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Original Article

MOLECULAR DOCKING AND ADMET PREDICTION OF 5-BENZYLOXYTRYPTOPHAN AS A POTENTIAL RADIOPHARMACEUTICAL KIT FOR MOLECULAR IMAGING OF CANCER

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ABSTRACT

Objective: This *in silico* study aims to determine the inhibition effect of 5-BOTP with various bifunctional chelating agents (BFCA); NOTA, DOTA, TETA, CTPA, H2CB-DO2A, H2CBTE2A against the antiporter site of the LAT1.

Methods: The research method consisted of the binding mode of 5-BOTP and its derivatives with LAT1, the docking score, the analysis of preADMET, and the overview of Ro5 compatibility.

Results: The results showed that 5-BOTP-NOTA and 5-BOTP-DOTA had interactions with the gating residue (Phe252, Trp257, Asn258, and Tyr259) on the antiporter site of LAT1. 5-BOTP-NOTA and 5-BOTP-DOTA affinity are around-11.50 and-9.14 kcal/mol, respectively.

Conclusion: Based on this study, 5-BOTP-NOTA and 5-BOTP-DOTA are the new compounds that have the potential as a theranostic agent of cancer by inhibiting LAT1.

Keywords: 5-Benzyloxytryptophan, ADMET, Bifunctional chelating agents, LAT1, Molecular docking

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INTRODUCTION

The large-type amino acid transporter (LAT-1) is an antiporter of ubiquitous Na+and H+involved in the uptake of essential amino acids of the cells. LAT-1 is over-expressed in human cancer cells due to its increased demand for amino acids, but its distribution is limited to normal cells [1-4]. LAT-1 can distribute eight of the nine essential amino acids at somebody areas, such as the blood-brain barrier and placenta [5]. The overexpression of LAT-1 in cancer cells and the specific presence in cancer cells make LAT-1 become a promising cancer target for therapy and diagnostic (theranostic) molecules [6, 7].

Early detection and treatment of cancer in the human body are a big challenge. The Current method that can be used to diagnose and treat cancer is using radiopharmaceutical agents. The advantages of using radiopharmaceutical agents are high specificity, sensitivity, rapidness, and have no pain (like surgery) [8]. The component of radiopharmaceutical agent consists of a carrier substance that has an affinity with the target and also radionuclide that is complexed with the carrier (some are helped by bifunctional chelator). The radiopharmaceutical agent will carry the radionuclide to the target and emit γ-ray (for diagnostic purposes) and β-ray (for therapeutic purposes).

Based on *in silico* studies, previous research showed that 5-BOTP has a good affinity to the LAT-1, with the consensus score at 2.170 and the pharmacophore fit score at 46.53%. Besides that, 5-BOTP led to a complete and robust inhibition with IC50 values of 0.64±0.27 μM [9]. These results make 5-BOTP become a prospective carrier molecule of theranostic-radiopharmaceutical agent to target LAT-1 protein.

Although 5-BOTP has a good interaction with LAT-1, but 5-BOTP cannot be labeled directly with radiometal due to the incapability of the structure to complex the metal. Therefore, the addition of a chelator structure to 5-BOTP is needed. Hence, 5-BOTP can be distributed to the cancer cells through the interaction with LAT-1 as target protein. Changes in the chemical structure of 5-BOTP (due to the conjugation of chelator) have the potential to change the pharmacological activity of the molecule. This research aimed to describe changes in molecular interaction in order to know its molecular activity against LAT-1. Besides that, the absorption, distribution, metabolism, excretion, and toxicity (ADMET) of 5-BOTP derivatives have also been evaluated through *in silico* studies.

Experimental

Hardware and software

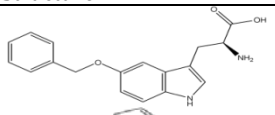
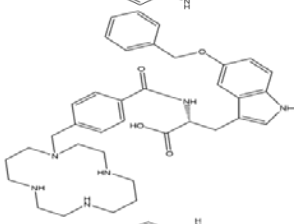
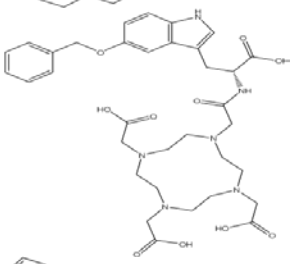
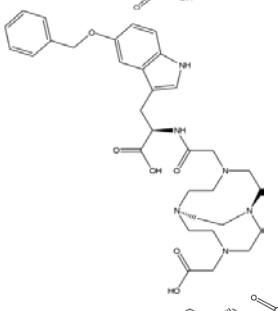
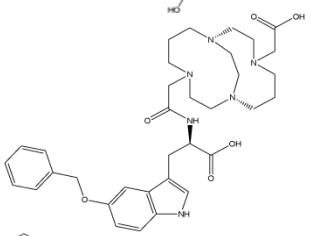
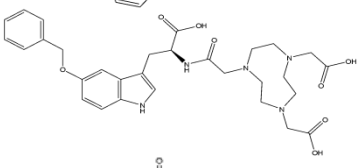
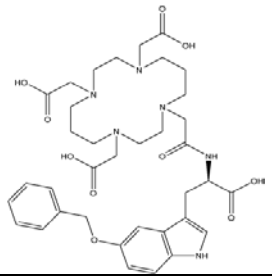
A portable personal computer with 4-cores 8-threads Intel Core i7-7700HQ, 2.80 GHz CPU with GTX 950M GPU and 8 GB RAM was employed and equipped with the following software for *in silico* studies:

1. The ChemDraw Ultra 12.0 and ChemOffice 2010 programme for drawing 2D structures and convert drawn structures into 3D structures,
2. LigandScout Advanced 4.1 software for ligand-protein interaction,
3. AutoDock 4.2.6 and AutoDockTools 1.5.6 programs for molecular docking simulations,
4. BIOVIA Discovery Studio 2017 R2 Client for visualization of PDB complex, the bond between ligands and receptors, and geometry optimization.
5. VNN-ADMET to predict the pharmacokinetics and toxicity of drugs.

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, 5-benzyloxytryptophan based) was drawn and has been optimized using ChemOffice 2010 and ChemDraw Ultra 12.0 (table 1).

Table 1: The structure of 5-BOTP and its derivatives, 5-BOTP was modified by conjugating its primary amines with carboxylate at various BFCH

Compounds	Empirical formula	Structure
5-BOTP	$C_{18}H_{18}N_2O_3$	
5-BOTP-CTPA	$C_{36}H_{46}N_6O_4$	
5-BOTP-DOTA	$C_{34}H_{44}N_6O_{10}$	
5-BOTP-H2CB-DO2A	$C_{32}H_{42}N_6O_6$	
5-BOTP-H2CB-TE2A	$C_{34}H_{46}N_6O_6$	
5-BOTP-NOTA	$C_{30}H_{37}N_5O_8$	
5-BOTP-TETA	$C_{36}H_{48}N_6O_{10}$	

Molecular docking simulation

LAT-1 and all ligands were docked using AutoDock 4.2.6 and ligands with the lowest bond energy (ΔG) to 6IRT were selected and each interaction was further characterized by Biovia Discovery Studio.

Ligand-based 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.

RESULTS

Table 2: Validation of computational method of 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

Table 3: Molecular docking parameters of 5-BOTP and its derivatives, 5-BOTP-NOTA and 5-BOTP-DOTA is the best candidate for radiopharmaceutical kit for radiotheranostic compound while the rest molecules are excluded due to its worst docking performance

Compound	ΔG (kcal/mol)	Ligand-receptor interaction	
		Hidrogend bond	Van der waals
5-BOTP	-8.05	Ser66, Gly65, Ser338, Ile63, Lys204, Thr62, Gly61	Ly67, Tyr259, Ala253, Ser342, Val339, Gly341, Ile64, Ser144, Leu260, Gly255, Gly256, Phe400
5-BOTP-CTPA	-6.68	Glu562, Lys533, Asp392, Ser308	Arg626, Glu303. Glu534, Ser307, Asn561, Thr302, Gly80, Leu76, Phe394, Ile393, Glu309
5-BOTP-DOTA	-9.14	Gly65 (2.91 Å), Gly255(2.43 Åand 2.56 Å), Thr345(1.99 Å), Trp257(2.82 Å), Trp405(2.23 Å), Asn404(3.09 Åand 2.98 Å), Ser144(3.54 Åand 3.77 Å)	Gly67, Gln145, Ala253, Ile63, Ser338, Gly341, Leu260, Glu136, Arg141, Asn258, Leu251, Tyr254
5-BOTP-H2CB-DO2A	-8.49	Ser66, Ser338, Lys204, Ser144, Gly337, Gly65, Thr62	Gly256, Tyr259, Phe400, Gly255, Gly67, Ile68, Tyr289, Ile63, Ile64, Ser342. Gly341, Ser334, Gln197, Gly61
5-BOTP-H2CB-TE2A	-10.38	Ala253, Ile64, Tyr289, Gly65, Gly255, Ile68, Ser144	Leu260, Gly61, Thr62, Val148, Ser342, Gln145, Glu136, Arg141, Ile140, Gln197, Lys204, Ile63, Gly341, Ser338, Ser66, Gly67, Gly256, Tyr254, Asn404
5-BOTP-NOTA	-11.50	Gly65(2.71 Å), Gly67(2.86 Å), Arg141(3.01 Å), Gln145(2.09 Å), Ser338(2.52 Å), Ser144(2.41 Å), Tyr259(2.51 Å), Gly256(3.12 Å),	Ile64, Thr62, Ile63, Phe252, Leu251, Trp257, Trp405, Phe400, Gly255, Asn404, Ser66, Asn258, Glu136, Ala253, Ser342, Thr345
5-BOTP-TETA	-3.98	Ala253, Gly67, Tyr289, Lys204, Gly255, Val148, Ser143, Gly61, Thr62, Gly341, Ser338	Ser334, Ile68, Leu260, Ser66, Ile147, Gly65, Ser342, Ile63, Ile64, Gly256, Tyr254, Thr345, Gln145, Phe400, Ile140

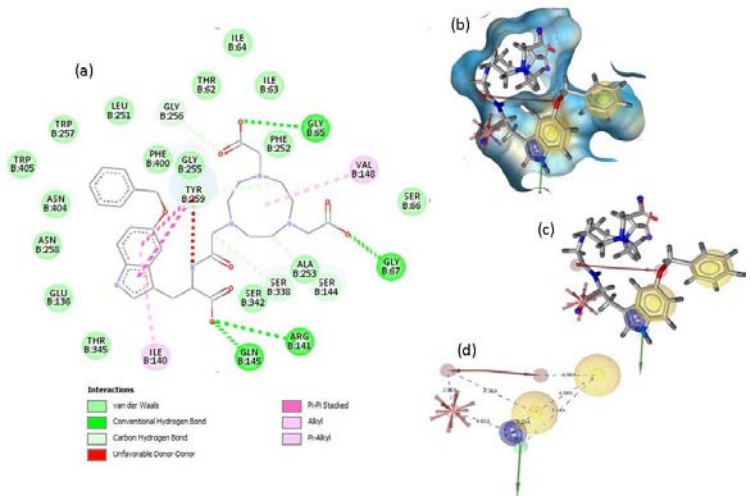


Fig. 1: Visualization of the best-docked ligand with LAT-1. (A) Interaction of LAT-1 amino acid with 5-BOTP-NOTA. (B) Interaction of LAT-1 amino acid with 5-BOTP-DOTA. (C) Information about mode of interaction

Table 4: ADMET Prediction of 5-BOTP and its derivatives, all of 5-BOTP derivatives fulfil the ADMET parameters except 5-BOTP-CTPA

Compounds 5-BOTP-	Absorption	Distribution		Metabolism						Toxicity	
	HIA (%)			HLM	CYP Inhibitor						
		PPB (%)	BBB		1A2	3A4	2D6	2C9	2C19	DILI	hERG Blocker
5-BOTP	90.32	67.17	No	No	No	No	No	No	No	No	No
-CTPA	89.67	26.50	Yes	No	No	No	No	No	No	No	No
-DOTA	70.27	16.62	No	No	No	No	No	No	No	No	No
-H2CB-DO2A	90.15	18.71	No	No	No	No	No	No	No	No	No
-H2CB-TE2A	90.94	13.97	No	No	No	No	No	No	No	No	No
-NOTA	84.54	21.73	No	No	No	No	No	No	No	No	No
-TETA	75.22	12.02	No	No	No	No	No	No	No	No	No

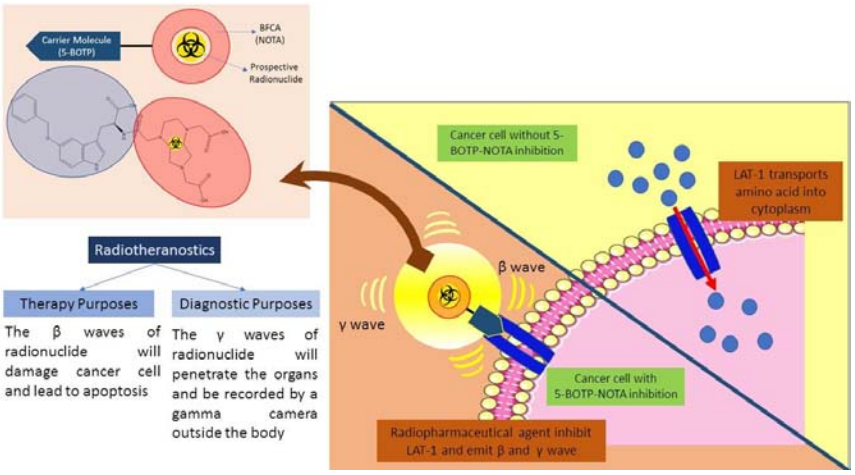


Fig. 2: Graphical abstract of 5-BOTP and its conjugate as a prospective radiopharmaceutical kit through LAT-1 inhibition

DISCUSSION

The electron microscopy structure of LAT-1 was complex with BCH and 4F2Hc (PDB ID: 6IRT) selected for molecular docking studies of 5-BOTP and its derivatives. The specifications of 6IRT are the experimental resolution (3.5 Å), and it is expressed by Homo sapiens. 6IRT was chosen due to its well-defined domain of active site with the lowest resolution compared with any other PDB ID in the database (http://www.rscb.org) as of January 2021. Before molecular docking was conducted, BCH and 4F2Hc were deleted from the LAT-1 structure and re-docked to verify that the molecular docking software can reproduce the agonist conformation of BCH. This validation is also commonly used in molecular docking simulation as followed by several studies [10, 11]. The result is valid when an RMSD value obtained is≤2.0 Å [12]. The best -docked BCH conformation showed an RMSD of 1.827 Å compared to the original LAT-1 conformation (table 2).

The structure of 5-BOTP and its derivatives are shown in table 1. Modified structures of 5-BOTP are obtained from the combination of various BFCH. All the chelators used in the modification each has carboxylate group. Hence, the possible site in 5-BOTP to be a center of conjugation is the amine structure so that 5-BOTP can form amide bonds between the primary amines in the 5-BOTP structure and the carboxylate through SN1 reaction.

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 of each 5-BOTP derivative have been done by energy minimization calculations using the Austin Model (AM1) semi-empirical method.

Targeting the key residues (gating amino acid) in the transport region of LAT-1 is the main inhibition strategy to inactivate the LAT-1 antiporter activity. The gating amino acid of LAT-1 is presented by Tyr117, Phe252, Trp257, Asn258, Tyr259, and Arg348.[2] The hydrogen bond formed between amino acid residue and ligand are pivotal for inhibition and stability, thus showed a significant role in drug-receptor interaction. Besides that, the distance between the hydrogen-bond donor and acceptor (the shorter, the stronger) determine the strength of a hydrogen bond [10, 13].

Table 3 shows the molecular docking parameters of six ligands. 5-BOTP-NOTA performed as the best-docked molecule (ΔG=-11.50 kcal/mol), and it has the ability to bind Tyr259 through a moderate carbon-hydrogen bond (radius= 2.51 Å), and also can bind with Phe252, Trp257, and Asn258 through Van der Waals interaction (fig. 1A) which acts as the gating residue of antiporter region of LAT-1.

Another prospective radiopharmaceutical agent is 5-BOTP-DOTA. Based on the docking result (table 3), 5-BOTP-DOTA can bind with two gating residues (Trp257 and Tyr259) through hydrogen bond interaction and also through Van der Waals interaction with Asn258 (fig. 1B). The Gibbs free energy of 5-BOTP-DOTA is-9.14 kcal/mol. These results showed prominent results of docking studies of 5-BOTP derivatives.

Other 5-BOTP (-CTPA,-H2CB-DO2A,-H2CB-TE2A, and-TETA) are excluded from drugs candidate due to their worst Gibbs score, and they cannot interact with gating residue, which has a pivotal role in the antiporter mechanism of LAT-1. Based on molecular docking simulation, 5-BOTP-NOTA and 5-BOTP-DOTA is the best candidate for radiopharmaceutical kit for radiotheranostic compound, while the rest molecules are excluded. The mechanism of 5-BOTP derivatives in targeting LAT-1 as a radiopharmaceutical kit is summarized in fig. 2.

Table 4 shows that 5-BOTP-NOTA and 5-BOTP-DOTA has good Human Intestinal Absorption (HIA) with value around 84.54% and 70.27%, respectively. The substitution of BFCA (NOTA and DOTA) decreases the number of HIA in 5-BOTP. However, the decreasing of HIA value is tolerated because the range is still in a good qualification—70-100% [14]. Besides that, those molecules have a good distribution parameter as well as drug metabolism, in which the compound does not penetrate BBB and is not a CYP inhibitor. The results suggest 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. Based on toxicity parameters, 5-BOTP-NOTA and 5-BOTP-DOTA cannot stimulate Drug-induced Liver Injury (DILI) and is not a hERG blocker. Based on the results of ADMET predictions, 5-BOTP-NOTA and 5-BOTP-DOTA are the radiopharmaceutical kits that have a good ADME parameter and can avoid toxic effects resulting from the use of the drugs.

CONCLUSION

Molecular docking and ADMET structure-based prediction studies revealed 5-BOTP-NOTA and 5-BOTP-DOTA 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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