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Molecular Docking and Drug-Likeness for the Identification of Inhibitory Action of Acetogenins from *Annona muricata* as Potential Anticancer against Hypoxia Inducible Factor 1 Alpha

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Hypoxia inducible factor 1 alpha (HIF-1α) regulates cell growth and differentiation which is implicated in human cancers. HIF-1α activates its cascade carcinogenesis mechanism in cancer cells. It is well-understood that signaling is initiated by HIF-1± receptor. Overexpression of HIF-1α is associated with several different human cancers, including breast cancer, lung cancer and colon cancer. Thus, HIF-1α becomes potential target of therapeutic approach in developing HIF-1α inhibitors. The aim of this research is to investigate potential inhibitors which are known as Acetogenins (AGEs) isolated from *Annona muricata* against HIF-1α. In order to achieve this goal, chemical structures of all compounds were retrieved from PubChem database. Molecular docking was performed by AutoDock Vina program and the resulting binding modes were analyzed with AutoDock Tools program. Among all the compounds, murihexocin A showed the best binding modes compared to other two inhibitors based on the lowest binding energies (LBE = -7.9 kcal/mol) as high as gefitinib. This was indicating that murihexocin A has favorable interaction with the essential amino acid residues at catalytic site of HIF-1α. Drug-likeness calculation of AGEs were also performed. These in silico results could be beneficial as a compound model for further studies in-vitro and in-vivo.

Keywords: HIF-1α, *Annona muricata*, inhibitor, acetogenins, molecular docking, drug-likeness.

Hypoxia inducible factor-1 (HIF-1) is a transcription factor that controls the expression of gene that involved in tumorigenesis and metastases of malignancies [1, 2]. The HIF-1α level could be enhanced within breast oncogénesis, and it closely related with other tumor biomarkers [3]. HIF-1α plays an important role in binding the consensus sequence 52 -RCGTG-32 (which R is purine) at the response elements of hipoxia to target genes [4]. The transcription process of various genes are activated by HIF-1, including glycolytic enzymes, gluconeogenesis, mediating glucose transporters, growth factors, high-energy phosphate metabolism, heme metabolism, iron transport, erythropoiesis,

synthesis of nitric oxide, and regulation of vasomotor. Therefore, HIF-1 possibly promotes the tumor cell viability in hypoxic circumstances [5, 6].

Hypoxia induces tumor cell proliferation, metastasis, and the rate of cell apoptosis [7]. Moreover, HIF-1 is considered as a starting point of angiogenic process in tumor cells by transcription activation of cancer-related gene, such as vascular endothelial growth factor (VEGF) gene [8]. The level of HIF-1α escalates the pathological stage which is higher in poorly differentiated lesions than in well-differentiated lesions [3]. The enhanced levels of HIF-1α are strongly related with high proliferation and enhanced ER as well as VEGF expressions [9]. Therefore, the high level of HIF-1α has potency in associating with further massive tumors [3].

Natural bioactive compounds which are derived from plants have been used for maintaining health and remedies in many years. The phytochemical constituents in plants have been a critical pipeline for the discovery of bioactive substances in pharmaceutical field [10]. *A. muricata* or Graviola has been greatly pressumed to have valuable natural products that play important role in affecting anticancer activity [11]. *A. muricata* leaves have been used to investigate of numerous numbers of human diseases, including cancers [10]. The highly constituents screening are most possibly affected by its major bioactive components known as annonaceous Acetogenins (AGEs) [12]. Many studies reported that isolated AGEs from different extracts of the plants have significant antiproliferative effects against various cancer cell lines [10]. However, some of these studies have defined the staple mechanism of action. Recent in-vitro studies showed inhibition action of ethyl acetate extract from *A. muricata* leaves combating lung cancer cells (A549) and colon cancer cells (HCT-116 and HT-29) [10, 13]. The leaf extract was capable of inducing colon carcinoma and lung cancer cells apoptosis by way of mitocondrial route. This antiproliferative effect was associated with cell cycle involved in the G1 phase. Moreover, the migration and invasion of colon cancer cells were significantly halted by the leaf extract [14-16].

The aim of this research is to determine the inhibition mechanism by bioactive compounds of *A. muricata* interact with HIF-1α. To study the binding interactions of bioactive compounds with HIF-1α through molecular docking methods. Computational methodologies have become a crucial component in drug discovery program, which involves identification to lead optimization. Molecular modeling is one of the methodologies primarily used as hit identification tool when only structure of target and its active or binding site are available [17]. Docking method is an energy-based scoring function which identifies the energetically most favorable ligand conformation that binds to the target [18].

METHOD

Protein structure preparation

The amino acid sequence of HIF-1α (Entry PDB code : 4z1v) was retrieved from RSCB Protein Database [19]. The attached ligand in the protein structure was removed from the binding site and saved to a new file format: pdbqt. The Gasteiger charges and the solvation condition were added to the protein structure using the AutoDockTool [20].

Ligand structures preparation

Ligands which are AGEs consisting of eight 3D structures of natural bioactive compounds originally belong to *A. muricata* and one anticancer drug for molecular docking experiments and their conformational energy were minimized by using MMFF94 force field. 8 molecules of AGEs. The molecule structures are retrieved from PubChem database (Fig. 3). The structures were scored based on their physicochemical properties under Chemicalize (ChemAxon) and Molsoft platforms [21, 22]. These physicochemical properties are important for developing drug candidate in every stages from design to pre-clinical study.

Drug likeness analysis of *A. muricata* bioactive compounds

3D structures of Cinchona alkaloids were analyzed using a program based on the physicochemical properties, Molsoft Drug - Likeness. Determination of physicochemical properties is important in the development of drug candidates in all stages ranging from study design through pre- clinical trials [22].

Molecular docking of HIF-1α and *A. muricata* bioactive compounds

The three dimension structure of protein and ligands were prepared in pdb format. Molecular

docking simulation was run by Autodock Vina (Vina, The Scripps Institute) [23]. The AutodockTools (ADL) was utilized in minimizing energy and adding the partial charges of polar hydrogens of receptor (protein). The ligands were prepared with flexible torsion angles and the protein was prepared in a static (rigid) form. Furthermore, protein and ligands were kept in pdbqt formats which suitable for docking simulation. The affinity binding were calculated as total intermolecular energies (kcal/mol) which involved hydrogen bond, Van Der Walls force, desolvation and electrostatic energies. On the other hand, the apropriate torsion angles of ligand is also induced as internal ligand energy. The docking program evaluated the lowest binding energy (LBE) to obtain the best binding mode. The Root-Mean-Square Deviation (RMSD) which less than 2.0 Å was scored during running docking program.

RESULTS

Bioactive compounds isolated from *A. muricata* , including annomuricin A, annomuricin B, annomuricin C, annomuricin E, annomutacin, murihexocin A, murihexocin B, murihexocin C, and gefitinib (Figure 1) were docked into binding pocket of HIF-1α.

The lowest binding energy (LBE) to the target protein was murihexocin A (-7.9 kcal/mol). The binding interactions between *A. muricata* bioactive compounds with of HIF-1α binding pocket residues were analysed as shown in Table 1.

From docking result, murihexocin A compound interacted hydrophobically with Thr-183, Trp-296, Tyr-102, His-199, Gln-147, Gln-203, Glu-202, Ser-184, Pro-235, Gln-239, Tyr-103, and Ile-281 in the binding pocket of HIF-1α.

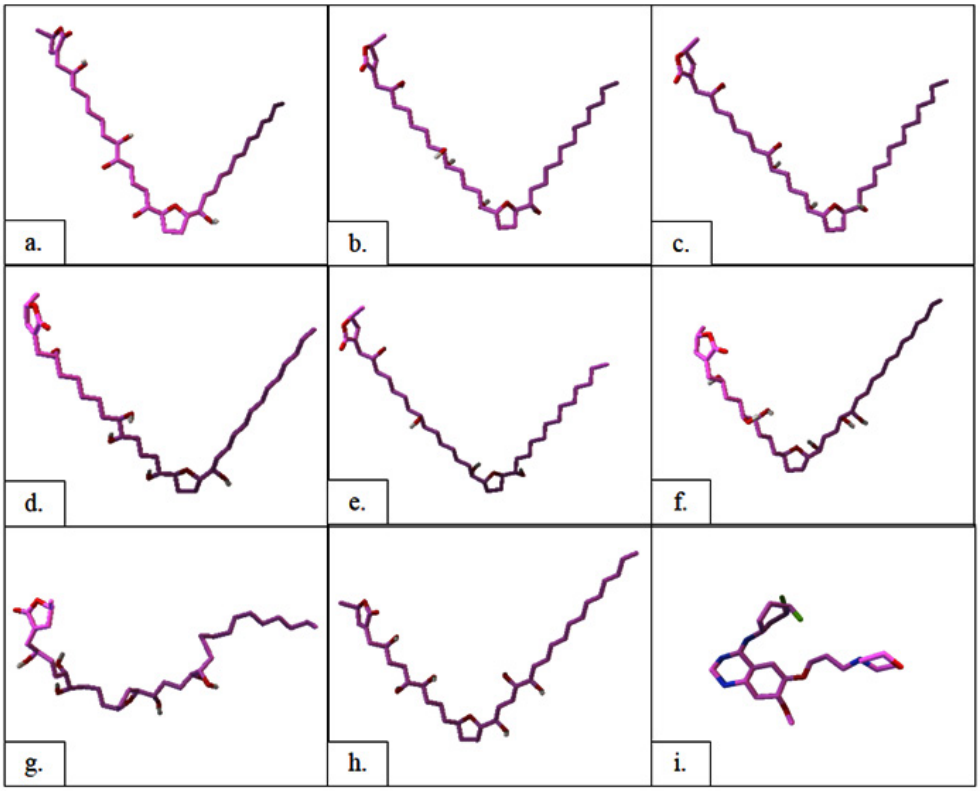


Fig. 1. AGEs compound of *A. muricata* leaves: a. annomuricin A; b. annomuricin B; c. annomuricin C; d. annomuricin E; e. annomutacin; f. murihexocin A; g. murihexocin B; h. murihexocin C; i. gefitinib

The molecular interaction of murihexocin A with HIF-1α is illustrated in Fig. 2. Murihexocin A interacted with HIF-1α was stabilized by hydrogen bond between nitrogen atom of carboxamide group of Ser-184 side chain with hydrogen from hydroxyl group of murihexocin A.

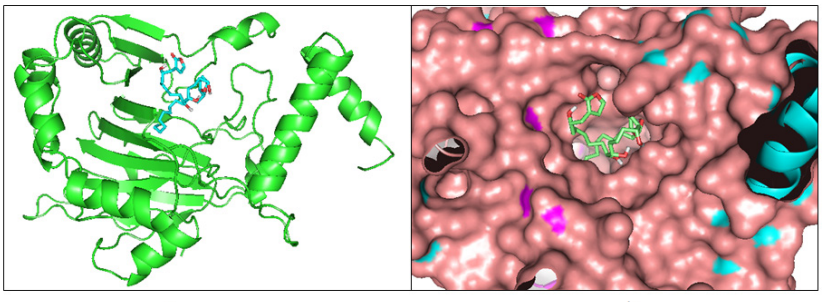
Drug likeness properties of *A. muricata* bioactive molecules and comercial anticancer drug molecule, gefitinib were calculated using Molsoft Drug – Likeness program.

DISCUSSIONS

The analysis of molecular docking has shown that the selected bioactive compounds interacted at similar site as triterpene with a different binding mode. The calculated lowest binding energy (LBE) values of the protein-ligand complexes are exhibited in Table 1. LBE is combined energy of the intermolecular energy and the free energy torsion which indicating the likeable interactions and strong binding with

Table 1. Binding interactions between *A. muricata* bioactive compounds with of HIF-1± binding pocket residues

Compound	LBE (kcal/mol)	H-bonding	Hydrophobic Interaction with HIF-1α residues
Annomuricin A	-6.7	3	Tyr-93, Tyr-102, Asp-104, Leu-186, Leu-188, Gln-147, His-199, Phe-207, Ile-281, Asp-201, Arg-238, Gln-239, Trp-296, His-279
Annomuricin B	-7.2	2	Thr-149, Thr-183, Ser-184, Leu-186, Trp-296, Ile-281, Gln-203, Asp-201, Arg-238, His-199, Gln-239, Tyr-102, Thr-196, His-279
Annomuricin C	-7.1	2	Ser-184, Asn-294, Asn-205, Trp-296, Tyr-93, Tyr-102, Arg-238, Asp-104, Asp-237, Gln-239, Tyr-103
Annomuricin E	-7.3	2	Trp-296, Gln-203, Asp-201, Arg-238, Asp-237, Pro-235, Gln-239, Tyr-102, Ile-281, Leu-188, Asn-294, Thr-196, Tyr-103, Thr-196, Lys-214
Annomutacin	-6.9	0	Thr-183, Ser-184, Gln-203, Trp-296, Leu-186, Arg-238, His-199, Tyr-102, His-279, Thr-196, Ile-281, Gln-147, Trp-296, Ser-184
Murihexocin A	-7.9	1	Thr-183, Trp-296, Tyr-102, His-199, Gln-147, Gln-203, Glu-202, Ser-184, Pro-235, Gln-239, Tyr-103, Ile-281
Murihexocin B	-6.7	0	Tyr-93, Asp104, Tyr-102, Trp-296, Leu-166, Ser-184, Arg-238, Asp-201, Gln-203, Glu-202, Tyr-93, Asp-104, Gln-239
Murihexocin C	-7.6	2	Gln-147, Thr-196, His-199, Gln-203, Arg-238, His-279, Asn-294, Ser-184, Phe-207, Gln-203, Trp-296, Leu-188
Gefitinib	-7.9	2	Gln-147, Thr-196, His-279, Asn-294, Ser-164, Gln-203, Arg-238, Gln-239, Tyr-102, Tyr-103, Phe-207



a. b.

Fig. 2. a. Complex interaction between murihexocin A and HIF-1α in ribbon-stick form; b. Complex interaction between murihexocin A and HIF-1α in HIF-1α binding pocket

Table 2. Drug likeness properties of *A. muricata* bioactive molecules and commercial anticancer drug molecule

Compound	Drug Likeness	Log P	Molecular weight (g/mol)	TPSA (A^2)	Stereocenter number**	Violation of Lipinski's Rule**
Annomuricin A	-0,08	5.65	646.40	28.29	0	1
Annomuricin B	0.04	5.7	628.88	65.52	0	1
Annomuricin C	-0,99	4.81	648.42	112.24	0	1
Annomuricin E	-0.24	4,33	646.47	28.29	0	0
Annomutacin	0.04	4.81	647.45	65.52	0	0
Murihexocin A	-0,99	4,33	628.88	112.24	0	0
Murihexocin B	-0.24	4.81	648.41	28.29	0	0
Murihexocin C	0.04	4,33	648.40	65.52	0	0
Gefitinib	-0.24	4,33	446.15	56.07	0	0

main amino acid residues at the binding pocket of the receptor. On the other hand, LBE also performed the intermolecular energy which was calculated based on the set of total energy which involved hydrophobic interaction, hydrogen bond interaction, electrostatic potential and desolvation free energy.

In order to find the best lead as anticancer agent from *A. muricata* bioactive compounds, we evaluated drug likeness properties of eight AGEs compounds compared with one commercial anticancer drug compound, gefitinib. It was found that all bioactive compounds had one violation of Lipinski's rule of five, based on molecular weight. All molecular weight was above 500 g/mol, which means too big as a drug. However, according to docking result, murihexocin A was the best lead as anticancer agent and it could be used as a model for further analysis both in-vitro and in-vivo.

Approximately 133 acetogenins (AGEs) from different medicinal plants, such as *Annona muricata, Annona squamosa* Linn., *Asiminatriloba* (paw paw), and *Cherimolia* were reported have in-vitro anticancer activities against various cancer cell lines. Some AGEs such as asimin, asiminecin, asiminocin, and asiminacin have shown exceptionally high cytotoxicity for malignancies in three major tissues: breast, lung, and colon. Moreover, in-vivo data have been documented along with the tumor cell types, animal used, route of administration and dosage information. Some AGEs including annonacin, desacetyluvaricin, bullatacin, and bullatalicin have demonstrated significant in-vivo tumor growth inhibitory activities [24-26].

CONCLUSION

Medicinal plants play important roles in the development of modern therapeutic agents. This study conclusively demonstrated that *A. muricata* was a good natural source of various phytochemical constituents. On the basis of our results, it can be concluded that the annonaceous AGEs were powerful phytochemicals found in *A. muricata*, which offers protective effect against cancer. In agreement with the lowest binding energy, annomuricin A, annomuricin B, annomuricin C, annomuricin E, annomutacin, murihexocin A, murihexocin B, murihexocin C and gefitinib were found -6.1, -7.2, -7.1, -7.3, -6.9, -7.9, -6.7, -7.6 and -7.9 kcal/mol, respectively. Murihexocin A showed similar LBE value with gefitinib. Thus, murihexocin A was selected to be the best lead as anticancer agent in silico. These in silico results could be beneficial as a compound model for further experimentally in-vitro and in-vivo assays to elucidate the exact mechanism of inhibitory activity and to examine its potential therapeutic effects.

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