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PHARMACEUTICAL SCIENCE
Research Article

Augmentation of Caspase-3 Activity to Induced Apoptosis by Novel 1,3,4-Thiadiazole Derivatives: A Potential Bioinformatics Hypothesis to Treatment of the Cancer

Vinit Raj[1], Amit Rai[1], Arvind Kumar[2], Vinod Kumar[2] and Sunny Singh[1]*

[1]*Department of Pharmaceutical Sciences, Babasaheb Bhimrao Ambedkar University, India*

[2]*Department of Pharmaceutical Sciences, S. D. College of pharmacy and vocational studies, India*

***Corresponding Author:** Sunny Singh, Department of Pharmaceutical Sciences, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Rai Bareli Road, Lucknow-226025, India.

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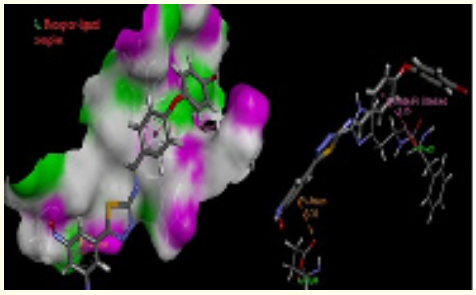
Abstract

Cancer is an abnormal cell growth with spread to other parts of the body. Whereas the life of normal cell maintained by an apoptosis pathway which is facilitated by caspases. Caspases are a family of cysteine-aspartyl proteases which show significant roles in Apoptosis. However, Apoptosis is the principle mechanism of tumor cell death by chemotherapy. In this study, we measured in-silico cellular caspase-3 activation by designing novel 1,3,4-thiadiazole derived as well as compared with the Fluorouracil as an anticancer drug with the help of Autodock bioinformatic software. On the basis of Binding affinity, ADMET properties and high bioactivity scores a new lead has been selected and whose stability with the binding site of caspase-3 was predicted by MD (Molecular dynamics) simulation for 5 ns. All Bioinformatics parameters supported our hypothesis as well as shown potent binding affinity as compared than the standard drug (-8.95 kcal/mol and -4.88 kcal/mol, respectively).

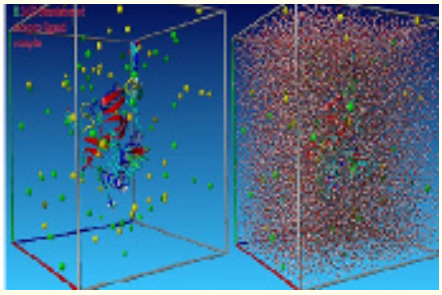
However, further studies, like synthesis, in-vivo evaluation and mechanism of action of these compounds are necessary to support this hypothesis. These titled compounds emerged as a lead for caspase-3 drug screening for future.

Keywords: *Anticancer; Caspase-3; Docking studies; MD simulation and ADMET properties*



a

Receptor-ligand complex.



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MD Simulation of receptor-ligand complex

Introduction

Cancer is the uncontrolled faster cell growth and live longer than normal. Therefore, either increased activity of antiapoptotic proteins or decreased activity of proapoptotic proteins can involve to the enlargement of cancer [1]. In this way, we can say disturb the normal programmed cell death by apoptosis pathway. Therefore, apoptosis is derived from an ancient Greek means "leaves falling from a tree". It has an essential role in controlling cell number in many developmental and physiological settings and in chemotherapy-induced tumour-cell killing by two major pathways: the death-receptor-induced extrinsic pathway and the mitochondria-apoptosome-mediated apoptotic intrinsic pathway. Both of these pathways lead to caspase augmentation and cleavage of specific cellular substrates. Caspases are intensified in a cascade-like fashion. Initiator or upstream caspases (caspases 8, 9, and 10) can activate effectors or downstream caspases, including caspases 3, 6, and 7, which leads to orientation of apoptosis. Inducers of apoptosis have been used in cancer therapy. Intrinsic and extrinsic pathways can be activated separately, but activation of caspases seems central to most apoptotic pathways [2]. Hence, cancer is a burgeoning major public health issue worldwide and economic burden as well as a threat to society which is increasing the risk of human affliction. In 2008, 2•45 million people were examined which affected with cancer and 1•23 million died because of cancer in the 27 countries of the European Union (EU) [3]. From these surveys indicated that the most common cancer were breast, colorectal, lung, and prostate cancers founded. Another survey, particularly to emerging countries, China (1 350 695 000 people), India (1 236 686 732 people), and Russia (143 533 000 people) together account for nearly 40% of the world's population affected with cancer [4].

In this study, we focused on developing a new scaffold which can be able to have potent activation effect. During searching of new lead for caspase 3 target, we used 1, 3, 4-thiadiazole moiety as it has one hydrogen binding domain and two-electron donor system. Previous literature survey suggested that 1, 3, 4-thiadiazole is the important pharmacophore than other isomers for binding to the receptor and it has multiple pharmacological actions as well. This ring exhibited antimicrobial [5-6], anticancer [7], antanxiety, anti-depressant [8], anti-oxidant properties [9], anticonvulsant activity [10] and antitubercular activities [11]. Thiadiazole ring expressed diverse biological activities, might be due to the presence of = N-C-S moiety [12].

In view of the above fact, the question arose whether 1, 3, 4-thiadiazole might be an important activator for caspase-3 target. To prove this hypothesis, docking studies was carried out between newly designed protein and prepared ligand to get interaction energy.

After that, pharmacokinetics parameters (ADME and toxicity) were also measured with that designed compound to rule out whether these compounds might be suitable for in vivo biological system. We hypothesized that these compounds may be lead target for caspase-3 and also suitable for in vivo screening in future.

Material and Methods

In this present study, National centre for Biotechnology Information (NCBI) and Protein Data Bank (PDB) were used as chemical sources. The software were used for experiment, tabulated in Table 1. Fluorourcil and series of 1,3,4-thidiazole derivatives structures were drawn through Chemdraw Ultra 10.0 (Figure 1) and their geometry was optimized six times with Gauss view 5.0.

Software	Purposes
Chemdraw Ultra 10.0 and open babel GUI.	For drawing the chemical structure and convert into PDB format.
Argus Lab software, Gauss view 5.0.	For optimizing the geometry of derivatives.
Autodock 4.0,Discovery studio and autodock 4.0.	For docking studies.
Molinspiron software toolkit, Med Chem Designer and Lasar toxicity prediction service.	For characterization of the derivatives.

Table 1: *Softwares used for modeling and their Purposes.*

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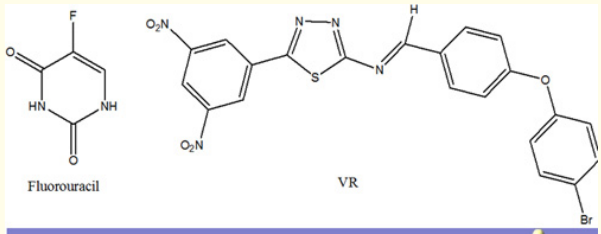

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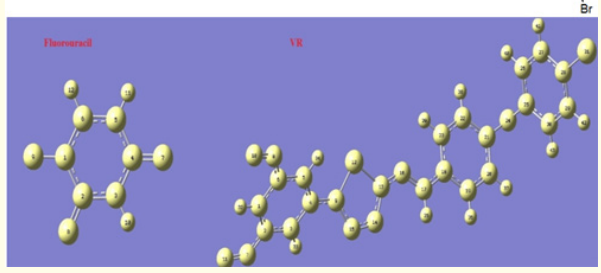

Figure 1: *Structures of standard and designed compound with optimized geometry.*

Homology of caspase-3

The amino acid sequence of human caspase-3 was retrieved from Gen Bank (accession number: 1QX3_AGI:37928084) in NCBI [13]. It consists of 257 amino acids. The caspase-3 was then subjected to a PSI-BLAST search in order to identify the homologous proteins. Hence, 100% identity was observed.

Figure 2: *3-Dimensional Structure Prediction of Caspase-3 Protein of Homo sapiens.*

>gi|37928084|pdb|1QX3|A Chain A, Conformational Restrictions In The Active Site Of Unliganded Human Caspase-3

SGISLDNSYKMDYPEMGLCIIINNKNFHKSTGMTSRSGTDVDAANLRETFRNLKYEVRNKNDLTREEIVELMRDVSKEDHSKRSSFVCVLLSH-GEEGIIFGTNGPVDLKKITNFFRGDRCRSLTGKPKLFIIQACRGTELDCGIETDSGVDDDMACHKIPVDADFLYAYSTAPGYYSWRNSKDGSWFIQS-LCAMLKQYADKLEFMHILTRVNRKVATEFESFSFDATFHAKKQIPCIVSMLTKELYFYHLEHHHHHH

Virtual Docking studies

Virtual screening of about 160 compounds of 1,3,4-thiadiazole derivatives have been performed by Pyrex virtual screening software. However, most active compound screened among of them which compound shown the better affinity with caspase-3. Additionally, best ligand selected then docking study was performed by using Autodock 4.0 along with Autodock Vina. Before the docking study, we identified the active site domain with the help of Dog site score/Castp acitive Site recognizer server of protein wherein the ligand showed the best configuration (Figure 3 and Figure 4). Keeping in view active site amino acid sequence, Grid box was set. Their binding affinity (kcal/mol) and count of probable hydrogen bonds also evaluated in the similar experiment.

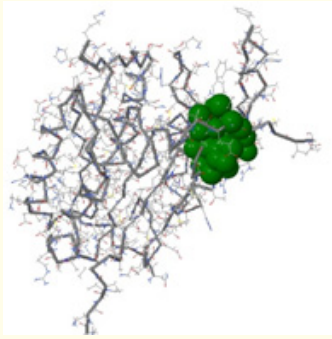

Figure 3: *Structures of standard and designed compound with optimized geometry.*

DoGSiteScorer: Active Site Prediction and Analysis Server

Pockets and descriptors have been calculated for XPRO:

Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Simple Score
P0	366.53	550.97	319.26	19.14	0.17
P1	336.13	565.21	396.21	17.64	0.16
P2	280.00	450.48	264.44	16.23	0.11
P3	222.34	312.08	229.59	14.79	0.07
P4	206.91	400.63	337.92	14.53	0.08
P5	130.37	313.76	186.81	10.66	0.00
P6	123.01	209.59	84.23	6.21	0.00
P7	112.51	342.62	224.59	7.43	0.00
P8	104.19	284.96	152.57	8.71	0.00

Clicking on the name of a single pocket opens a separate window containing further calculated pocket properties.

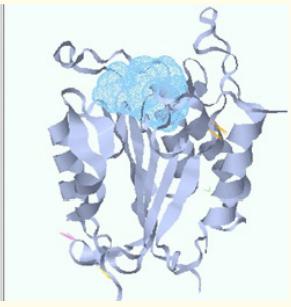

Figure 4: *Amino acid present in the active site are labeled with sky blue color.*

Prediction of pharmacokinetic properties

The designed compound assessed for pharmacokinetic properties through medchem designer software. Later, the pharmacokinetic parameters of the lead molecules analyzed, including their absorption, distribution, metabolism and excretion (ADME), using Molinspiration property online calculator [14]. The percentage of absorption (%ABS) was calculated using TPSA by the following formula: % ABS = 109-(0.345 × TPSA) [15].

Bioactivity prediction and Toxological comparative studies

For prediction of bioactivity and toxicological properties of titled compounds evaluated by Molinspiration property online calculator and Lasar toxicity prediction server. The designed derivative and original drug bioactivity predictions had been compared along with some selected activity GPCR (G-Protein coupled receptor).

MD Simulation study

Molecular dynamics simulation has been performed for the higher affinity complex with the help of Yasara tools which complex placed in a cubic box and filled with solvent (HOH) by applying AMBER 99 force field, temperature 298 K that controlled through rescale velocities and pressure reached 1.000 bar these parameters were applied in order to check the stability of their complex. Figure 5 shows the solvated structure when visualized in MD. After energy minimization of the solvated and electroneutral system its Potential Energy had been analyzed and plotted by using Sigma Plot 11.0 tools. MD simulation was run for 5.0 ns. The following parameter evaluated including: (i) Complex binding energy vs time which indicated that complex stability under the MD simulation (Figure 6), (ii) Potential energy of comsplex with respective time (Figure 7) and (iii) Average RMSD (Root Mean Square Deviation) graph which indicated convergence of the simulated structure towards an equilibrium state with respect to a reference structure (starting structure) (Figure 8)

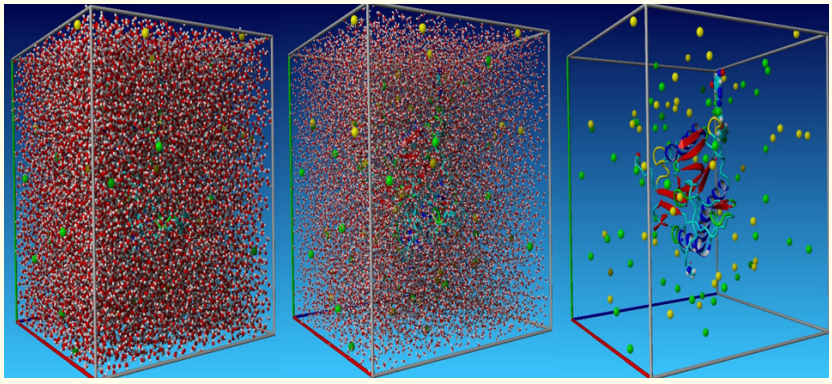

Figure 5: *Shows solvated structure visualized in MD simulation. Here red color represents the solvent as well as yellow green bolls shown the sodium and chloride which neutralized the environment into the cubed. Protein shown in greenish-yellow-red-blue color and ligand shown as white-skein blue color.*

Compound binding Energies[kj/mol]

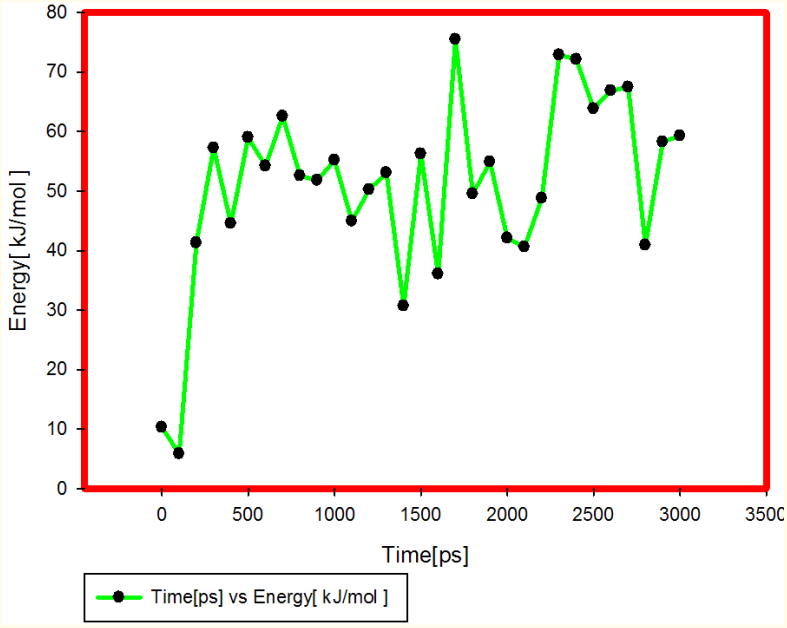

Figure 6: *Shown the Time vs compound binding energy which indicated that the energy stabilized at 1000 ps.*

Root mean square distance (RMSD) of the backbone of the structure

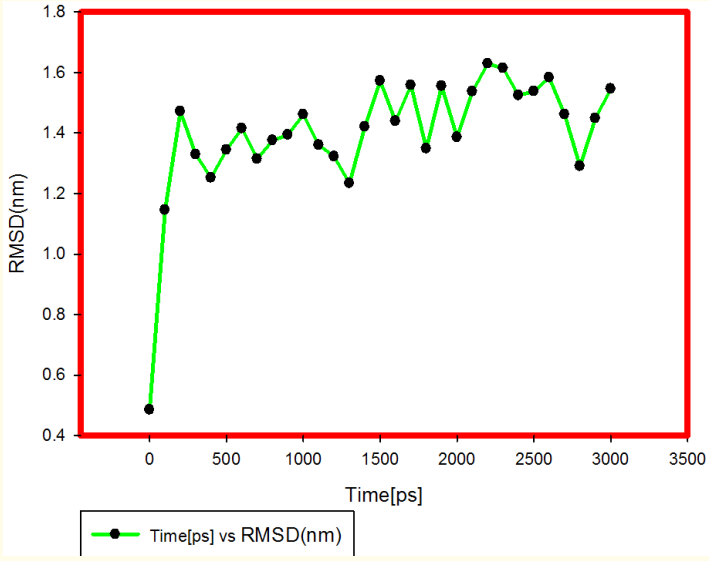

Figure 7: *Root mean square distance (RMSD) of the backbone of the structure simulated over 5ns nanosecond.*

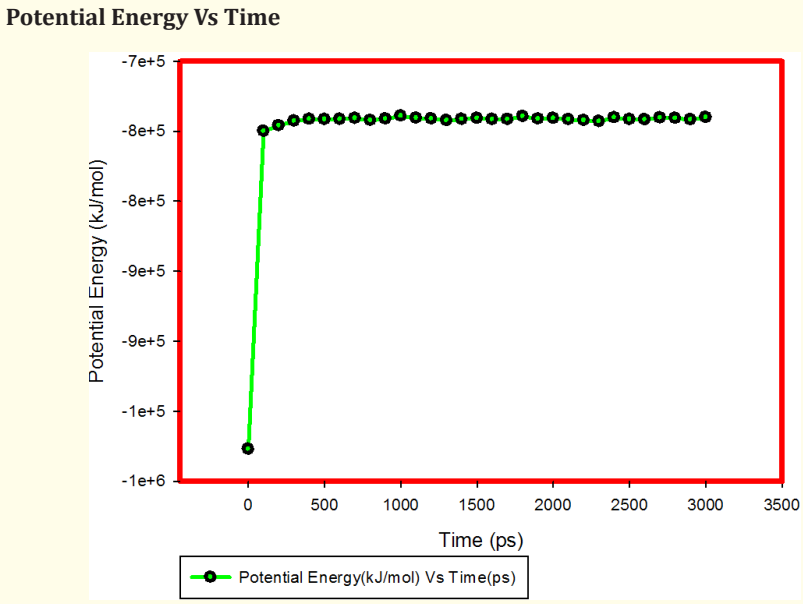

Figure 8: *Shows Time (3500ps) Vs Potential Energy which indicated that very small fluctuation observed.*

Results and Discussion

Docking studies

Docking study of designing compound was performed with caspase-3. We detected the active site domain with the help of Dog site/castP active Site recognizer server of protein where the ligand showed the best configuration. Later, Grid box was set according to an active site sequence of amino acid. Their binding affinity (kcal/mol) and count of probable hydrogen bonds were evaluated (Table 2) through docking studies. Docking images of fluorouracil and VR with the target receptors was shown in (Figure 9). Fluorouracil and Compound VR exhibited good binding properties with caspase-3 receptor (-4.8 and -8.9 kcal/mol, respectively). Addition, the interaction of fluorouracil and VR to the receptor has concluded that TRP 206 and TRP 204 common essential amino acids, which may be involved in enhancing the efficacy of caspase-3. Hence, this observation could be attributed as potential anticancer with caspase-3 mimetic/facilitator mode of action.

Ligand	Receptor (PDB:1QX3)	Affinity (Kcal/Mol)	Amino Acids Involved in Interactions	H- bonds	Pi bonds
Fluorouracil	Caspase-3	-4.8	LEU A 168, ASP A 169, SER A 198 , THR A 199, ALA A 200, TYR A 203, TYR A 204, SER A 205, TRP A 206, LYS A 260, GLN A 261, IL E A 262	3	6
VR	Caspase-3	-8.9	TYR A 204, TRP A 206, ARG A 207, ASN A 208, ASP A 211, TRP A 214, GLN A 217, GLU A 246, PHE A 247, GLU A 248, SER A 249, PHE A 250, SER A 251, PHE A 256	0	2

Table 2: *Binding affinities of standard drug and designed compound.*

a)

b)

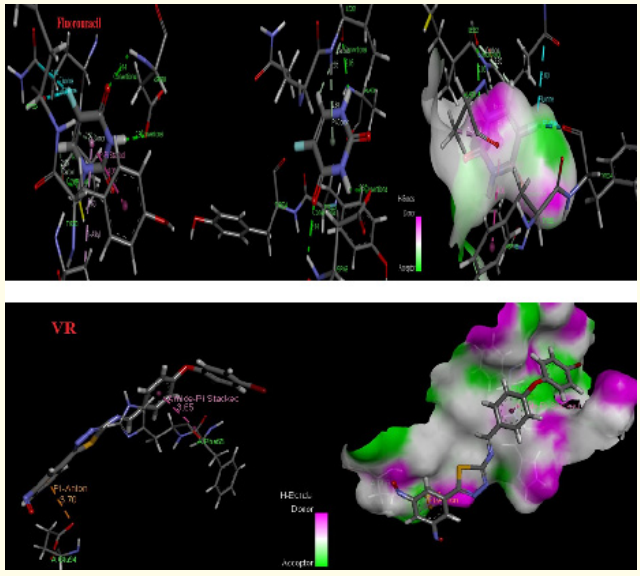

Figure 9: *Docking images (a) Fluorouracil and (b) VR with caspase-3, the green color dotted line shows hydrogen bonding and yellowish, light blue or whitish dotted line show Pi Donor, Acceptor and Alkyl bond respectively with amino acids involved in binding poses.*

Prediction of ADME properties

The ADME properties of the designed compounds were assessed by evaluating their physicochemical properties using the medchem designer software. Their molecular weights were < 500 Da; they had < 5 hydrogen bond donors and < 10 hydrogen bond acceptors, and logP values of < 5 (Table 3). These properties are within the acceptable range of Lipinski's rule of five. Furthermore, the pharmacokinetic parameters of the lead molecules were analyzed, including their ADME using Molinspiration property online calculator and Lasar toxicity prediction server. For the designed compound, the partition coefficient (QPlogPo/w) and water solubility (QPlogS) values, the %ABS for the compound approximately 72%. These pharmacokinetic parameters are well within the acceptable range defined for human use, thereby indicating their potential as drug-like molecules.

Oral bioavailability of all designed compounds were calculated theoretically which lied in accepted range (more than 70%). Oral bioavailability essential key for the pharmacokinetic profile of the compound to enhance the pharmacological activity. These all theoretically parameter of the designed VR compound supported our hypothesis.

MlogP, Moriguchi estimation of logP. S+ log P logP calculated using Simulations Plus' highly accurate internal model; S+logD, logD at user-specified pH (default 7.4), based on S+logP;n-OHNH donor, Number of Hydrogen bond donor protons; M_NO, Total number of Nitrogen and Oxygen atoms; T_PSA, Topological polar surface area in square angstroms; Rule Of Five, Lipinski's Rule of Five: a score indicating the number of potential problems a structure might have with passive oral absorption;miLog P, logarithm of compound partition coefficient between n-octanol and water; log D, logarithm of compound distribution coefficient; n-ROTB, number of rotatable bonds; MV, molecular volume; n-ON acceptor, number of Hydrogen bond acceptor protons.

S No		Rule	Fluorouracil	VR
1	S + log P	−2.0 to 6.5	-0.701	3.229
2	S + log D	–	-0.918	3.229
3	M log P	–	-0.005	2.731
4	T_PSA	–	65.92	106.23
5	n-OHNH donor	< 5	2.000	0.000
6	M_NO	–	4.000	8.000
7	Rule of 5	≤ 1	0.000	0.000
8	% ABS (% of absorption)	_	86.26	72
9	MV	–	96.913	360.45
10	n-ON acceptor	< 10	4	8
11	n-ROTB	–	2	7
12	M. Wt.	< 500	130.079	494.33

Table 3: *The theoretical ADME properties of fluorouracil and VR.*

Bioactivity prediction and Toxological comparative studies

In this study, for prediction of bioactivity and toxicological properties of titled compounds was also determined in our study. From all calculated parameters, it can be observed that titled compound expressed less affinity to GPCR (G-Protein coupled receptor) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease enzyme inhibitor and the toxicological as compared to fluorouracil. The Bioactivity and Toxological data are given in Table 4 and 5.

S No	Receptors	Fluorouracil	VR
1	GPCR ligand	-2.60	-0.74
2	Ion channel Modulator	-1.95	-0.73
3	Kinase inhibitor	-2.61	-0.10
4	Nuclear receptor ligand	-3.04	-0.65
5	Protease inhibitor	-3.15	-0.59
6.	Enzyme inhibitor	-3.15	-0.24

Table 4: *Score of bioactivity prediction of fluorouracil and thiadiazole derivative.*

Computational details

A computational study for prediction of ADME properties of title compound was performed. The percentage of absorption (%ABS) was calculated using TPSA. From all these parameters, it can be observed that titled compound exhibited good ADMET properties. None of the compounds violated Lipinski,s parameters, making them potentially promising agents for anticancer durg. From the MD simulation study of compound VR shown the stability of complex at 5 ns with average en -50.416 (kJ/mol). In addition, the complex didn't show more fluctuation in potential energy in respectively with time. Whereas, binding energy of compound at 0 ps time was founded -977086.922 Kg/mol which decrease up to -740059.004 Kg/mol at 3500 ps under MD simulation. The complex shows the stability near 1000 ps. However, the RMSD of the backbone structure shown the stability near 2000 ps. These findings suggested that the complex structure of VR with caspase-3 shown the best stable fitting (affinity) in the MD simulation study.

S No	DSSTox Toxicity Origin	Fluorouracil	VR
1.	DSSTox Carcinogenic Potency DBS MultiCellCall:	Carcinogen: 0.000	Carcinogen: 0.00766
2.	DSSTox Carcinogenic Potency DBS Mutagenicity:	non-mutagenic: 0.000	Mutagenic: 0.0217
3.	DSSTox Carcinogenic Potency DBS Rat:	non-carcinogen: 0.000	non-carcinogen: 0.00869
4.	Kazius-Bursi Salmonella mutagenicity:	non-mutagenic: 0.000	Mutagenic: 0.133
5.	FDA v3b Maximum Recommended Daily Dose mmol: 0.0152722115276765	0.136	0.107
6.	DSSTox Carcinogenic Potency DBS SingleCellCall:	carcinogen: 0.000	Carcinogen: 0.0157
7.	EPA v4b Fathead Minnow Acute Toxicity LC50_mmol:	0.728	0.178
8.	DSSTox ISSCAN v3a Canc:	Carcinogen: 0.000	Carcinogen: 0.839
9.	DSSTox Carcinogenic Potency DBS Hamster:	Carcinogen: 0.0831	non-carcinogen: 0.167
10.	DSSTox Carcinogenic Potency DBS Mouse:	carcinogen: 0.000	non-carcinogen: 0.0114

Table 5: *Topological comparative studies of fluorouracil and thiadiazole derivative.*

Conclusion

Virtual screening approach was used in our study to identified ligand against caspase-3 protein. According to exist literature and analysis of the results from the our research of the virtual docking and computational study indicated that the designed novel 1,3,4-thiadiazole derivative has potent activation effect on caspase-3 receptor as potential anticancer target as well as treatment of a number of life threading disease. The compound displayed significant binding affinity compared than fluorouracil. Hence, compound VR has shown significant efficacy as well as the complex stability in the MD simulation. The other parameters like toxicity, ADME and oral bioavailability of screened hit lead showed similar trends. The docking study data strongly support the assumption that caspase-3 may be involved in anticancer activity of 1, 3, 4-thiadiazole derivative. However, the interaction of compound with the receptor has concluded that TRP 206 and TRP 204 common essential amino acids those may be involved in enhancing the efficacy of caspase-3. Hence, this observation could be attributed that the compound VR shown as potential anticancer activity with caspase-3 with mimetic/facilitator mode of action.

However, further studies, like synthesis, *in-vivo* evaluation and mechanism of action of the compound is necessary to support this hypothesis. The titled compound emerged as a lead for caspase-3 drug screening for future.

Acknowledgements

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