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Computational study of Arginyl-Glycyl-Asparagine, predicted CD44 targeting agent to be used inPeptide mediated Drug Delivery System

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ABSTRACT: Poor survival rate of patients suffering from cancer has a strong relationship with cancer stem cell marker CD44 and its variants, heterogeneously overexpressed on cell surface.The success of chemotherapy is reliant on precised delivery of therapeutics to the malignant target. Modern drug delivery approaches are utilizing the concept of active targeting using polymeric/inorganic nanocarriers decorated with ligand that has a strong affinity towards the receptor target.Peptide targeted drug/biological macromolecules delivery is a proved and promising approach. In this work docking study of a synthetic short peptide Arginyl-Glycyl-Asparagine(Arg-Gly-Asn) to CD44 has been performed. From the Computational simulation result it is noticeable that the peptide ligand can bind to the site of the receptor similar to that of it's natural ligand hyaluronic acid with considerable affinity. The result reveals that Arg-Gly-Asn can be a novel CD44 targeting peptide to be explored after functionalizing in drug delivery systems.

KEY WORDS: CD44, Hyaluronic acid, Synthetic peptide, Docking, Arginyl-Glycyl-Asparagine

I. INTRODUCTION

Tumor resistancy to drug internalization is one of the key problem of successful treatment.To overcome such barriers in a clever manner can be accomplished effectively by active targeting where an assorment of ligands are exploited to target specific antigens or receptors overexpressed in cancer cells. Aptamers, antibodies, antibody fragments, proteins, amino acids, peptides, polysaccharide etc. are conjugated on the surface of nanocarriers encapsulating the potent chemotherapeutic cargo(s).Efficient targeting is reliant on vast number of factors among which high affinity towards desired target, optimal ligand stability and low immunogenicity play the pivotal roles. Peptides are of bleeding edge interest due to its higher permeation potentiality than high molecular weight antibodies or proteins and more patient compatibility. Significance of peptide is not bound to target tumor rather than some targeting peptides for example Cilengitide [cyclo (RGDfN (Me)V-)], Disruptin (SVDNPH)etc.exert tumor growth inhibitory or suppression activities[1-2]. This reveals to develop peptide conjugated targeting drug delivery systemhaving synergistic action, more significant in multiresistant cancer.According to the mode of action or pharmacological target peptides can be classified into diverse group like tumor vascular system targeting peptides(blood vessel/lymphatic vessel), cell penetrating peptides, macrophage targeting peptides etc. [3]. Although peptides are less susceptible to metabolizing enzymes than proteins, modification of peptide chain terminus, polyethylene glycol conjugation, cyclization, oligomerization or using peptides with unnatural amino acid sequence can enhance the half-life[4]. The flexibility of peptides give the horde of opportunities to attach imaging agents, radioisotopes in its architechture facilitating to develop efficient diagnostic tools. Paramagnetic quantum dots(pQDs) labeled with cyclic Asn-Gly-Arg (cNGR) peptide shown to possess specificity for tumorangiogenicvessels and 3-fold higher detection power than non-peptide conjugated pQDs by molecular MRItechnique[5]. Additionally the simplicity in peptide synthesis processes and cheapness is one of the major concern in the developing nations.

CD44(Cluster of differentiation-44), a transmembrane glycoprotein is associated with varities of malignancies like lung cancer, gallbladder cancer, breast cancer, ovarian cancer, prostate cancer, colon cancer, pancreatic cancer, colorectal cancer and many others.Level of CD44 receptor and it's different isoform with varying cancer type has been shown to instigate EMT (Epithelial-Mesenchymal-Transition) and associated metastasis, cancer cell plasticity.

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Moreover the malignant cells that go through non-motileepithelial to migratorymesenchymal cell transition exhibits enhanced resistance to chemotherapy and apoptosis[6-7].Therefore active targeting of CD44 has been taken into account to block the multiple cell signalling pathways of cancer progression with more speficity and to achieve increased intracellular concentration of drugs/therapeutic molecules.Recent advancements in CD44 targeting is based on neutralizing antibodies, peptide mimetics, CD44 decoys, aptamers although very few peptide mediated targeting strategies have been explored to deliver anticancer agents or therapeutic macromolecules to the CD44 overexpressed tumor cells. A urokinase derived peptideKPSSPPEE, known as A6 peptide when functionalized to polymerosomal anticancer drug exhibit A6 peptide density based antitumor effectivity with remarkable specificity and faster cellular internallization in CD44-positive multiple myeloma[8]. A bio-probe containing RP-1 peptide has been evaluated for the diagnosis of gastric cancer along with prognosis prediction at the early-stage, resulted high sensitivity[9].Based on different evidences of successful clinical interventions mediated by tumor specific peptide-functionalized therapeutic cargo delivery systems and peptide based diagnostic tools it can be stated that there is more need to look into new peptides that can be either natural or synthetic nature.Here L-Arginyl-glycyl-L-asparagine (Arg-Gly-Asn) or Arginyl-glycyl-asparagine tripeptideof molecular weight 345.36 g/molhas been investigated for its affinity towards the CD44 receptor by docking study which has not been reported before. TheArg-Gly-Asn shares 2 similar amino acids in peptide sequence to that of RGD peptide /Arginyl-glycyl-aspartic acid (Arg-Gly-Asp); the most generally utilized endothelial restricting tripeptide with high selectivity towards multiple integrins for tumor-targeting and combination therapy[10].

The primary ligand of the CD44 receptor is hyaluronic acid(HA/hyaluronan); however the mechanism of its binding to the ubiquitous CD44 receptor protein is activation state dependent[11]. After binding to the receptor domain HA incite conformational changes that permit binding of several adaptor proteins/cytoskeletal elements which enact signaling pathways prompting cell adhesion, multiplication, migration and invasion[12]. Therefore here HA has been selected as a control to compare binding affinity of test-peptide molecule.

The result of computational analysis of Arginyl-Glycyl-Asparagine as a targeting agent is not a conclusive report rather than a hypothetical prediction as there is need of more stringent screening by in-vitro and in-vivo evaluation.

II. EXPERIMENTAL METHODS:

Receptor and Ligand: Hyaluronan binding domain of human CD44 structure[PDB ID: 1POZ] has been downloaded from RCSB Protein Data Bank (PDB) [13] where the structure has no mututation. Structure of CD44 target protein was visualized by PyMOL (Figure 1). Arginyl-Glycyl-Asparagine wasdownloaded from PubChem [compound CID: 25252933][14]. Hyaluronic acid [PubChem CID: 24847767] was used as control which is a reported ligand of human CD44[15]. Hyaluronic acid 2D structure obtained was converted into 3D structure prior to docking.

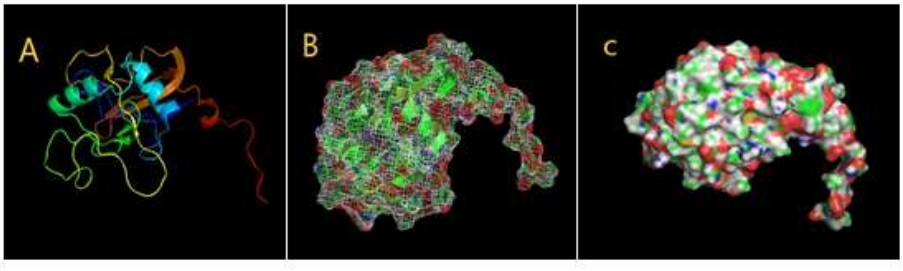

Figure 1. Visualization of target protein 1POZ structure by different representations: A. Biological assembly B. Mesh structure C. Surface structure

Molecular Docking: Docking study has been carried out using Autodock tools (AutoDockTools-1.5.6 and AutodockVina). Before docking both receptor protein and ligand peptide was prepared for docking to avoid errors. SDF file of ligand was converted into PDB file format by PyMOL and followed by conversion into PDBQT file format using AutoDockTools (ADT). Protein was

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downloaded in PDB format and therefore it was converted to PDBQT directly. ADT read pdbqt format during docking command and can generate target files. Before generation of Protein (receptor) PDBQT file, water molecules were deleted from the structure, polar hydrogens and Kollman charges were added to the molecule.Gasteiger charge was assigned to both ligand and receptor.To recognize the binding site blind docking was performed. The Grid box size was set to 36, 42 and 62 along the x, y and z axes respectively with spacing of 1Å and the center was 2, 1, -10 along x,y,z axes[16]. The energy range has been used 3 and exhaustiveness was 8. All other parameter were set to default. Docking complex with 9 different modes were generated which were visualized with ADT tools, PyMOL visualizer as well as Protein Ligand Interaction Profiler(PLIP)[17].

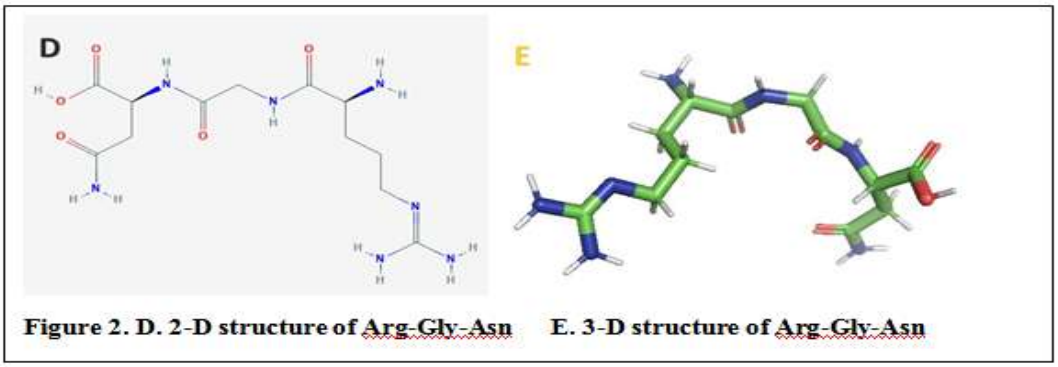

Figure 2. D. 2-D structure of Arg-Gly-Asn E. 3-D structure of Arg-Gly-Asn

III. RESULTS AND DISCUSSION

9Poses were generated after docking simulation.Conformation-1 and conformation-2 of ligand has been shown to associate with highest affinity value of -6.0 kcal/mol (Table-1a). Root mean square deviation between the 1st and 2nd conformation is 27.575Å.The binding pocket related to the 1st conformation matches to the hyaluronic acid binding site[Figure 5].

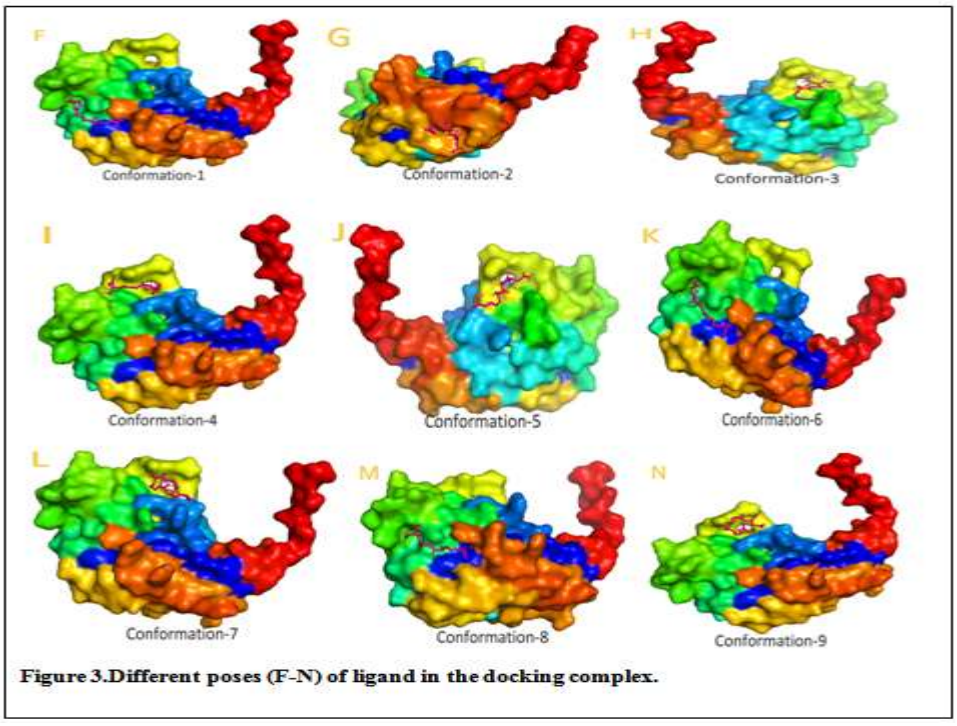

Figure 3.Different poses (F-N) of ligand in the docking complex.

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Table 1a. Output result of Arg-Gly-Asnpeptide docking with receptor

Conformation /Pose	Affinity (kcal/mol)	Distance from RMSD lower Boundary(□)
1	-6.0	0.000
2	-6.0	27.575
3	-5.6	23.571
4	-5.6	18.273
5	-5.6	23.705
6	-5.5	1.552
7	-5.5	20.276
8	-5.4	1.808
9	-5.4	20.850

Table 1b. Output result of hyaluronic acid docking with receptor

Conformation/ Pose	Affinity (kcal/mol)	Distance from RMSD lower Boundary(□)
1	-7.0	0.000
2	-7.0	1.167
3	-6.8	18.742
4	-6.7	3.295
5	-6.6	1.825
6	-6.5	3.242
7	-6.5	1.492
8	-6.5	25.146
9	-6.5	23.789

PLIP showed that the 1st pose of ligand Arg-Gly-Asn interacts to the receptor protein by forming 10 hydrogen bonds and 1 hydrophobic interaction [figure-4].There was no pi-pi or pi-cation interaction.90A ARG and 92A HIS amino acids have formed salt bridges to the carboxylate group of ligand peptide. 91A ILE of receptor protein has participated in hydrophobic interaction with the ligand.

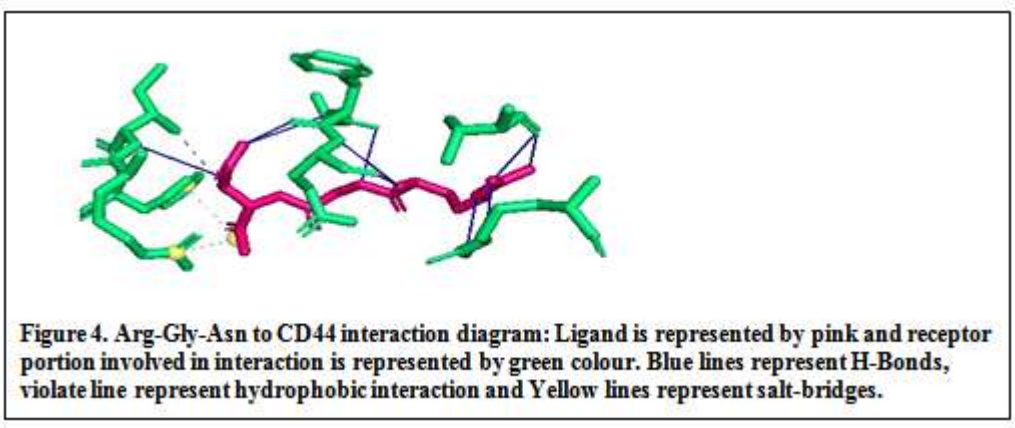

Figure 4. Arg-Gly-Asn to CD44 interaction diagram: Ligand is represented by pink and receptor portion involved in interaction is represented by green colour. Blue lines represent H-Bonds, violate line represent hydrophobic interaction and Yellow lines represent salt-bridges.

Some of the amino acid residuesof the receptor involved in the interaction to the Arg-Gly-Asn peptide are similar to that of the amino acid residues interacts with Hyaluronic acid (Table-3).

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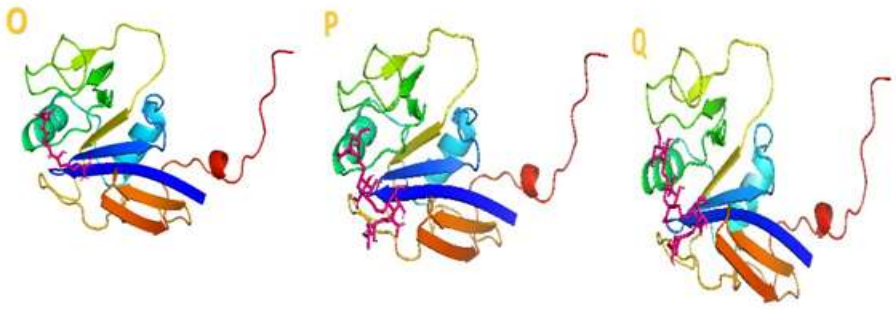

Figure 5. Docking complex with highest binding affinity of ligand to the receptor

O. Arg-Gly-Asn docked to CD44 [1POZ] P. HA (conformation-1) docked to CD44 [1POZ]. Q. HA (conformation-2) docked to CD44[1POZ]

Table 2. Details of hydrogen bond interactions between Arg-Gly-Asn and receptor

SL NO.	RESIDUE	AMINO ACID	DISTANCE H-A	DIATANCE D-A
1	27A	THR	2.27	3.10
2	27A	THR	2.14	3.00
3	73A	GLY	2.50	3.05
4	74A	PHE	3.25	3.91
5	74A	PHE	2.18	2.97
6	75A	GLU	3.27	3.89
7	75A	GLU	3.32	3.91
8	91A	ILE	2.88	3.69
9	150A	ARG	2.80	3.24
10	150A	ARG	2.82	3.32

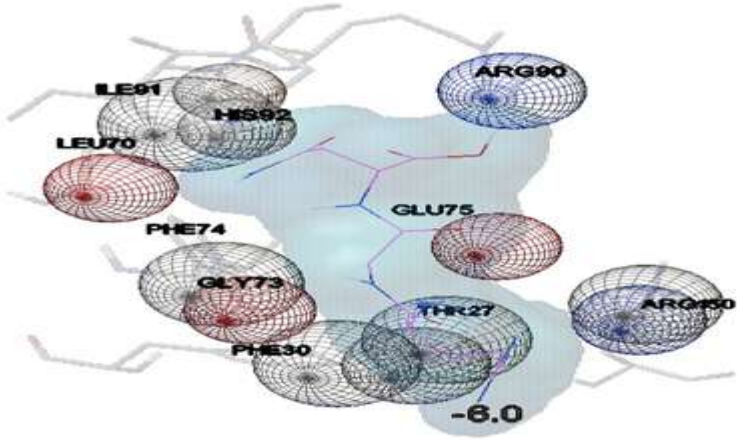

Figure 6. 3-D interaction diagram of peptide ligand Arg-Gly-Asn with receptor amino acids.

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Table 3. Amino acids(AA) of CD44 receptor protein participated in different interaction with ligand

LIGAND	AA IN HYDROGEN BOND	AA IN HYDROPHOBIC INTERACTION	AA IN SALT BRIDGE FORMATION
HA (Conformation-1)	LEU70, ILE72, PHE74, GLU75, ILE91, HIS92, GLU127, CYS129, ARG150	GLU75	ARG90, HIS92
HA (Conformation-2)	THR27, PHE74, GLU75, ARG90, GLU127, CYS129, ARG150, GLY152	LEU70, GLU75, ILE91	HIS92
Arg-Gly-Asn (Conformation-1)	THR27, GLY73, PHE74, GLU75, ILE91, ARG150	ILE91	ARG90, HIS92

IV. CONCLUSION

In this study it is predicted thatArginyl-Glycyl-Asparagine peptide has affinity to the hyaluronan binding domain of CD44 receptor and number of hydrogen bonding as well as hydrophobic interaction exist which can contribute to receptor-ligand complex stabilization.The resulted hit from the docking study need to be subjected to pharmacological evaluation to validate its affinity and potency to proof the suitability of the peptide to be used as targeting agent.

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CONFLICT OF INTEREST There is no conflict of interest.

REFERENCES

[1]. Reardon D A.; and Cheresh, D.: Cilengitide: A Prototypic Integrin Inhibitor for the Treatment of Glioblastoma and Other Malignancies. Genes & Cancer. 2011; 2(12): 1159–1165. DOI: 10.1177/ 194760191 2450586.

[2]. Ahsan, A.; Ray, D.; Ramanand, S. G.; Hegde, A.; Whitehead, C.; Rehemtulla, A.; … and Nyati, M. K.: Destabilization of the Epidermal Growth Factor Receptor (EGFR) by a Peptide That Inhibits EGFR Binding to Heat Shock Protein 90 and Receptor Dimerization. Journal of Biological Chemistry. 2013; 288(37): 26879–26886. DOI:10.1074/jbc.m113.49228.

[3]. LeJoncour, V.; and Laakkonen, P.: Seek & Destroy, use of targeting peptides for cancer detection and drug delivery.Bioorg Med Chem. 2018 Jun 1; 26(10):2797-2806. DOI:10.1016/j.bmc.2017.08.052. Epub 2017 Sep 1. PMID: 28893601.

[4]. Werle, M.; and Bernkop-Schnu¨rch, A.: Strategies to improve plasma half life time of peptide and protein drugs. Amino Acids. 2006; 30: 351–367. DOI 10.1007/s00726-005-0289-3.

[5]. Oostendorp, M.;Douma, K.;Hackeng, TM.; Dirksen, A.; Post, MJ.; van Zandvoort, MA.; and Backes, WH. : Quantitative molecular magnetic resonance imaging of tumor angiogenesis using cNGR-labeled paramagnetic quantum dots. Cancer Res. 2008 Sep 15;68(18):7676-83. DOI: 10.1158/0008-5472.CAN-08-0689. PMID: 18794157.

[6]. Zhao,S.;Chen, C.;...and Freeman, J. W. : CD44 Expression Level and Isoform Contributes to Pancreatic Cancer Cell Plasticity. Invasiveness,and Response to Therapy, Clin Cancer Res. 2016 June 7; 22(22):5592–604. DOI: 10.1158/1078-0432.CCR-15-3115.

[7]. [7].Wang,J.; Wei,Q.; Wang,X.;.....and Luu,H H.: Transition to resistance: An unexpected role of the EMT in cancer chemoresistance.

Genes & Diseases. 2016; 3(1):3-6. DOI:10.1016/j.gendis.2016.01.002.

[8]. Gu,W.; An,J.; Meng,H.; Yu,N.;....and Zhong,Z.:CD44-Specific A6 Short Peptide Boosts Targetability and Anticancer Efficacy of PolymersomalEpirubicin to Orthotopic Human Multiple Myeloma.Adv. Mater. 2019; 1904742. DOI: 10.1002/adma.201904742.

[9]. Li1,W.; Jia2,H.; Wang,J.;...and Lu,S.: A CD44-specific peptide, RP-1, exhibits capacities of assisting diagnosis and predicting prognosis of gastric cancer. Oncotarget. 2017; 8(18):30063-30076. DOI:10.18632/oncotarget.16275.

[10]. Hou, J.; Diao, Y.; Li, W.; Yang, Z.; Zhang, L.; Chen, Z.; and Wu, Y.: RGD peptide conjugation results in enhanced antitumor activity of PD0325901 against glioblastoma by both tumor-targeting delivery and combination therapy. International Journal of Pharmaceutics. 2016; 505(1-2):329–340. DOI:10.1016/j.ijpharm.2016.04.017.

[11]. Alaniz, L.; Cabrera, P. V.; Blanco, G.; Ernst, G.; Rimoldi, G.; Alvarez, E.; and Hajos, S. E.: Interaction of CD44 with Different Forms of Hyaluronic Acid. Its Role in Adhesion and Migration of Tumor Cells. Cell Communication & Adhesion. 2002; 9(3):117–130. DOI:10.1080/15419060214522.

[12]. Chen, C.; Zhao, S.; Karnad, A. and Freeman, J. W.: The biology and role of CD44 in cancer progression: therapeutic implications. Journal of Hematology& Oncology. 2018; 11:64. DOI: 10.1186/s13045-018-0605-5.

[13]. Teriete, P.; Banerji, S.; Noble, M.; .... and Jackson, D.G.:Structure of the Regulatory Hyaluronan Binding Domain in the Inflammatory Leukocyte Homing Receptor CD44. Mol Cell. 2004; 13:483-496. DOI: 10.1016/s1097-2765(04)00080-2.

[14]. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 25252933, Arginyl-glycyl-asparagine; [cited 2021 Feb. 28]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Arginyl-glycyl-asparagine.

[15]. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 24847767, Hyaluronic Acid; [cited 2021 Feb. 28]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Hyaluronic-Acid.

[16]. Sargazi,A.; Kamali,N.; Shiri, F.; and Majd, M. H.:Hyaluronic acid/polyethylene glycol nanoparticles for controlled delivery of mitoxantrone, Artificial Cells, Nanomedicine, and Biotechnology. 2017; 500-509. DOI:10.1080/21691401.2017.1324462.

[17]. Salentin, S.; Schreiber, S.; Haupt, V. J.; Adasme, M. F.; and Schroeder, M.: PLIP: fully automated protein-ligand interaction profiler. Nucl. Acids Res. 2015 July; 43 (W1): W443-W447. DOI: 10.1093/nar/gkv315.

[18]. Daina, A.; Michielin, O.; and Zoete,V.: SwissADME: a free web tool to evaluate pharmacokinetics, Drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports. 7:42717. DOI: 10.1038/srep42717.