RESEARCH ARTICLE

An *in silico* Appraisal to Identify High Affinity Anti-Apoptotic Synthetic Tetrapeptide Inhibitors Targeting the Mammalian Caspase 3 Enzyme

Seema Kelotra[1]*, Meeta Jain[2], Ankit Kelotra[2], Ish Jain[4], Srinivas Bandaru[3], Anuraj Nayarisseri[4], Anil Bidwai[5]

Abstract

Apoptosis is a general phenomenon of all multicellular organisms and caspases form a group of important proteins central to suicide of cells. Pathologies like cancer, Myocardial infarction, Stroke, Sepsis, Alzheimer's, Psoriasis, Parkinson and Huntington diseases are often associated with change in caspase 3 mediated apoptosis and therefore, caspases may serve as potential inhibitory targets for drug development. In the present study, two series of synthetic acetylated tetrapeptides containing aldehyde and fluromethyl keto groups respectively at the C terminus were proposed. All these compounds were evaluated for binding affinity against caspase 3 structure. In series 1 compound Ac-DEHD-CHO demonstrated appreciable and high binding affinity (Rerank Score: -138.899) against caspase 3. While in series 2 it was Ac-WEVD-FMK which showed higher binding affinity (Rerank Score: -139.317). Further these two compounds met ADMET properties and demonstrated to be non-toxic.

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Introduction

Caspase, initially called interleukin 1β converting enzyme (ICE), was identified not because of its involvement in cell death, but because it was responsible for cleaving (Miao et al., 2011) and thereby activating interleukin 1β, a pro-inflammatory cytokine (Leist et al., 2011), later caspases were accomplished to be the key executioners of the cell death program (Denault et al., 2002; Danial et al., 2004; Gomez et al., 2005; Turk et al., 2007; Noy et al., 2010). The evidence of involvement of caspases in cell death comes from the pioneering study wherein the inhibitors designed against caspases prevented worm cells from killing themselves. The above observation also added to the speculation that because the mechanisms of programmed cell death in the worm were similar to those for apoptosis of mammalian cells (Li et al., 2012; Sankari et al., 2012), caspase inhibitors could presumably prevent the death of mammalian cells as well (Hengartner et al., 2000).

During the last decade, major progress has been made to further understand caspase structure and function, providing a unique basis for drug design (Lavrik et al., 2005; Le et al., 2006; Takai et al., 2012; An et al., 2013, Zou et al., 2013; Liu et al., 2014). Caspases belong to the family of cysteinyl aspartate-specific proteases (Chai et al., 2001; Belizario et al, 2008), are activated through proteolysis at specific asparagine residues that are located within the prodomain, the p20 and p10 subunits (Shiozaki et al., 2004; Arockiaraj et al., 2013) This results in the generation of mature active caspases that consist of the heterotetramer $p20_2$-$p10_2$. Their vital role in apoptosis makes caspases potential targets for drug development.

Most of the synthetic peptide caspase inhibitors were developed based on the tetrapeptide caspase recognition motif (Fischer et al., 2005, Kuhn et al., 2011). Therefore, the selectivity of inhibitors matches the caspase substrate specificities, the introduction of an aldehyde group at the C terminus of the tetrapeptide results in the generation of reversible inhibitors (Powers et al., 2002; Grawert et al., 2012), whereas a fluoromethyl ketone (fmk), a chloromethyl ketone (cmk) (Shi et al., 2002; Huang et al., 2001), or a diazomethyl ketone (dmk) (Concha et al., 2002) at this position irreversibly inactivates the enzyme.

In the view of above, we have proposed two series of 25 synthetic acetylated tetrapetides compounds flanked by aldehyde or fluromethyl ketone at C terminus and observed their binding affinity, drug likeness and tested for toxicity, which we anticipate can form potent inhibitor targeting caspase 3.

*[1]M.B Khalsa College, [2]School of Biochemistry, Devi Ahilya University, Takshashila Campus, [4]In silico Research Laboratory, Eminent Biosciences, [5]Index medical College, Indore, [3]Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad, India *For correspondence: seemakelotra@gmail.com*

Materials and Methods

Selection of tetrapeptide compound

In the study we proposed two series of 25 tetrapeptide compounds anticipated to have appreciable inhibitory potential against caspase 3. In one of the series all the tetra peptides were flanked by acetyl group at N terminus and aldehyde group at C terminus. In the second series the compounds were same as in series one, except the C terminus was flanked by fluromethyl group. The series of tetrapeptide compounds selected in the study and their corresponding sequence is shown in Table 1.

Structure preparation and minimization

The twenty five tetrapeptide with their unique sequence was built using Accelyrys Dicovery Studio 3.0 visualization software. Further at the N terminal the acetyl, and at C terminal aldehyde groups (for series 1 compound) and fluromethyl ketone (for series2 compounds) groups were added to the tetrapeptide sequence with Marvin Sketch 5.6.0.2.

The three-dimensional structure of caspase-3 [PDB: 1RHK] (Becker et al., 2004) was retrieved from the Protein Data Bank. Before docking of the compounds, the protein was optimized and prepared by removing all bound crystal water molecules and adding hydrogen bonds. Explicit hydrogen was created and bond orders, hybridizations and charges were assigned wherever missing. The resulting structure was saved in .pdb format for docking studies.

Flexible molecular docking of tetrapeptide compounds

Molecular Docking Program-Molegro Virtual Docker 2010.4.0 provided a flexible platform for docking 25 tetrapepyide compounds belonging to both the series. The structure based virtual screening of compounds was based on rerank score which is the mathematical representation for ligand-protein affinity. Rerank score is based on MolDock scoring function (MolDock Score) derived from the Piecewise Linear Potential (PLP) scoring functions(Vuree et al., 2013) (Nayarisseri et al., 2013). Docking parameters were set to 0.20 Å as grid resolution, maximum iteration of 1500 and maximum population size of 50. Energy minimization and hydrogen bonds were optimized after the docking. Simplex evolution was set at maximum steps of 300 with neighborhood distance factor of 1. After the ligand was docked the total energy was minimized using Nelder Mead Simplex Minimization (using non-grid force field and H bond directionality). Binding affinity and interactions of the compound with receptor was evaluated on the basis of the internal electrostatic, hydrogen bond interactions and sp2-sp2 torsions. On the basis of rerank score, best compound in each series with optimal binding affinity was selected against caspase.

Table 1. Combinations of Tetrapeptide Compounds Belonging to Each Series

Tetrapeptide sequence	Series 1 Ac-TETRAPEPTIDE -CHO	Series 2 Ac-TETRAPEPTIDE -FMK
DEGD	Ac-DEGD-CHO	Ac-DEGD-FMK
DEPD	Ac-DEPD-CHO	Ac-DEPD-FMK
IEVD	Ac-IEVD-CHO	Ac-IEVD-FMK
YEVD	Ac-YEVD-CHO	Ac-YEVD-FMK
WEVD	Ac-WEVD-CHO	Ac-WEVD-FMK
LEVD	Ac-LEVD-CHO	Ac-LEVD-FMK
IVVD	Ac-IVVD-CHO	Ac-IVVD-FMK
DVVD	Ac-DVVD-CHO	Ac-DVVD-FMK
YVVD	Ac-YVVD-CHO	Ac-YVVD-FMK
WVVD	Ac-WVVD-CHO	Ac-WVVD-FMK
LVVD	Ac-LVVD-CHO	Ac-LVVD-FMK
IEPD	Ac-IEPD-CHO	Ac-IEPD-FMK
WEPD	Ac-WEPD-CHO	Ac-WEPD-FMK
LEPD	Ac-LEPD-CHO	Ac-LEPD-FMK
IVPD	Ac-IVPD-CHO	Ac-IVPD-FMK
DVPD	Ac-DVPD-CHO	Ac-DVPD-FMK
YVPD	Ac-YVPD-CHO	Ac-YVPD-FMK
WVPD	Ac-WVPD-CHO	Ac-WVPD-FMK
LVPD	Ac-LVPD-CHO	Ac-LVPD-FMK
DHPD	Ac-DHPD-CHO	Ac-DHPD-FMK
DHVD	Ac-DHVD-CHO	Ac-DHVD-FMK
DQPD	Ac-DQPD-CHO	Ac-DQPD-FMK
DEHD	Ac-DEHD-CHO	Ac-DEHD-FMK
EQVD	Ac-EQVD-CHO	Ac-EQVD-FMK
ESVD	Ac-ESVD-CHO	Ac-ESVD-FMK

Lipinski's drug-likeness and toxicity screening

Lazar an online server (Hardy et al., 2010) was used to predict toxicity and Lipinski filter were applied to test the drug-likeness of the compounds.

Results

Analysis of ligand binding affinities

Evident from rerank scores (Table 2), both Series 1 compounds (Acetyl Tetrapeptide Aldehyde) and 2 (Acetyl Tetrapeptide Fluro Methyl Ketone) demonstrated appreciable binding affinity against Caspase 3. A closer

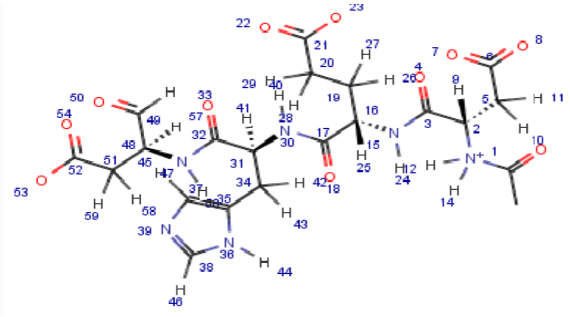

Figure 1. Structure of Ac-DEHD-CHO

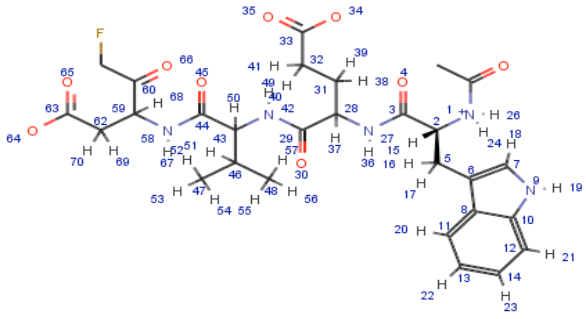

Figure 2. Structure of Ac-WEVD-FMK

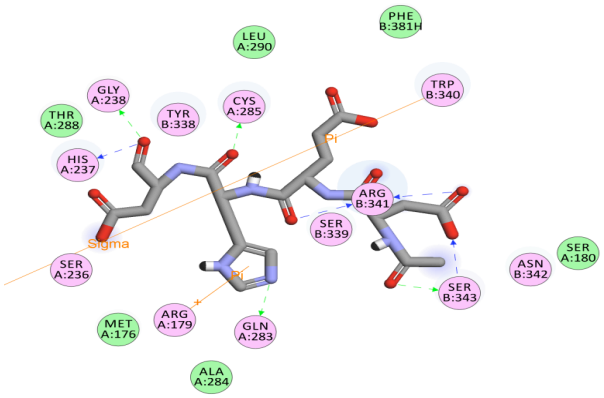

Figure 3. Interactions of Ac-DEHD-CHO with Amino Acids of Caspase 3 Structure

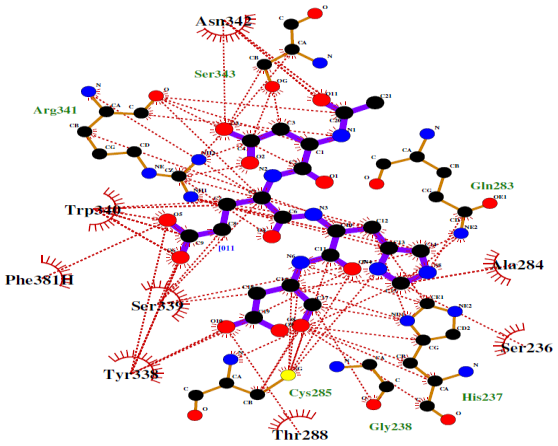

Figure 4. Hydrophobic Interactions of Ac-DEHD-CHO with Amino Acids of Caspase 3 Structure

perusal into the rerank scores showed that Ac-DEHD-CHO (Figure 1) had high binding affinity (Rerank Score: -138.899) in Series 1 compounds while, in case of series 2, Ac-WEVD-FMK (Figure 2) demonstrated higher binding affinity (Rerank Score: -139.317). Though both Ac-DEHD-CHO and Ac-WEVD-FMK had almost similar binding affinity, the latter though not significant, but had marginally higher binding affinity.

Pharmacophoric identification of Ac-DEHD-CHO and Ac-WEVD-FMK

Ac-DEHD-CHO forms six hydrogen bonds with His 237, Gly 238, Cys 285, Gln 179, Gln 283 and two H bonds with Ser 343. The Pi interactions are observed between Trp 340 and Arg 179. Further the compound shows electrostatic interactions with Gly 238, Tyr 338, Cys 285, Trp 340 Asn 342, Ser 343, Gln 283 and vander Waals interactions with Met 176, Ala 284, Thr 288, Leu 280, Phe 381, Ser 180 and Ala 284 (Figure 3). The hydrophobic interactions are observed between Trp 340, Phe 381, Ser 339, Tyr 338, Asn 342, Ala 284 (Figure 4). The electrostatic interactions and Solvent accessibility surface area of Caspase 3 on Ac-DEHD-CHO are shown in Figure 5 and 6.

In the series 2, Ac-WEVD-FMK demonstrated higher binding affinity and it is also marginally greater than Series1 Ac-DEHD-CHO. Keen investigation on ligand receptor interactions shows that seven hydrogen bonds are formed in the caspase with residues *viz,* three H-bonds with Arg 341, two H bonds with Arg 179 and one each with Ser 339 and His237. Further the good affinity between receptor ligand is reflected from electrostatic interactions between Ser 343 and 339, Asn342, Arg 179,

Table 2. Binding Affinity Score (Rerank score) of Tetrapeptides Compounds Belonging to Each Series

	SERIES 1 Ac TetrapeptideCHO			SERIES 2 Ac TetrapeptideFMK		
RANK	Ligand CHO	MolDock Score	Rerank Score	Ligand FMK	MolDock Score	Rerank Score
1	Ac-DEHD-CHO	-190.393	-138.899	Ac-WEVD-FMK	-214.81	-139.317
2	Ac-DQPD-CHO	-177.604	-134.938	Ac-EQVD-FMK	-195.605	-133.597
3	Ac-YVPD-CHO	-178.424	-131.259	Ac-DHVD-FMK	-182.815	-125.66
4	Ac-LEVD-CHO	-187.452	-125.434	Ac-WEPD-FMK	-181.681	-125.453
5	Ac-WVPD-CHO	-183.538	-124.146	Ac-WVVD-FMK	-179.818	-122.32
6	Ac-DEPD-CHO	-177.4	-122.442	Ac-IEPD-FMK	-185	-121.892
7	Ac-YEVD-CHO	-187.525	-121.725	Ac-LEPD-FMK	-173.747	-121.529
8	Ac-IEPD-CHO	-169.777	-120.828	Ac-DEPD-FMK	-195.454	-118.804
9	Ac-LVVD-CHO	-193.223	-119.368	Ac-DQPD-FMK	-199.298	-117.012
10	Ac-DEGD-CHO	-158.669	-115.103	Ac-YEVD-FMK	-181.662	-116.972
11	Ac-WEVD-CHO	-176.406	-114.521	Ac-DEGD-FMK	-174.705	-116.742
12	Ac-IEVD-CHO	-164.686	-113.23	Ac-DEHD-FMK	-172.631	-116.19
13	Ac-DHPD-CHO	-176.542	-112.432	Ac-DVPD-FMK	-176.204	-114.906
14	Ac-DVVD-CHO	-157.135	-112.209	Ac-YVPD-FMK	-170.073	-114.6
15	Ac-YVVD-CHO	-144.974	-110.05	Ac-WVPD-FMK	-178.346	-113.41
16	Ac-WEPD-CHO	-172.443	-109.648	Ac-ESVD-FMK	-150.467	-110.45
17	Ac-DVPD-CHO	-155.111	-107.951	Ac-IEVD-FMK	-160.573	-109.695
18	Ac-LEPD-CHO	-165.295	-105.345	Ac-LVVD-FMK	-158.336	-105.82
19	Ac-EQVD-CHO	-172.987	-104.727	Ac-DVVD-FMK	-157.295	-105.073
20	Ac-WVVD-CHO	-162.023	-103.995	Ac-IVPD-FMK	-151.839	-104.925
21	Ac-DHVD-CHO	-145.789	-96.3371	Ac-YVVD-FMK	-162.784	-104.819
22	Ac-LVPD-CHO	-146.923	-96.3042	Ac-IVVD-FMK	-149.273	-102.398
23	Ac-IVPD-CHO	-144.165	-93.4167	Ac-DHDD-FMK	-157.165	-101.233
24	Ac-IVVD-CHO	-139.929	-78.1558	Ac-LEVD-FMK	-179.309	-97.8763
25	Ac-ESVD-CHO	-125.882	-75.7934	Ac-LVPD-FMK	-158.191	-82.2746

Ser 236 Gly 238 & 287, Ala 284, Met 176 and Thr 177. Vander Waals contacts are observed between Trp 340, Glu 239, Thr 288 Phe 381 and Tyr 338 (Figure 7). The hydrophobic interactions are observed between Phe 381, Tyr 338, Met 176, Ser 339 & 236 and Trp 340 (Figure 8). The electrostatic interactions and solvent accessible surface area of caspase 3 on ligand binding is shown in Figure 9 and 10 respectively.

Drug likeness and toxicity screening.

Both the compounds Ac-DEHD-CHO and Ac-WEVD-FMK passed through Lipinski and Vebers filters implying the compounds are drugs likely. Further the compounds were screened for toxicity by Lazar Toxicity screening program. Both the compounds proved to be non toxic (Table 3).

Since both the compounds are non-toxic and passed

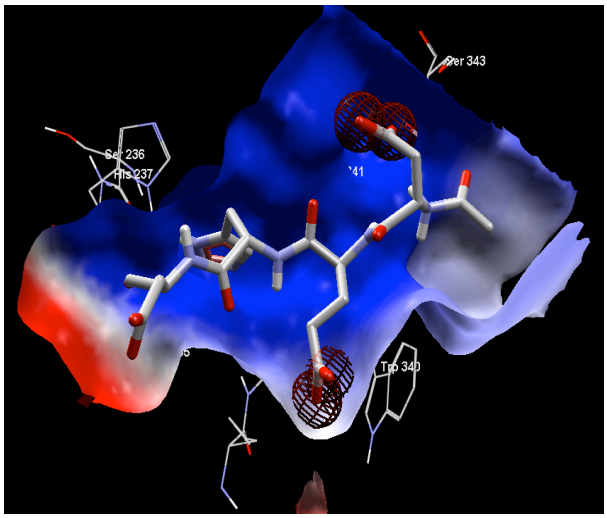

Figure 5. 5Electrostatic Interactions of Ac-DEHD-CHO with Caspase 3 Structure

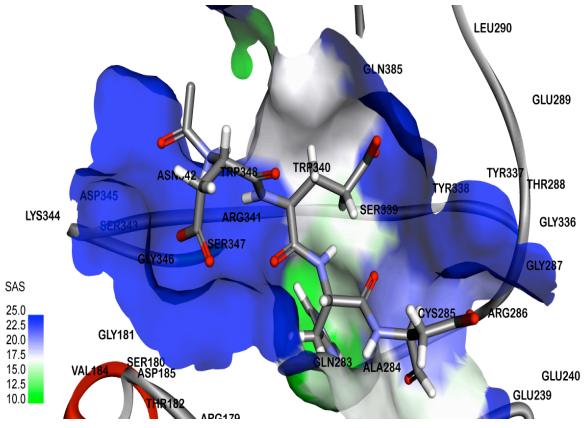

Figure 6. Solvent Accessible Surface Area of Caspase 3 upon Binding of Ac-DEHD-CHO

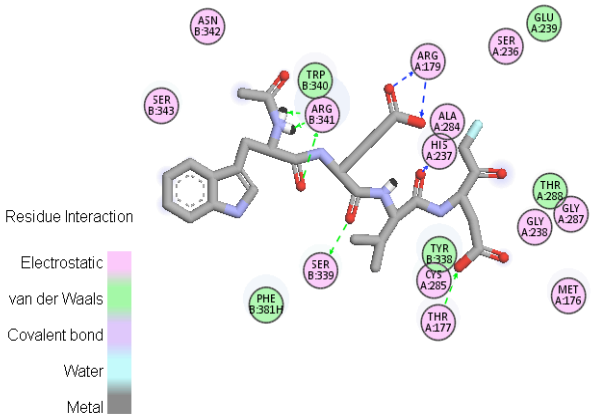

Figure 7. Interactions of Ac-WEVD-FMK with Amino Acids of Caspase 3 Structure

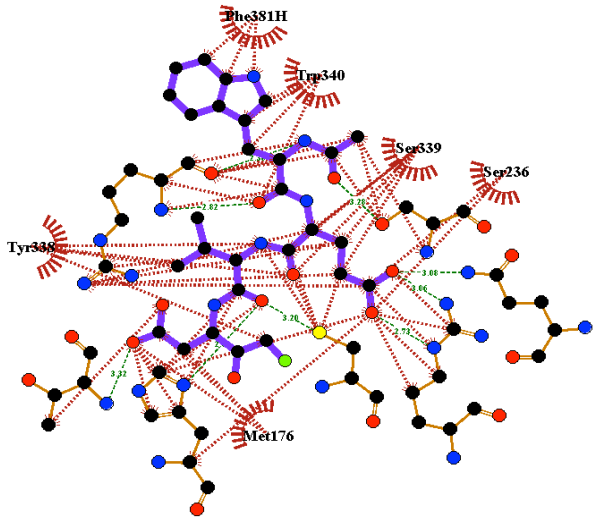

Figure 8. Hydrophobic Interactions of Ac-WEVD-FMK with Amino Acids of Caspase 3 Structure

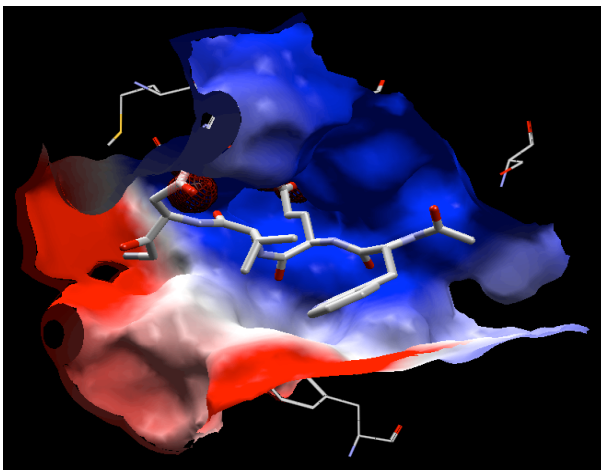

Figure 9. Electrostatic interactions of Ac-WEVD-FMK with Caspase 3 Structure

Table 3. Toxicity Prediction of Compounds Provided by LAZAR Toxicity Prediction Server

Compound	DSSTox Carcinogenic PotencyDBS MultiCellCall	Kazius-Bursi Salmonella mutagenicity	DSSTox Carcinogenic PotencyDBS Mouse
Ac-DEHD-CHO	non-carcinogen	non-mutagenic	non-carcinogen
Ac-WEVD-FMK	non-carcinogen	non-mutagenic	non-carcinogen

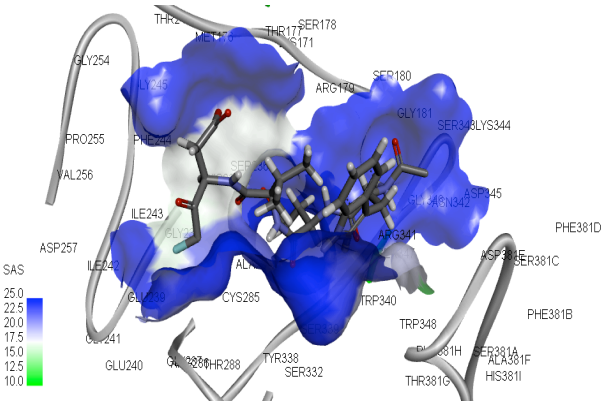

Figure 10. Electrostatic Interactions of Ac-WEVD-FMK with Caspase 3 structure

drug likeness rules and further shows good affinity, the compounds now can be designated as a potent inhibitor nevertheless, need to be tested *in vitro* for further drug development.

Discussion

Caspase inhibitors have now surfaced as important targets to prevent the death of mammalian cells and the research is now speeding up to bring down needless apoptotic events that kills the normal cells. The tetrapeptide caspase inhibitors have taken their stand in potentially inhibiting caspases from triggering cell death. Supplementing to the ongoing research, we in possible attempt proposed two series of 25 acetylated tetrapeptide compounds flanked by Aldehyde or Fluromethyl keto groups. Compound Ac-DEHD-CHO and Ac-WEVD-FMK showed remarkable interactions with caspase 3, further which proved to be non toxic. We anticipate that these two compounds can be put to pharmacodynamic and pharmacokinetic studies in way ahead for successful inhibition of pointless apoptotic events.

Acknowledgements

All the structures were optimized in MarvinSketch 5.6.0.2, (1998-2011, Copyright© ChemAxon Ltd). Flexible Molecular docking of the compounds was achieved through in Molegro Virtual Docker 2010.4.0.0. LAZAR toxicity web server was used for in silico toxicity prediction. Drug likeness of the compound was detected by LIPINSKI filters server of supercomputing facility for Bioinformatics & Computational Biology, Indian Institute of Technology, New Delhi. Accelrys Discovery Studio® Visualizer 3.5.0.12158 (Copyright© 2005-12, Accelyrs Software Inc.) was used for visualization purpose.

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