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Original Article

THE MOLECULAR INTERACTION AND ADMET PREDICTION OF MODIFIED JPH203 AS A POTENTIAL RADIOPHARMACEUTICAL KIT FOR MOLECULAR IMAGING OF CANCER: AN *IN SILICO* RESEARCH

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ABSTRACT

Objective: In this study, various types of pharmacokinetic modifying linkers and chelators are combined with JPH203 to obtain the best-docked molecule for prospective radiopharmaceutical kits.

Methods: AutoDock 4.2.6 and AutoDockTools 1.5.6 programs was used to do the molecular docking simulation and ADMET prediction was done using VNN-ADMET to predict the pharmacokinetics and toxicity of the ligand.

Results: The result of this study showed that JPH203-linker K-NOTA has the best affinity with a docking score of about-10.7 kcal/mol and shows hydrogen interaction with Tyr259, which acts as key residue of the active site.

Conclusion: Based on the results, JPH203-linker K-NOTA has good potential as a radiopharmaceutical kit of cancer.

Keywords: JPH203, LAT-1, Molecular docking, ADMET, *In silico*

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INTRODUCTION

Cancer is a condition of abnormal cellular growth and proliferation. The cancer cells invade the surrounding tissue and can metastasize to other parts of the body. Currently, cancer is the second leading cause of death in the world (18.1 million cases of cancer in the world and 9.6 million cases of cancer deaths). Globally, one in six deaths is caused by cancer and 70% of cancer deaths occur in middle to low-income country [1].

Early detection and treatment of cancer is a big challenge. Recently, the cancer treatment paradigm has shifted towards personalized medicine with the discovery of targeted drugs, where a variety of new targeted drugs were developed clinically, but this has not made cancer treatment fulfill the expected pharmacological effect due to the heterogeneity of biological markers. These biomarkers are ideal drug targets in the future [2, 3]. Much attention has been pointed to the target molecules that are suitable for cancer diagnosis and therapy, one of these molecules is LAT-1 [4, 5].

LAT-1 can distribute eight of the nine essential amino acids to certain body areas such as the placenta and blood-brain barrier so that LAT-1 is said to be one of the main proteins in cell growth and development [6]. In addition, LAT-1 has overexpression and is highly selective against cancer cells, but its distribution is limited to normal cells [7]. The overexpression of LAT-1 in cancer cells and its ability to deliver substrates makes LAT-1 become a promising target for therapy and diagnostic (theranostic) molecules (fig. 1) [8, 9].

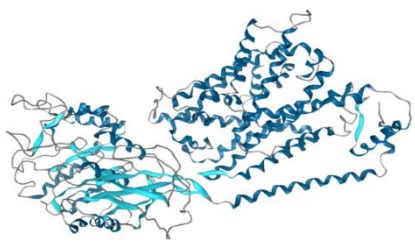


Fig. 1: The structure of large amino acid transporter type-1 (LAT-1) complexes with 4F2Hc [9]

Various compounds that have inhibitory activity on LAT-1 (e. g. JPH203), has a function as a tyrosine analog that can bind to LAT-substrates and showed a selective inhibiting effect on LAT-1 based on its *in vitro* and *in vivo* assays in mice with HT-29 tumors (colon cancer). JPH203 was also effective to inhibit various types of cancer [6]. So that JPH203 is promising to be a new anti-cancer agent [9].

Currently, a tool used in cancer diagnosis and therapy has been developed, namely theranostic radiopharmaceuticals, which are able to identify and treat various diseases (e. g. cancer, infectious diseases) [10]. The developments of theranostic radiopharmaceuticals need the ligands to bind with radionuclide and some require a bifunctional chelator to stabilize the metal-ligand complex [11]. To optimize the stability and activity of the radiopharmaceutical kit, we develop the various combination of JPH203 (carrier agent), pharmacokinetics modifier linker, and bifunctional chelator, which is docked to the LAT-1 structure through *in silico* studies. Nowadays, there has been no study that has been docked modified JPH203 with linker and chelator against LAT-1 in the development of radiopharmaceutical kit formulation.

MATERIALS AND METHODS

Hardware and software

A personal computer with 6-cores 12-threads AMD Ryzen 5 2600, 3.4 GHz CPU with Radeon RX 570 GPU and 16 GB RAM was employed and equipped with the following software for in silico studies:

1. The ChemOffice 2010 and ChemDraw Ultra 12.0 software (PerkinElmer Inc., downloaded from http://www.cambridgesoft.com) for drawing 2D structures and convert them into 3D structures,

2. AutoDock 4.2.6 and AutoDockTools 1.5.6 programs (The Scripps Research Institute, downloaded from http://www.autodock.scripps.edu) for molecular docking simulations,

3. BIOVIA Discovery Studio 2017 R2 Client (Dassault Systèmes, downloaded from http://www.accelrys.com/) for visualization of PDB complex, the bond between ligands and receptors, geometry optimization, and overlays during the validation process,

4. VNN-ADMET to predict the pharmacokinetics and toxicity of radiopharmaceutical kits.

Designing radiopharmaceutical structures

The 3D structure of the LAT-1 was downloaded from Protein Data Bank (PDB) (http://www.rscb.org, PDB ID: 6IRT). As a result of electron microscopy, LAT-1 (6IRT) was complexed with BCH molecules. The separation was performed using the BIOVIA Discovery Studio 2017 R2 Client. The 3D structure of the ligands (Radiopharmaceutical Kits, JPH203 Based) was drawn and has been optimized using ChemOffice 2010, and ChemDraw Ultra 12.0 (table S1, Supplementary Data).

Validation of docking method

The validation of molecular docking was conducted through a "re-docking" procedure of the native ligand of 6IRT to its active site. The procedure is valid if the root mean square deviation (RMSD)<2.0 Å [12, 13]. The results of validation process is shown in table 1.

Molecular docking simulation

LAT-1 and all ligands were docked using AutoDock 4.2.6 and AutoDockTools 1.5.6 programs. Ligands with the lowest bond energy (ΔG) to 6IRT were selected and each interaction was further characterized by Biovia Discovery Studio. The ligands used were the modified structure of JPH203 with linkers and chelators that were used to bind radionuclides to form a complex.

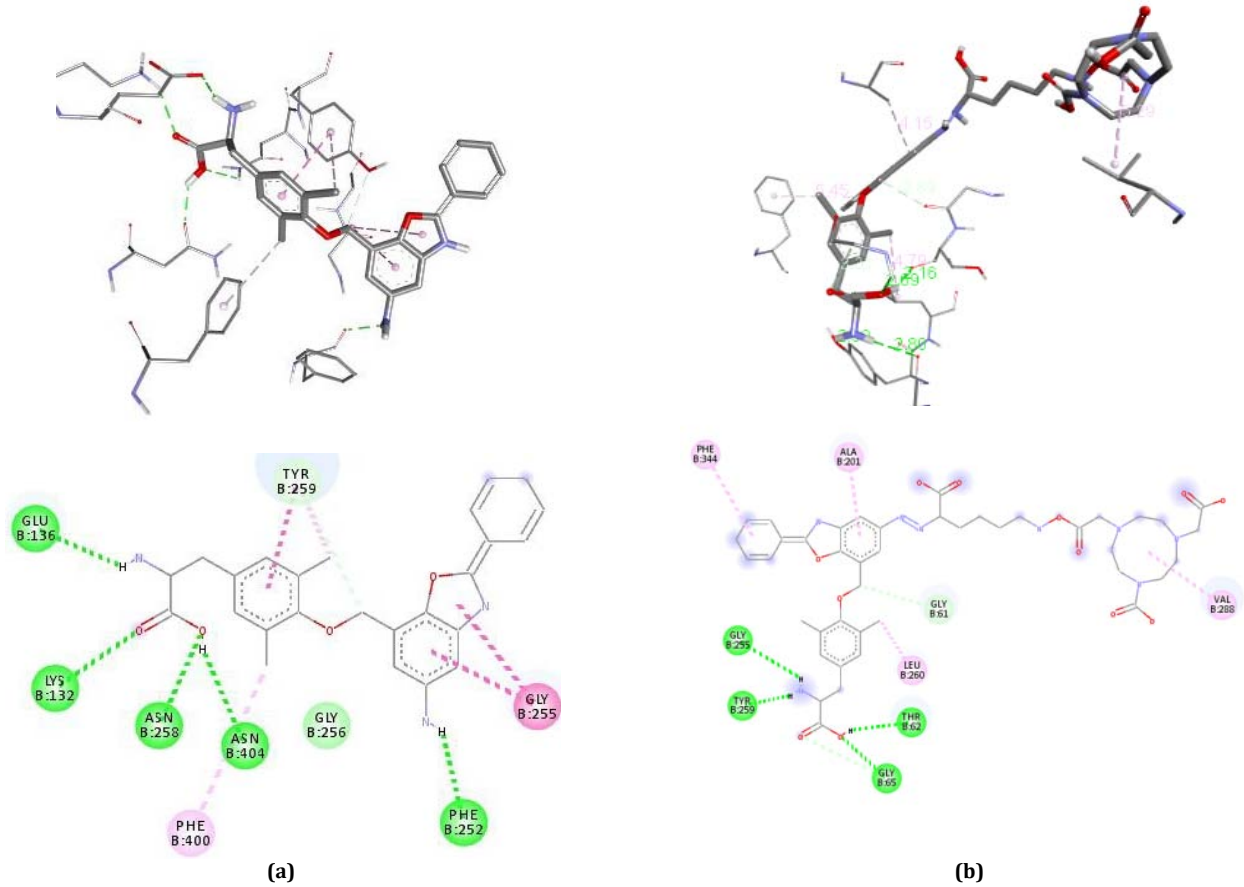
ADMET prediction

ADME and Toxicity predictions including drug-induced liver injury (DILI), cytotoxicity (HepG2), HLM, CYPs inhibitor, blood-brain barrier (BBB), p-gp inhibitor, p-gp substrate, cardiotoxicity, mitochondrial toxicity (MMP), Mutagenicity (AMES), maximum recommended therapeutic dose (MRTD) and significant descriptors of drug properties such as mutagenicity, toxicological dosage level for different tissues, and pharmacologically relevant properties of the compounds were predicted using web applications at vNN-ADMET (https://vnnadmet.bhsai.org/vnnadmet/login.xhtml) by Schyman [14].

RESULTS

Table 1: Validation of docking method to the LAT-1

Native ligand	Grid address (x,y,z)	ΔG (kcal/mol)	RMSD (Å)	Interaction (H-Bond)
N1	(155.98, 120.75, 190.07)	-3.11	4.832	Arg452, Asn507, Ser505, Ala448, Leu520, Ser519, Gln 513, Met508
N2	(147.38, 154.55, 206.56)	-4.52	4.608	Ser383, Asp385, Gln388, Asn382, Ser404
N3	(143.61, 177.30, 186.04)	-3.99	3.878	Asn366, Ser395, Ser359, Glu363
N4	(120.89, 152.65, 188.29)	-3.24	4.479	Asn425
N5	(146.32, 143.11, 134.34)	-5.25	1.827	Gly67, Ser66, Ile64, Tyr289, Gly255, Phe252
N6	(123.02, 140.49, 124.78)	-3.37	3.505	-
N7	(125.27, 129.23, 119.46)	-1.85	3.277	Cys496



(a) **(b)**

Fig. 2: Visualization of the docked ligand with LAT-1 (a) Interaction of LAT-1 amino acid with JPH203 as a lead compound (b) Interaction of LAT-1 amino acid with JPH203-K linker-NOTA as the best-docked molecule

Table 2: Molecular docking parameters of JPH203 and Its derivatives

Ligand			ΔG	KI (μM)	Hydrogen interaction
JPH203	**Linker**	**Chelator**	**(kcal/mol)**		
	-	-	-9.4	1.2E-07	Glu136, Lys132, Asn258, Asn404, Phe252
	E linker	NOTA	-10.2	3.3E-08	Asp198, Gly61, Ser66, Tyr259,
		DOTA	-10	4.6E-08	Phe344, Gln197
		TETA	-10.6	1.7E-08	Thr194, Gln197, Gly61, Gly65, Gly255, Gly67, Ser66
		CTPA	-10	4.6E-08	Gly255, Ser338, Gln197, Thr62
		H2CB-DO2A	-10.2	3.3E-08	Phe344, Leu343
		H2CB-TE2A	-9	2.5E-07	Thr154, Thr62, Lys204, Gly61, Gln197
	K linker	NOTA	-10.7	1.4E-08	Thr62, Gly65, Tyr259, Gly255
		DOTA	-10.1	3.9E-08	Ala202, Ile63, Thr62
		TETA	-9.2	1.8E-07	Phe344, Thr194, Gln197
		CTPA	-9.5	1.1E-07	Gly337, Thr62, Gly61
		H2CB-DO2A	-9.2	1.8E-07	Thr62, Lys204
		H2CB-TE2A	-10.5	2E-08	Gly65, Gly255
	PEG4 linker	NOTA	-9.8	6.4E-08	Gln197, Thr62
		DOTA	-8.8	3.5E-07	Asp198
		TETA	-9.7	7.6E-08	Thr62, Gln197
		CTPA	-9.6	9E-08	Leu343
		H2CB-DO2A	-10.1	3.9E-08	Leu205, Gly337, Gln197, Thr62
		H2CB-TE2A	-10	4.6E-08	Gly255, Gln197

Table 3: ADMET prediction of JPH203 and its derivatives

Query	DILI	Hepar Cyto-toxicity	HLM	Cyp1A2 Inh	Cyp3A4 Inh	Cyp2D6 Inh	Cyp2C9 Inh	Cyp2C19 Inh	BBB	P-gp Inh	P-gp Subs	hERG Blocker	MMP	AMES	MRTD (mg/day)
JPH203	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	No	No	No	293
JPH203-E-CTPA	Yes	No	Yes	No	No	No	No	No	Yes	Yes	Yes	Yes	No	No	324
JPH203-E-DOTA	Yes	No	Yes	No	No	No	No	No	No	No	Yes	Yes	No	No	346
JPH203-E-DO2A	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	No	No	No	347
JPH203-E-TE2A	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	No	No	No	344
JPH203-E-NOTA	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	Yes	No	No	319
JPH203-E-TETA	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	Yes	No	No	327
JPH203-K-CTPA	Yes	No	Yes	No	No	No	No	No	Yes	No	Yes	Yes	No	No	314
JPH203-K-DOTA	No	No	Yes	No	No	No	No	No	No	No	Yes	Yes	No	No	393
JPH203-K-DO2A	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	No	No	No	336
JPH203-K-TE2A	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	No	No	No	335
JPH203-K-NOTA	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	Yes	No	No	310
JPH203-K-TETA	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	Yes	No	No	329
JPH203-PEG4-CTPA	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	Yes	No	No	385
JPH203-PEG4-DOTA	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	Yes	No	No	414
JPH203-PEG4-DO2A	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	No	No	No	424
JPH203-PEG4-TE2A	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	No	No	No	418
JPH203-PEG4-NOTA	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	Yes	No	No	391
JPH203-PEG4-TETA	Yes	No	No	No	No	No	No	No	No	Yes	Yes	Yes	No	No	408

DISCUSSION

The electron microscopy structure of LAT-1 (in complex with BCH inhibitor and 4F2Hc, PDB ID: 6IRT) was selected for molecular docking studies of JPH203 and its derivatives. The specifications of 6IRT are the experimental resolution (3.5 Å), and it is expressed by *Homo sapiens.*

BCH and 4F2Hc that were contained in the selected microscopy structure of LAT-1 must be extracted from the LAT-1 structure and

re-docked to verify that the molecular docking software can reproduce the agonist conformation of the native ligand (N3). The result is valid if the obtained Root Mean Square Deviation (RMSD) value is ≤2.0 Å [7]. The best-docked N3 conformation showed an RMSD of 1.827 Å compared to the previous pose (table 1).

The modified structure of JPH203 and its derivatives are shown in table S1 (Supplementary Data). Modified structures of JPH203 are obtained from the combination of various PKM (Pharmacokinetics Modifying Linkers) and BFCA (Bifunctional Chelating Agent). The 2D structure of ligands was drawn with ChemDraw Ultra 12.0 and converted to 3D by Chem3D Ultra 12.0. The predominant conformational ligand structures (the correct geometry and conformation) of each modified JPH203 has been determined by energy minimization calculations using the Austin Model (AM1) semi-empirical method.

JPH203 and its derivatives have a ΔG value greater than-5.5 kcal/mol. However, only two molecules of JPH203 derivatives that can bind with key amino acid residue (gating residue) that is JPH203-Linker E-NOTA and JPH203-Linker K-NOTA by interacting with Tyr259 through hydrogen bond interaction with H-amine of JPH203 in JPH203-K-NOTA structure. The results showed in table 2.

Table 1 shows molecular docking parameters of 18 ligands and JPH203 as the lead compound. JPH203-K linker-NOTA is performed as the best-docked molecule (ΔG=-10.7 kcal/mol) and it can bind Tyr259 through hydrogen bond (fig. 2), which acts as the gating residue of LAT-1 transport activity.

Targeting the key residues (gating residues) in the active site of LAT-1 is the main inhibition strategy to inactivate the LAT-1 proteolytic function. The gating residues of transport activity of LAT-1 is presented by Tyr117, Phe252, Trp257, Asn258, Tyr259 and Arg348 [15]. The hydrogen bonds between protein side chains and drug are fundamental for inhibition and stability, thus play a significant role in drug-receptor interaction. The distance between the hydrogen-bond donor and acceptor (the shorter, the stronger) determine the strength of a hydrogen bond [16]. The result obtained from the docked modified structures of JPH203, indicates that all of our molecules meet the molecular docking parameter (minus value showed a spontaneous complection between the ligands and its target). However, only two molecules of JPH203 derivatives that can bind with key amino acid residue (gating residue) that is JPH203-Linker E-NOTA and JPH203-Linker K-NOTA by interacting with Tyr259 through Hydrogen Bond Interaction with H-amine of JPH203 in JPH203-K-NOTA structure. The details of the result can be seen in table 2.

JPH203-K linker-NOTA (fig. 2) was identified as the best-docked molecule (ΔG=-10.7kcal/mol) and it can bind Tyr259 through hydrogen bond, which acts as the gating residue of LAT-1 transport activity. Based on the previous research, Tyr259 inhibition decreases 80% transport activity of LAT-1 [13]. Therefore, the molecular interaction of JPH203-K linker-NOTA is promising for developing new radiopharmaceutical kits, while the rest of the molecules are excluded. Besides that, JPH203-K linker-NOTA showed a better inhibition constant (Ki= $1.4x10^{-8}$μM) than its lead compound (Ki=$1.2x10^{-7}$μM). These results showed that molecular docking parameters of JPH203-K linker-NOTA have a better docking parameter than the lead compound.

Table 3 shows the ADMET Prediction of the modified structure of JPH203. Most of all the molecules have good distribution and metabolism parameters. JPH203-K linker-NOTA does not penetrate BBB and is not a CYP inhibitor, has a much saver MRTD compared to other compounds (310 mg/day), but the compound has relatively unsafe toxicity parameters, especially hepatotoxicity (DILI parameters) and has cardiotoxic effect.

Table 3 shows results that JPH203-E-NOTA and JPH203-K-NOTA show better ADMET parameters (yellowed) than JPH203 itself as lead compound and DOTATE (the comparison compound, FDA approved). Based on the results of ADMET predictions, the JPH203-K linker-NOTA compound has a good distribution parameter as well as drug metabolism where the compound does not penetrate BBB and is not a CYP inhibitor. This suggests that this radiopharmaceutical kit candidate will not have CNS effects in the brain and will not experience drug damage before the drug reaches the receptors. However, the JPH230-K linker-NOTA compound has relatively unsafe toxicity parameters, especially hepatotoxicity (seen in DILI parameters), and has a cardiotoxic effect. Fortunately, these toxicity parameters can be negligible because radiopharmaceutical kits are generally required in very small quantities so that the toxic effects of the drug can be avoided. Other toxicity parameters possessed by these compounds are in the safe ranges, namely mutagenicity (AMES) and mitochondrial toxicity. The JPH203-K linker-NOTA compound also has a much safer MRTD compared to other compounds (310 mg/day); this shows that this radiopharmaceutical kit has the potential to avoid toxic effects resulting from the use of drugs in large quantities much smaller.

CONCLUSION

Molecular docking and ADMET structure-based prediction studies revealed JPH203-K linker-NOTA met the criteria as candidates of LAT-1 carrier drug for radiopharmaceutical kit in cancer therapy and or diagnostic.

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AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

Declared none

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