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Molecular Insights of Hyaluronic Acid as Potential Source of Polymer-Drug Conjugate in the Target-Mediated Treatment of Cancer

Gnanendra Shanmugam^a, Rajesh Salem Varadharajan^b, Desika Prabakar^c, Syed Mohammed^d, Sathiyapriya Renganathan^e, Murano Erminio^{f,g} and Vincent Aroulmoji^{e*}

^aDepartment of Biotechnology, Yeungnam University, Gyeongsan, South Korea

^bDepartment of Botany, Vivekanandha College of Arts and Science, Tiruchengode, Tamilnadu, India

^cBioinformatics Division, Origene Biosolutions, Salem, Tamilnadu, India

^dDepartment of Chemistry, Sadakathullah Appa College, Tirunelveli, Tamilnadu, India

^eCenter for Research & Development, Mahendra Educational Institutions, Namakkal, Tamilnadu, India

^fProtos Research Institute, Via Flavia 23/1, 34148, Trieste, Italy

^gNealys srl, Via Flavia 23/1, 34148, Trieste, Italy

aroulmoji@gmail.com

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The naturally occurring polysaccharide hyaluronic acid (HA) is a major component of the extracellular matrix and is found over expressed in many cancer cells. Hyaluronic acid is reported to be a potential carrier for drug delivery with the dual advantage of accumulation at the tumor site and receptor-mediated uptake. The use of drugs conjugated with macromolecules was shown to improve the drug pharmacokinetic profile. The various biological potentials such as biodegradability, biocompatibility, non-toxicity, hydrophilicity and non-immunogenicity, together with the availability of various chemical groups that allow the conjugation of drugs, put forward HA as a potential choice for the development of drug conjugates. In this context, the present study is focused to provide, through docking studies, insights on the activity of cancer drugs such as methotrexate, 3',5'-dichloromethotrexate and ornithine-methotrexate and their activity against the receptor caspase-1, which is a well-established drug target in the treatment of cancer. The docking study envisages that the usage of methotrexate properly conjugated to the natural polysaccharide HA might serve as a potential drug to effectively treat some cancer diseases.

Keywords: Hyaluronic acid, Methotrexate, Cancer, Docking studies, Drug-conjugate.

INTRODUCTION

The naturally occurring polysaccharide hyaluronic acid (HA) is a major component of the extracellular matrix and, additionally, it is found in the synovial fluid of joints and scaffolding that comprises cartilages. Peculiarly, one of its cellular receptors (CD44) it is found over expressed in many cancer cells [1,2]. Structurally, HA consists of β -(1 \rightarrow 4)-linked D-glucopyranuronic acid and β -(1 \rightarrow 3)-linked 2-acetamido-2-deoxy-D-glucopyranose. Its basic disaccharide repeating unit presents a carboxyl group at C-5' and two free hydroxyl groups at the C-2' and C-3' positions in the β -D-GlcpA and two hydroxyl groups at C-4 and C-6 position in the β -D-GlcpNAc moiety; chemical and enzyme-catalyzed reactions at some of these positions have led to a wide range of derivatives [3-5].

The well documented physico-chemical and biochemical characteristics such as network-forming and viscoelastic and polyelectrolytic behaviour of HA signifies its importance in contributing the biochemical properties of living tissues and also put forward this polymer as a potential nano carriers for the development of new anticancer drug-conjugates [6]. HA appears to be important in cell-cell interactions and takes part in regulating cell behavior during various morphogenic processes in the body [7]. The role of this natural polysaccharides in diseases such as various forms of cancers, arthritis and osteoporosis has led to the development of both biomaterials for surgical implants and drug conjugates for targeted delivery [8-10]. The use of drugs conjugated with macromolecules improves their pharmacokinetic

profile. The various biological potentials such as biodegradability, biocompatibility, non-toxicity, hydrophilicity and non-immunogenicity together with the presence of chemical groups favoring the conjugations with drugs put forward HA as a potential choice for carrier macromolecules to be employed for the development of drug conjugates.

In cancer disease, some cells display uncontrolled growth, invasion and sometimes metastasis. These malignant properties differentiate cancer from benign tumors, which are self-limited, do not invade or metastasize [11]. The development of cancer is generally considered a multistep process driven by carcinogen-induced genetic and epigenetic damage in susceptible cells, which as a result, gain selective growth damage. Subsequently, cells may undergo clonal expansion as the result of activation of proto-oncogenes and/or inactivation of tumor suppressor genes [12].

Nowadays, various types of cancers are reported spreading according to various mechanisms. The most frequently diagnosed cancers are bone, brain, breast, colon and skin cancers. There are many known causes of cancer like exposure to chemicals, drinking excess alcohol, excessive exposure to sunlight, and genetic differences, to name a few [13]. The caspase are a family of cysteine proteases that are one of the main executors of the apoptotic process and exist within the cell as inactive pro-forms or zymogens. These zymogens can be cleaved to form active enzymes following the induction of apoptosis [14]. The origin of cancer involves deregulated cellular proliferation and the suppression of

apoptotic processes, ultimately leading to tumor establishment and growth. Several lines of evidence point toward the family of proteins playing a role in oncogenesis, via their effective suppression of apoptosis. The central mechanisms of apoptotic suppression appear to be through direct caspase and pro-caspase inhibition.

Thus, the present study is focused to provide insights, through docking studies, on the activity of cancer drugs such as methotrexate, 3',5'-dichloromethotrexate and ornithine-methotrexate and their activity against the receptor of caspase-1, a well established drug target in the treatment of cancer.

RESULT AND DISCUSSION

Globally, it is estimated that deaths from cancer will raise to 13.1 million in 2030 [15,16] with lung, stomach, liver, colon and breast cancers being the most prominent causes for cancer deaths each year. Even though a number of advancing techniques have been put forward in delineating the molecular and cellular biology of cancer cell, the urgency in the treatment of metastatic diseases remains challenging. Due to certain limitation such as low specificity and efficacy, and high toxicity of anticancer drugs, great effort is devoted to the research of improved therapeutics based on molecular targeted approaches as alternatives to treat malignant tumor cells.

In most of the target-based therapeutics, the drugs are made to differentiate the malignant and healthy tissues on the basis of their physiological and biochemical differences. Most prominently, the poor intra-tumor lymphatic drainage and the vascular leakage are exploited by employing passive drug delivery systems that allow large molecules, such as polymeric conjugates [17], liposomes [18], micro-particles, and nanoparticles [19], to accumulate at the tumor site. In addition to this, due to the biochemical differences of cancerous and normal tissues, the usage of conjugated drugs may be proposed to selectively target over-expressed tumor specific receptors. Interestingly, evidence was provided that the conjugation of drugs with macromolecules enhances the pharmacokinetic profiles of the drugs themselves [20]. Furthermore, the sustained high intra-tumor drug levels and its lower plasma concentrations are driven by the slow release of the drug from the macromolecular carrier. This process can be effectively achieved by employing specific macromolecules with biodegradable linkage and suitable spacer, which can hold a potent anticancer agent that can preferentially be released within the tumor tissue [21]. The natural polysaccharide hyaluronic acid (HA) has the dual advantage of a potential accumulation at the tumor site and of a receptor-mediated uptake. Thus the anticancer activity of methotrexate, 3',5'-dichloromethotrexate, ornithine-methotrexate and its HA-drug conjugates HA-methotrexate, HA-3',5'-dichloromethotrexate and HA-ornithine-methotrexate against caspase-1, a well-established drug target in the treatment of cancer, was explored through docking studies.

The origin of cancer involves the deregulation of the cellular proliferation and the suppression of apoptotic processes that ultimately leads to the establishment of tumors and cell growth; caspase-1, as the main executor of the apoptotic process, exists within the cell as inactive pro-form or zymogen [22]. Since the central mechanisms of apoptotic suppression appear to involve direct caspase and pro-caspase inhibition, the choice of caspase-1 as potential cancer target is significantly worthy to be explored as alternative therapeutic choice of drugs that can exhibit their efficacy through the delivery by a polymer-drug conjugate. The crystal structure of the human caspase-1 [23] in complex with malonate,

determined by X-ray crystallography resolved at 1.80^Å with PDB ID: 1SC3, is shown in Figure 1. The drug target interactions between methotrexate, 3',5'-dichloromethotrexate, ornithine-methotrexate and HA-drug conjugates such as HA-methotrexate, HA-3',5'-dichloromethotrexate and HA-ornithine-methotrexate were explored through docking studies.

Ligands: The 2D structures of potential anticancer drugs such as methotrexate, 3',5'-Dichloromethotrexate and Ornithine-methotrexate and the HA-drug conjugates HA-methotrexate, HA-3',5'-dichloromethotrexate and HA-ornithine-methotrexate sketched in ACD ChemsSketch along with the HA dimer and HA-peptide linker (Phe-Phe used in HA-drug conjugates) are shown in Figure 2.

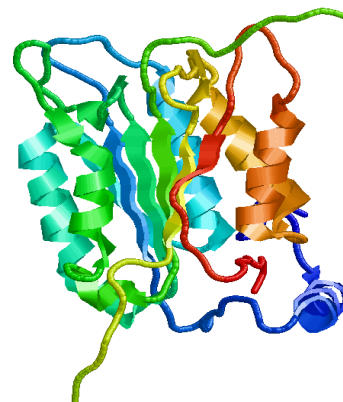


Figure 1: Crystal structure of the human caspase-1 (PDB ID: 1SC3).

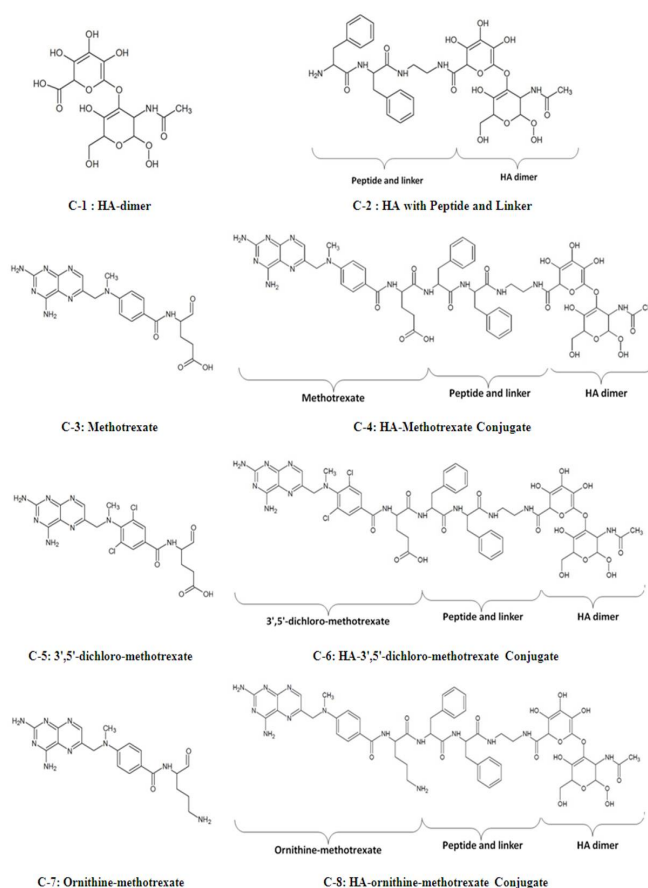


Figure 2: 2D structures of HA-dimer, HA-peptide linker, anti-cancer drugs and HA-drug conjugates used in docking studies.

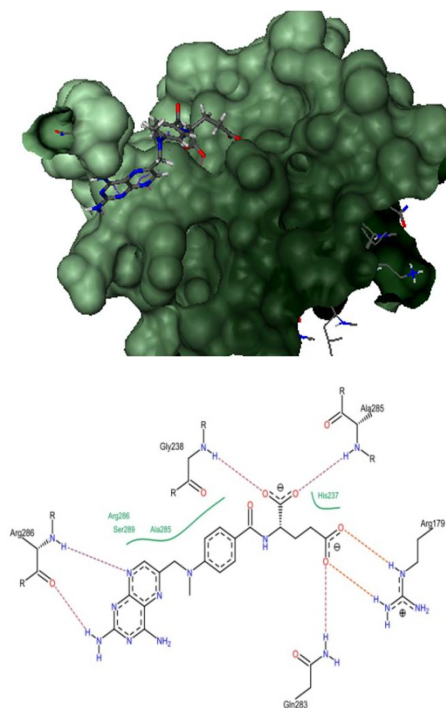


Figure 3: Docking complex and interactions of methotrexate within the active site of human caspase-1 (Dock score: -26.9011 kJ/mol).

Docking interactions of methotrexate: The compound methotrexate docked within the active site of human caspase-1 exhibited the dock score of -26.9011 kJ/mol. It was observed that the compound occupied the ligand binding site of malonate as in the crystal structure of human caspase-1 by favoring the H-bond interactions and non-bonded interactions. The amino acids Arg179, Gly238, Gln283, Ala285 and Arg286 favored the H-bond interactions and the non-bonded interactions are supported by His237, Ala285, Arg286 and Ser289. The docking complex and docking interactions of methotrexate within the active site of human caspase-1 is shown in Figure 3.

Docking interactions of 3',5'-dichloromethotrexate: The compound 3',5'-dichloromethotrexate docked within the active site of human caspase-1 exhibited the dock score of -21.6994 kJ/mol. It was observed that the compound occupied the ligand binding site of human caspase-1 by favoring the H-bond interactions with amino acids such as Arg179, Gly238, Gln283 and Ala285 while non-bonded interactions were exhibited by His237, Ala285, Arg286 and Ser289. The docking complex and docking interactions of 3',5'-dichloromethotrexate within the active site of human caspase-1 is shown in Figure 4.

Docking interactions of ornithine-methotrexate: The compound ornithine-methotrexate docked within the active site of human caspase-1 exhibited the dock score of -17.5100 kJ/mol. It was observed that the compound occupied the ligand binding site of malonate as in the crystal structure of human caspase-1 by favoring the H-bond interactions and non-bonded interactions. The amino acids Arg179, Gly238, Gln283, Arg286 and Ser289 favored the H-bond interactions and the non-bonded interactions are supported by His237, Gln283, Ala284, Ala285 and Arg286. The docking complex and docking interactions of ornithine - methotrexate within the active site of human caspase-1 is shown in Figure 5.

Docking interactions of HA: The compound HA docked within the active site of human caspase-1 exhibited the dock score of

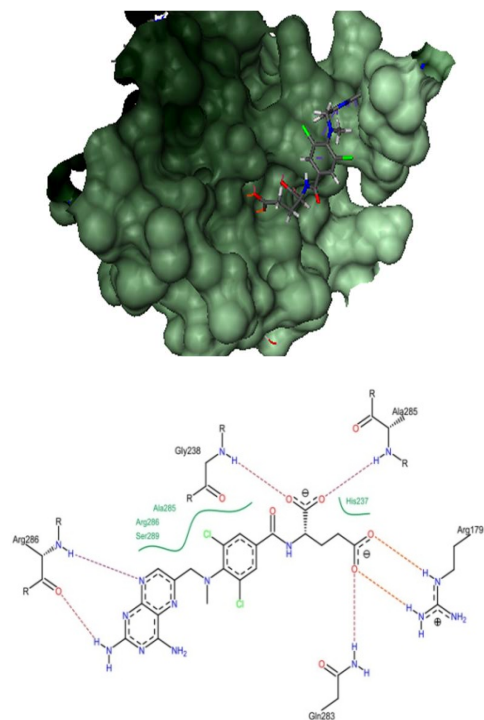


Figure 4: Docking complex and interactions of 3',5'-dichloromethotrexate within the active site of human caspase-1 (Dock score: -21.6994 kJ/mol).

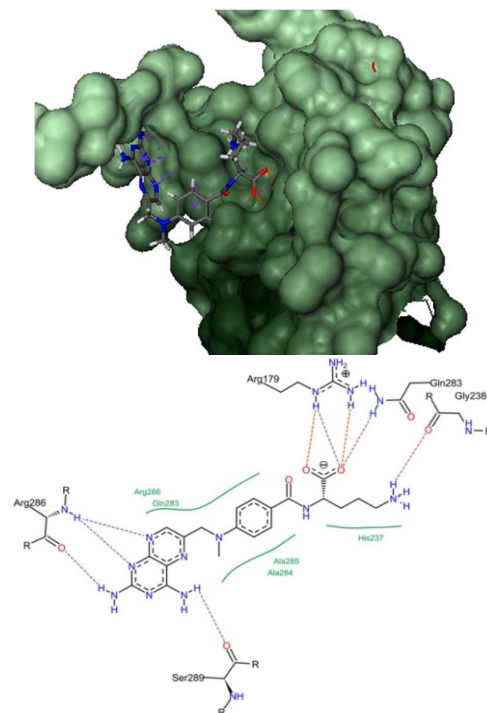


Figure 5: Docking complex and interactions of ornithine-methotrexate within the active site of human caspase-1 (Dock score: -17.5100 kJ/mol).

-24.4711 kJ/mol. It was observed that the compound partially occupied the ligand binding site of malonate as in the crystal structure of human caspase-1 by favoring the H-bond interactions and non-bonded interactions. The amino acids Arg179, Gly238, Gln283, Ala285 and Gly287 favored the H-bond interactions while non-bonded interactions are supported by His237, Gly238, Ala284 and Ala285. The docking complex and docking interactions of HA within the active site of human caspase-1 are shown in Figure 6.

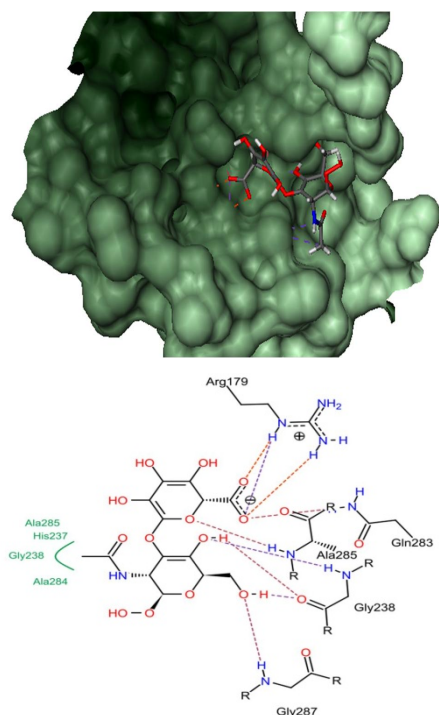


Figure 6: Docking complex and interactions of HA dimer within the active site of human caspase-1 (Dock score: -24.4711 kJ/mol).

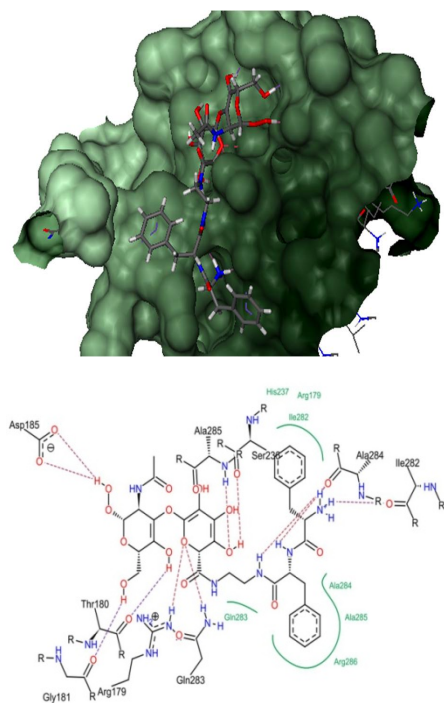


Figure 7: Docking complex and interactions of HA with Phe-Phe peptide linker within the active site of human caspase-1 (Dock score: -26.2475 kJ/mol).

Docking interactions of HA-peptide (Phe-Phe) linker:

The compound HA-Phe-Phe peptide docked within the active site of human caspase-1 exhibited the dock score of -26.2475 kJ/mol. It was observed that the compound occupied the ligand binding site of malonate as in the crystal structure of human caspase-1 by favoring the H-bond interactions and non-bonded interactions. The amino acids Arg179, Thr180, Gly181, Asp185, Ser236, Ile282, Gln283, Ala284 and Ala285 favored the H-bond interactions while non-bonded interactions are favored by Arg179, His237, Ile282, Gln283,

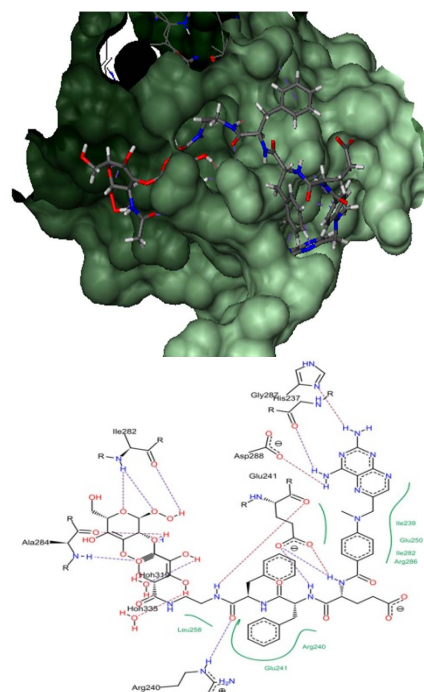


Figure 8: Docking complex and interactions of HA-methotrexate conjugate within the active site of human caspase-1 (Dock score: -34.3024kJ/mol).

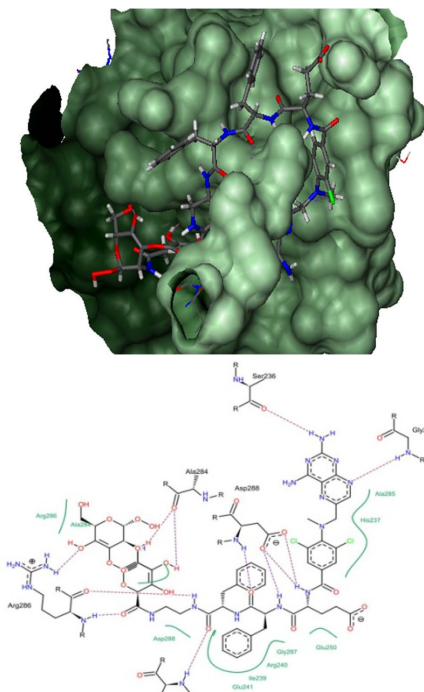


Figure 9: Docking complex and interactions of HA-3',5'-dichloromethotrexate conjugate within the active site of human caspase-1 (Dock score: -31.5882kJ/mol).

Ala284, Ala285 and Ala286. The docking complex and docking interactions of HA-peptide linker within the active site of human caspase-1 is shown in Figure 7.

Docking interactions of HA-methotrexate conjugate:

The compound HA-methotrexate conjugate docked within the active site of human caspase-1 exhibited the dock score of -34.3024kJ/mol. It was observed that the compound occupied the ligand binding site of malonate as in the crystal structure of human caspase-1 by favoring the H-bond interactions and non-bonded interactions. The amino acids Arg179, Gly238, Gln283, Ala285 and Arg286 favored the

Table 1: Docking interactions (H-bond & non-bonded) and respective dock scores of the compounds within the predicted binding pocket of caspase-1 (*H-bond interactions).

C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
-	-	-	-	-	-	-	*Glu171
-	-	-	-	-	-	-	Glu171
-	-	-	-	-	-	-	*Glu172
-	-	-	-	-	-	-	Arg178
*Arg179	*Arg179	*Arg179	-	*Arg179	-	*Arg179	*Arg179
-	Arg179	-	-	-	-	-	Arg179
-	*Thr180	-	-	-	-	-	-
-	*Gly181	-	-	-	-	-	*Gly181
-	-	-	-	-	-	-	*Ala182
-	-	-	-	-	-	-	Ala182
-	-	-	-	-	-	-	Glu183
-	-	-	-	-	-	-	*Glu183
-	-	-	-	*Arg286	-	-	*Val184
-	-	-	-	-	-	-	Val184
-	*Asp185	-	-	-	-	-	*Asp185
-	-	-	-	-	-	-	Asp185
-	*Ser236	-	-	-	*Ser236	-	-
-	-	-	*His237	-	-	-	-
His237	His237	His237	-	His237	His237	His237	His237
*Gly238	-	*Gly238	-	*Gly238	*Gly238	*Gly238	-
Gly238	-	-	-	-	-	-	-
-	-	-	Ile239	-	Ile239	-	-
-	-	-	*Arg240	-	-	-	-
-	-	-	Arg240	-	Arg240	-	-
-	-	-	*Glu241	-	-	-	-
-	-	-	Glu241	-	Glu241	-	-
-	-	-	Glu250	-	Glu250	-	-
-	-	-	Leu258	-	-	-	-
-	*Ile282	-	*Ile282	-	-	-	-
-	Ile282	-	Ile282	-	-	-	-
*Gln283	*Gln283	*Gln283	-	*Gln283	-	*Gln283	*Gln283
-	Gln283	-	-	-	-	Gln283	Gln283
-	*Ala284	-	*Ala284	-	*Ala284	-	-
Ala284	Ala284	-	-	-	Ala284	Ala284	-
*Ala285	*Ala285	*Ala285	-	*Ala285	-	-	-
Ala285	Ala285	Ala285	-	Ala285	Ala285	Ala285	Ala285
-	-	*Arg286	-	-	*Arg286	*Arg286	-
-	Arg286	Arg286	Arg286	Arg286	Arg286	Arg286	-
*Gly287	-	-	*Gly287	-	-	-	-
-	-	-	-	-	Gly287	-	-
-	-	-	*Asp288	-	*Asp288	-	*Asp288
-	-	-	-	-	Asp288	-	-
-	-	-	-	-	*Ser289	*Ser289	-
-	-	Ser289	-	Ser289	-	-	-
-24.4711	-26.2475	-26.9011	-34.3024	-21.6994	-31.5882	-17.5100	-28.7427

C-1: HA dimer; C-2: HA-peptide linker; C-3: methotrexate; C-4: HA-methotrexate conjugate; C-5: 3',5'-dichloro-methotrexate;
C-6: HA-3',5'-dichloro-methotrexate conjugate; C-7: ornithine-methotrexate; C-8: HA-ornithine-methotrexate conjugate.

H-bond interactions and the non-bonded interactions are supported by His237, Ala285, Arg286 and Ser289. The docking complex and docking interactions of HA-methotrexate conjugate within the active site of human caspase-1 are shown in Figure 8.

Docking interactions of HA-3',5'-dichloromethotrexate conjugate: The compound HA-3',5'-dichloromethotrexate conjugate docked within the active site of human caspase-1 exhibited the dock score ligand binding site of human caspase-1 by favoring the H-bond interactions with amino acids such as Arg179, Gly238, Gln283 and Ala285 while non-bonded interactions were exhibited by His237, Ala285, Arg286 and Ser289. The docking complex and docking interactions of HA-3',5'-dichloromethotrexate conjugate within the active site of human caspase-1 is shown in Figure 9.

Docking interactions of HA-ornithine-methotrexate conjugate: The compound HA-ornithine-methotrexate conjugate docked within

the active site of human caspase-1 exhibited the dock score of -28.7427 kJ/mol. It was observed that the compound occupied the ligand binding site of malonate as in the crystal structure of human caspase-1 by favoring the H-bond interactions and non-bonded interactions. The amino acids Arg179, Gly238, Gln283, Arg286 and Ser289 favored the H-bond interactions and the non-bonded interactions are supported by His237, Gln283, Ala284, Ala285 and Arg286. The docking complex and docking interactions of HA-ornithine-methotrexate conjugate within the active site of human caspase-1 is shown in Figure 10.

The docking interactions of HA dimer, HA-peptide (Phe-Phe) linker, methotrexate, 3',5'-dichloromethotrexate, ornithine-methotrexate, HA-methotrexate conjugate, HA-3',5'-dichloromethotrexate conjugate and HA-ornithine-methotrexate conjugate with their docking scores were given in Table 1.

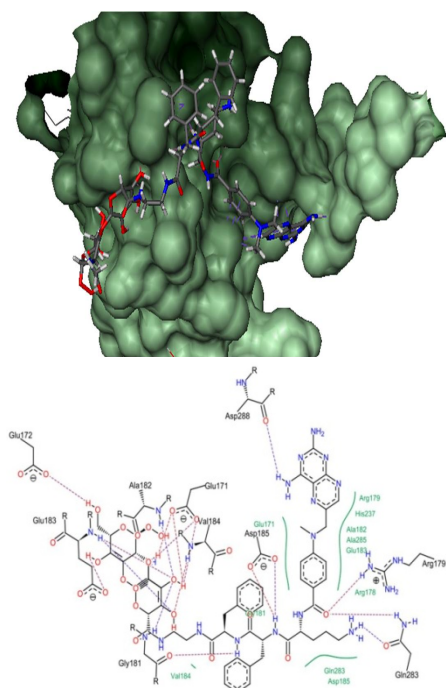


Figure 10: Docking complex and interactions of HA-ornithine-methotrexate conjugate within the active site of human caspase-1 (Dock score: -28.7427 kJ/mol).

Theoretically, it was observed that all the compounds exhibited better binding efficacy in terms of highest dock score, which significantly reveals the lowest binding energy of stable confirmation. The best dock score of -34.3024 kJ/mol was exhibited by HA-methotrexate conjugate, while the poor dock score of -17.5100 kJ/mol was obtained for ornithine-methotrexate interactions with caspase-1. It is note-worthy to mention that the dock score of anti-cancer drugs methotrexate, 3',5'-dichloromethotrexate and ornithine-methotrexate is poor when compared to the dockscore of the corresponding HA-drug conjugates HA-methotrexate, HA-3',5'-dichloromethotrexate and HA-ornithine-methotrexate, thus indicating that these compounds would certainly possess a better activity when employed as HA-drug conjugate. It is also worth to mention that the dock score of -24.4711 kJ/mol and -26.2475 kJ/mol, exhibited by HA dimer and HA-peptide linker molecules, are almost the same as the dock score of -26.7427 exhibited by methotrexate within the binding pocket of human caspase-1. This finding suggests that the HA-dimer and the use of a peptide linker for the design of HA-drug conjugate would also plays a major role in drug interactions.

The HA-drug conjugates designed to specifically target overexpressed CD44 in lung carcinoma cells as reported by Akima *et al.*, [24] reveals the uptake of a fluorescent HA conjugate, of HA-conjugated mitomycin C (MMC), and of epirubicin.

Similarly, HA-butyrate and HA-paclitaxel conjugates showed increased apoptosis activity [25, 26] and inhibited cell growth in vitro, with significant decrease in tumor burden in vivo [27]. However, only few studies on HA-drug conjugation were reported to be promising. In this regards, the docking study reported here indicates that H-bond interactions favored by Arginine (Arg179 and 286), Glycine (Gly238) and Glutamine (Gln283) plays a crucial role in binding the methotrexate, 3',5'-dichloromethotrexate, ornithine-methotrexate compounds with human caspase-1. While the amino acids Aspartic acid (Asp288) plays a crucial role in binding the HA-methotrexate, HA-3',5'-dichloromethotrexate and HA-ornithine-methotrexate compounds, the amino acid Arginine (Arg286) is

found to be crucial in favoring the non-bonded interactions with all the compounds.

These docking interactions imply that the keto group (C=O) of the compounds and the amino group (NH) on the amino acids of caspase-1 molecule favor the H-bond interactions. In line with this, the efficacy of the conjugation of methotrexate, 3',5'-dichloromethotrexate and ornithine-methotrexate with HA macromolecules would certainly envisages that, as anti-cancer agent, HA-methotrexate is a better choice and might be employed in the treatment of various cancers and also provides a suggestion for the selection of novel HA-based anti-cancer agents with more target specificity.

CONCLUSIONS: As the estimated deaths from cancer continues to raise to 13.1 million in 2030 with lung, stomach, liver, colon and breast cancers being the most prominent causes for cancer deaths each year, the identification of potential drug conjugate with dual advantages of accumulation at the tumor site and receptor-mediated uptake would serve as a potential alternative to effectively treat cancer. In this scenario, the naturally-occurring polysaccharide hyaluronic acid (HA), a major component of the extracellular matrix whose cellular receptor CD44 is over expressed in many cancer cells, was selected as a potential carrier of anticancer drugs to synthesize new selective, potent drug-conjugates. Thanks to its biological potentials such as biodegradability, biocompatibility, non-toxicity, hydrophilicity and non-immunogenicity, and the presence, in the macromolecular backbone, of chemical groups that may favor drug conjugations make HA a potential choice for the development of anticancer drug conjugates. Thus, in the present study the binding efficacy of the selected cancer drugs such as methotrexate, 3',5'-dichloromethotrexate and ornithine-methotrexate and their activity against the receptor caspase-I, were explored through docking studies. The docking study envisages that the Aspartic acid (Asp288) and Arginine (Arg286) plays a crucial role in favoring H-bond and non-bonded interactions with all the compounds. The binding efficacy of the methotrexate, 3',5'-dichloromethotrexate and ornithine-methotrexate and the HA conjugate of these drugs has revealed that the usage of methotrexate as HA-methotrexate conjugate might represent a potential alternative to effectively treat cancer through the caspase-1 pathway.

Methodology

Potential drug Target: The 3D structure of caspase-1, that plays a central role as an executor of the apoptotic process, is retrieved from PDB [28]. The crystal structure of the human caspase-1 in complex with malonate, determined by X-ray crystallography resolved at 1.80\AA with PDB ID: 1SC3 was selected as a potential drug target.

Binding Site determination: To determine the interactions of methotrexate, 3',5'-dichloromethotrexate, ornithine-methotrexate and HA-drug conjugates such as HA-methotrexate, HA-3',5'-dichloromethotrexate and HA-ornithine-methotrexate with crystal structure of the human caspase-1, the potential binding pockets were predicted by submitting the co-ordinate file to DoGsite scorer [29]. The potential binding sites with predicted high drug score value was considered as potential binding site for further docking studies.

Drugs and HA-drug conjugates: The 3D structures of specified cancer drugs such as Methotrexate, 3',5'-Dichloromethotrexate and Ornithine-methotrexate were retrieved in SDF format from Pubchem database [30]. Further, the 2D structures of HA-drug

conjugates such as HA-Methotrexate, HA-3',5'-Dichloromethotrexate and HA-Ornithine-methotrexate were sketched by using ACD ChemsSketch and their respective 3D structures were converted into SDF by using 'Online Smiles translator'.

Molecular Docking: All the compounds in SDF file format were docked with the amino acids in the predicted binding pocket of human caspase-1 by using the following parameters [31]: (i) default docking information's; (ii) base placement using triangle matching; (iii) scoring of full score contribution and threshold of 0,30 and No score contribution and threshold of 0,70;(iv) chemical parameters of clash handling values for protein ligand clashes with

maximum allowed overlap volume of 2.9 Å³ and intralegend clashes with clash factor of 0.6 and considering the hydrogen in internal clash tests; and (v) default docking details values of 200 for both the maximum number of solutions per iteration and maximum number of solutions per fragmentation.

Ligand- Receptor Interactions: The interactions of the compounds Methotrexate, 3',5'-Dichloromethotrexate, Ornithine-methotrexate and HA-drug conjugates such as HA-Methotrexate, HA-3',5'-Dichloromethotrexate and HA-Ornithine-methotrexate with human caspase-1 in the docked complex were analyzed by the pose-view of LeadIT [32].

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