned formats. pH was adjusted from 0-14 as required.

OSIRIS property explorer (version 2)

OSIRIS property explorer version 2 (which requires JAVA platform to run) was used in the present study. The structure of designed molecule when drawn in or when pasted in smiles format will show the results at right side with colour coding. A green colour indicates non- toxic and red indicates toxicity (Fig 2).

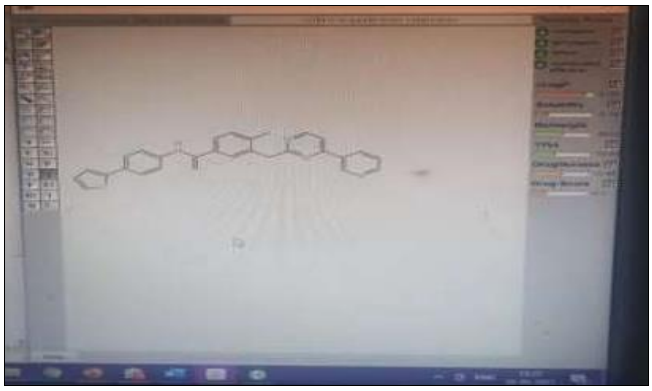


Fig 2: Molecular property prediction by OSIRIS property explorer

Molsoft property explorer (version v.3.7-2)

Molsoft property explorer version v.3.7-2 was used in the present study. The structure when drawn directly on the window or when inserted in Mol, Inchi, Smiles formats will calculate properties like MlogP, MlogS (Fig 3).

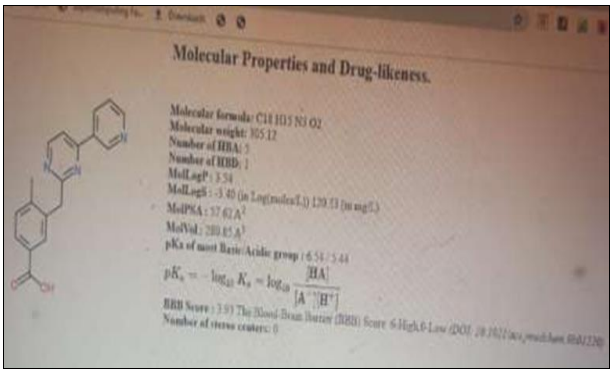


Fig 3: Molecular property prediction by Molsoft property explorer

Molinspiration property explorer

Molinspiration cheminformatic along with Java platform was used in the present study. The structure when drawn directly on the window or when inserted in SD, Smiles formats will calculate properties like MlogP, MlogS (Fig 4).

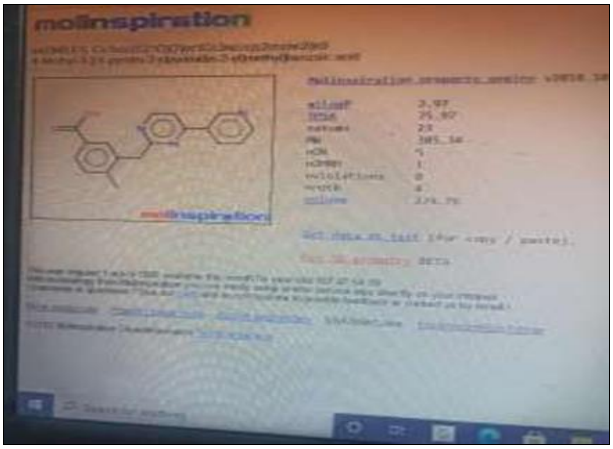
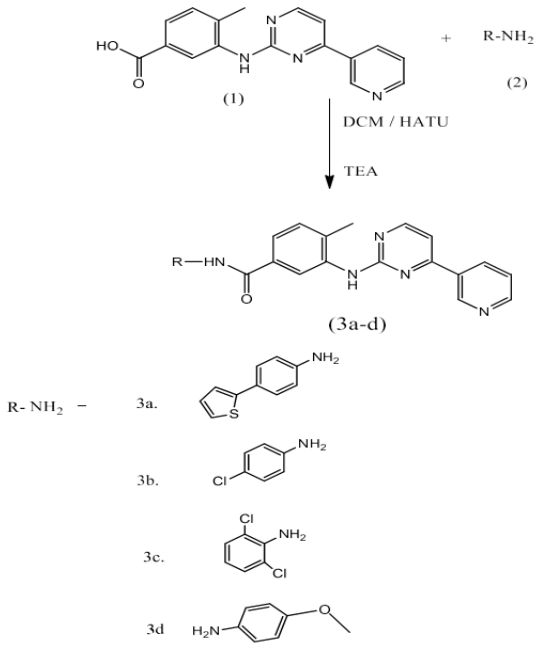


Fig 4: Molecular property prediction by Molinspiration property explorer

Scheme of synthesis



Scheme for synthesis of novel pyrimidine derivatives

Methodology involved in synthesis

Synthesis of compound 3a: Weigh 8.429 moles of NTK acid and 0.001 moles HATU in a separate, clean and dry beaker. Add 10 ml of DCM to the beakers and stir, and close with aluminium foil. Add HATU, NTK, 0.00168 moles of TEA in a conical flask and stir for 15 min. Then add 0.001 moles of 4-chloroaniline and excess of DCM is added and close with aluminium foil then continue stirring for 22-24 h. Monitor the reaction with TLC after 10 h till the reaction completes. Pour it into water, extract the reaction mixture with 10ml of DCM for 2-3 times. Evaporate the DCM layer and collect the product. Wash with sodium bicarbonate. Finally dry and weigh the product.

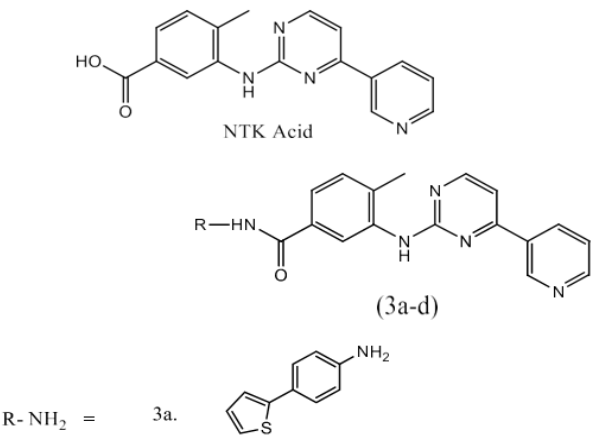
Synthesis of compound 3b: Collect two separate beakers and add 8.429 moles of NTK acid and 0.001 moles of HATU, add 10ml of DCM and stir well. Add 0.00168 moles TEA, NTK acid, HATU in a conical flask and stir for 15 min. Add 0.001 moles of 2,6-dichloroaniline and excess of DCM and continue stirring for 22-24 h. Monitor the reaction with TLC. Pour it into water, then extract the reaction mixture with DCM for 2-3 times. Collect the product and wash with 10% sodium bicarbonate and wash with water. Then dry and weigh it.

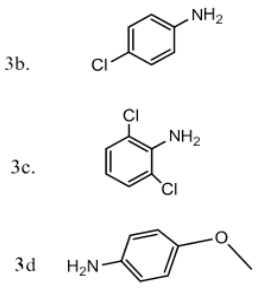
Synthesis of compound 3c: Accurately weigh 8.429 moles NTK acid, 0.001 moles HATU and mix with 10ml of DCM in separate beakers. Take conical flask add NTK acid and HATU and stir it for 5 min. Add 0.00168 moles of TEA and stir it for 15 min. Then add 0.001 moles of p-anisidine to the reaction mixture with excess amount of DCM and continue stirring for 22-24 h. Use TLC to monitor the reaction. Pour it into water, then extract with 10ml of DCM for 2-3 times. Evaporate DCM layer and collect the product. Wash with 10% sodium bicarbonate and water. Dry and weigh it.

Synthesis of compound 3d: Weigh 8.429 moles of NTK acid, 0.001 moles of HATU and 10 ml of DCM in separate beakers and stir. Add NTK acid, HATU, 0.00168 TEA, 0.001 moles of 4-thiophene 2-yl aniline and DCM in a conical flask and stir for 22-24 h. Check the reaction process by using TLC. Pour it into water, extract with 10ml of DCM for 2-3 times and then collect the product, wash with 10% sodium bicarbonate and water. Filter, dry and weigh the product.

Molecular properties for NTK acid and the newly designed molecules

NTK acid and their analogues (1-3d) were studied for their molecular properties by various software's and the results pertaining to them are presented in Table 1-4.





Scheme for the synthesis of novel derivatives

Molecular properties of the designed molecules by Lipinski's filters:

Table 1: Molecular property prediction by Lipinski's filters

Compound	Mass	HBD	HBA	logP	MR
1	306	2	6	3.09	86.02
3a	463	2	6	6.3	136
3b	415	2	6	5.3	118
3c	450	2	6	5.6	123
3d	411	2	6	4.8	128

Note: HBD-Hydrogen bond donors, HBA- Hydrogen bond acceptors, MR-Molar refractivity, logP- Partition coefficient.

All the newly designed NTK acid analogues obeyed the Lipinski's rule of five. The synthesized molecules were within the limits.

Molecular properties of the designed molecules by OSIRIS:

Table 2: Molecular property prediction by OSIRIS property explorer software (version 2)

Compound	Toxicity	CLogP	Solubility	M. Wt.	TPSA	D.L.	Drug score
1	No	2.6	-3.8	306	88	-3.4	0.4
3a	No	5.5	-6.9	449	108	2.9	0.18
3b	No	4.5	-5.6	401	79.8	1.9	0.4
3c	No	5.1	-6.43	435	79	1.9	0.2
3d	No	4.17	-5.32	411	89	0.5	0.43

Note: ClogP: Calculated logP; M. Wt.: Molecular weight; TPSA: Topological polar surface area; D.L.: Drug likeness

From the above results, all the newly designed NTK acid analogues are non- toxic, safer drug like molecules.

Molecular properties of the designed molecules by Molsoft

Table 3: Molecular property prediction by Molsoft software (Version: v.3.7-2)

Compound	M. Wt.	HBA	HBD	MLogP	MLogS	MLogSA	M. Vol.	S.C.
1	3.6	5	2	2.7	-2.82	66.24	276	0
3a	449	5	2	5.4	-5.7	62.3	410	0
3b	401	4	2	4.60	-4.7	61.6	355	0
3c	435.7	4	2	4.65	-4.7	60.2	371	0
3d	411.17	5	2	3.78	-3.90	68.42	390.95	0

Note: HBD: Hydrogen bond donars; HBA: Hydrogen bond acceptors; S.C.: Number of stereo centers; MlogP: Molecular logP; MlogS: Molecular logS; SA: Surface area; M.Vol.: Molar volume

The designed molecules properties are predicted by Molsoft.

Molecular properties of the designed molecules by Molinspiration:

Table 4: Molecular property prediction by Molinspiration software

Comp.	MilogP	TPSA	N-atoms	Mass	No. of O-H	No. of OH-NH	N-violation	NROTB	Vol.
1	3.2	88	23	306	6	2	0	4	270
3a	5.1	79	33	449	6	2	1	6	391
3b	4.4	79	29	401	6	2	0	5	343
3c	5.0	79	30	436	6	2	1	5	356
3d	4.25	89	31	411.46	7	2	0	6	371.76

Note: Clog P: Calculated milog P; TPSA: Topological polar surface area; NROTB: Number of rotatable bonds; Vol.: Volume; No. of OH-NH: Number of hydrogen bond donors; No. of O- N: Number of hydrogen bond acceptors

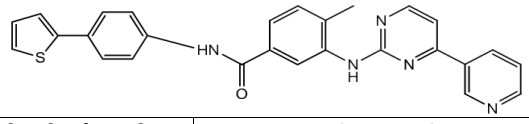
Thus, obtained results were demonstrated us to synthesize some new safer molecules.

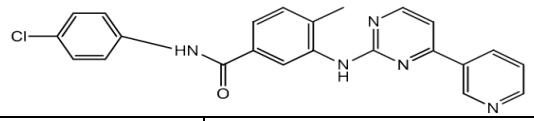
Chemistry of the synthesized compounds

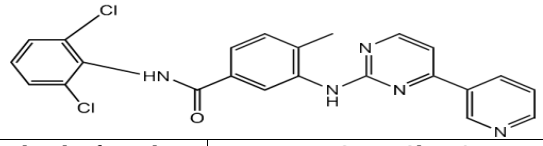
The new NTK acid based heterocyclic derivatives (3a-d) were synthesized by using appropriate synthetic methods as depicted in scheme.

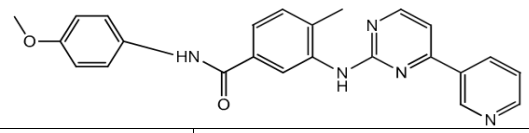
Physical data for the synthesized compounds:

The physical data of novel pyrimidine derivatives were described below.

3a	
	
Molecular formula	$C_{26}H_{19}N_5O_2$
Molecular weight	449
Solubility	DCM, THF
Percentage yield	83.3%
R_f	0.7 (Methanol:Ethylacetate - 1:1.5)

3b	
	
Molecular formula	$C_{22}H_{16}Cl_2N_5O_4$
Molecular weight	401
Solubility	DCM, THF
Percentage yield	79.04%
R_f	0.64 (Methanol:Ethylacetate – 1:1)

3c	
	
Molecular formula	$C_{22}H_{15}Cl_2N_2O$
Molecular weight	435.7
Solubility	DCM, THF
Percentage yield	90.19%
R_f	0.88 (Methanol:Ethylacetate – 1:1.5)

3d	
	
Molecular formula	$C_{24}H_{21}N_5O_2$
Molecular weight	411.12
Solubility	DCM, THF
Percentage yield	72.9%
R_f	0.6 (Methanol:Ethylacetate – 1:1)

Docking results

Name of the protein: BCR-ABL Tyrosine kinase

PDB ID: 3CS9

Docking of all the synthesized compounds in to the binding site 3CS9 and estimating the binding affinity of the complex is a significant part of the structure-based drug design process. It is an open-source software for drug discovery, molecular docking. The structural interactions between PDB with NTK acid and synthesized compounds were docked separately (Fig 5 to 9). Docking studies are commonly performed for predicting binding modes to proteins and their binding energies of ligands. Results revealed that all the ligands (3a-d) exhibited greater affinity than NTK acid for 3CS9. Ligand 3b and 3a exhibited more binding affinities -10.74 and -10.25 respectively.

Table 5: Molecular docking interactions of compounds 3a-d with 3CS9

S. No	Compound	Binding energy (C) (Kcal/Mol)/Dock score
1	1 (NTK acid)	-8.36
2	3a	-10.25
3	3b	-10.74
4	3c	-9.94
5	3d	-9.945

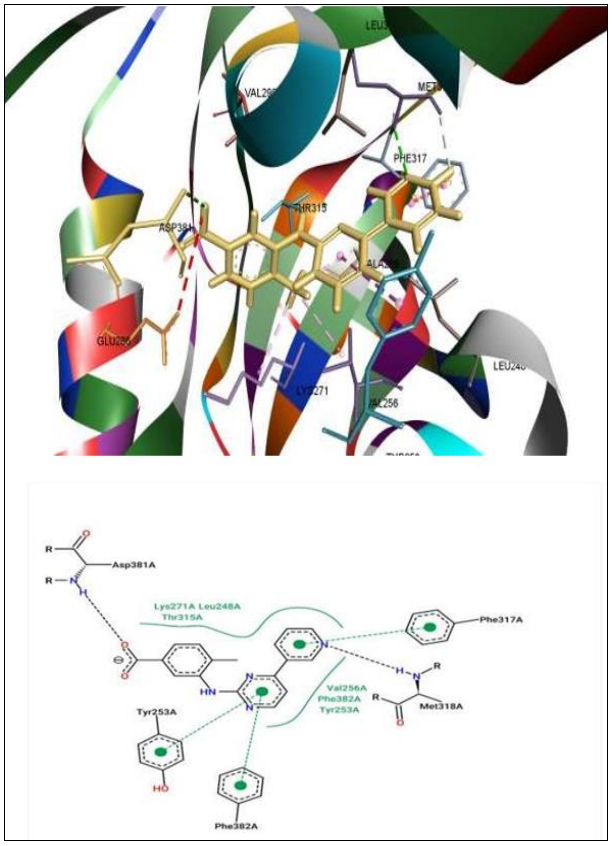


Fig 5: Docking interaction of compound 1 (NTK ACID) with 3CS9

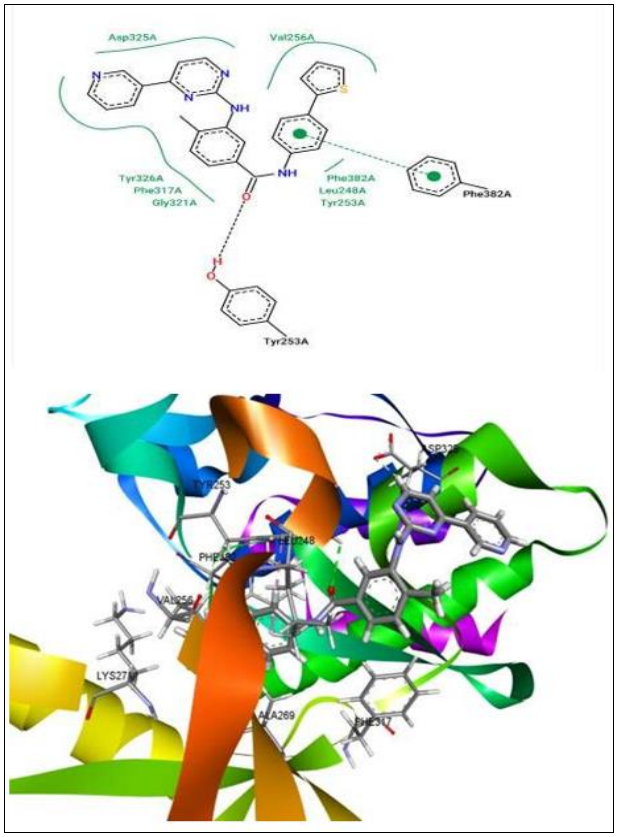


Fig 6: Docking interaction of compound 3a with 3CS9

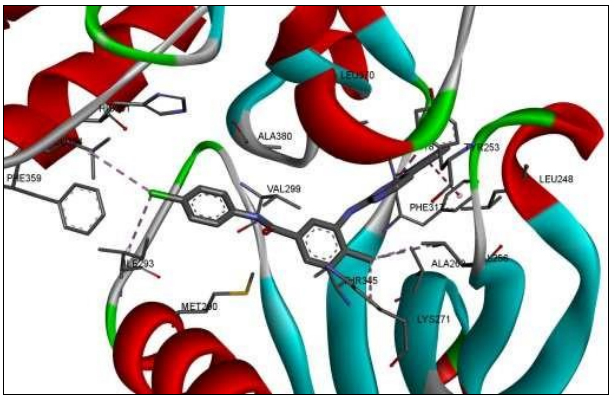


Fig 7: Docking interaction of compound 3b with 3CS9

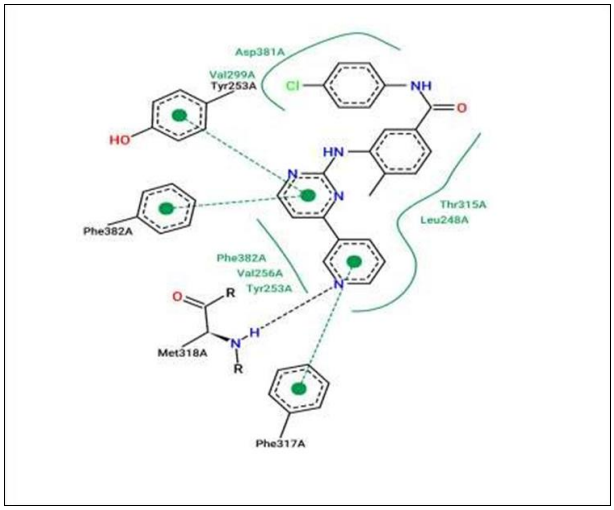


Fig 8: Docking interaction of compound 3c with 3CS9

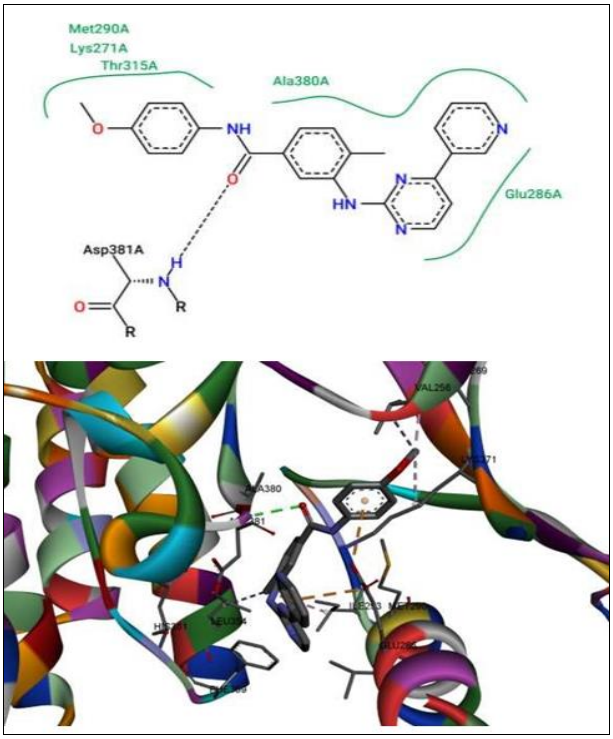


Fig 9: Docking interaction of compound 3d with 3CS9

Conclusion

Molsoft is building unique technologies for structure prediction that improves our understanding of the spatial organization of biological molecules and their interactions with each other, their biological substrates and drug-like molecules at the atomic level. Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. In the present study, the designing of all the scheme and molecules done in Chemdraw Ultra 12.0. The NTK acid, derivatives (3a-d) were studied for their molecular properties by various software and revealed that designed molecules are safer, non-toxic and drug like. Some derivatives were synthesized according to the scheme and obtained in better yields. And all the compounds were subjected to the docking studies. All the derivatives have shown more binding affinity than NTK acid with protein 3CS9. Out of these compounds, 3a and 3b has shown significant binding affinity among designed derivatives. Based on the present research results, the designed molecules can be further studied for its biological activity.

Acknowledgement

We express our indebtedness and sense of gratitude to the management of School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Medchal, Hyderabad, Telangana, India for providing the necessary equipment for research, praiseworthy inspiration, constant encouragement, facilities and support.

Declarations

Authors contribution

All authors contributed to experimental work, data collection, drafting or revising the article, gave final approval of the version to be published, and agreed to be

accountable for all aspects of the work.

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Competing interest statement
All authors declare that there is no conflict of interests regarding publication of this paper.

Additional information
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Ethical approval
Not required.

Consent
It is not applicable.

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