In-Silico Screening Compounds of *Brassica rapa* ssp. *chinensis* as Potential Inhibitor of Neutral Amino Acid Transporter B⁰AT1 as an Alternative Phenylketonuria Treatment

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ABSTRACT. Phenylketonuria (PKU) is known as a severe autosomal recessive disease caused by mutations in the expression enzyme, namely the PAH (Phenylalanine Hydroxylase) enzyme that causes the build-up of phenylalanine in the body. Untreated PKU affected brain damage and developmental problems. One of the strategies to reduce phenylalanine in the body is inhibiting B⁰AT1 activity using carotenoid and terpenoids compounds from Bok choy (*Brassica rapa* ssp. *chinensis*). In this study, we evaluated the nine carotenoid and terpenoid compounds from Bok choy as B⁰AT1 inhibitors. Nine Bok choy compounds, including alpha-carotene, beta-carotene, dimethylallyl pyrophosphate, isopentenyl pyrophosphate, lutein, neoxanthin, violaxanthin, geranylgeranyl diphosphate, and zeaxanthin were downloaded from PubChem database, while the 3D structure of B⁰AT1 was retrieved from Protein Data Bank RCSB. The compounds and B⁰AT1 were prepared by PyRx 0.8 version and Discovery Studio ver 21.1.1, then docked with Hex 8.0.0 and analyzed using Discovery Studio ver 21.1.1. This screening implies that three terpenoid compounds dimethylallyl pyrophosphate, isopentenyl pyrophosphate, and geranylgeranyl diphosphate interacts in C domain of B⁰AT1 while six carotenoid compounds, alpha carotene, beta-carotene, lutein, neoxanthin, violaxanthin, and zeaxanthin interacts in A domain and have possibility to inhibit B⁰AT1, because it interact with same A domain and have a stronger binding energy than phenylalanine. Alpha carotene has a same residue with phenylalanine, Phe144, making it potentially greater than other compound as inhibitors. *Brassica rapa* ssp. *chinensis* is indeed good for consumption by people with phenylketonuria, but it is also necessary to do a further compound screening in other low-phenylalanine diet foods to know which one is better as alternative phenylketonuria treatment.

Keywords: alpa carotene, B⁰AT1, *Brassica rapa*, phenylalanine, phenylketonuria

INTRODUCTION

Phenylketonuria (PKU) is an inherited autosomal recessive congenital disease caused by a genetic mutation. Genetic mutations occur in a pair of alleles on chromosome 12 that encode genes for the expression of the PAH enzyme (Phenylalanine Hydroxylase), resulting in inactivation of the PAH enzyme and reduced performance of the enzyme in phenylalanine catabolism [1]. Deficiency of the PAH enzyme causes Phe build-up in the blood and brain. PKU sufferers cannot break down the amino acid phenylalanine into tyrosine, hence, the substance will accumulate in the body [2]. If left untreated, PKU sufferers will experience brain damage, intellectual disabilities, developmental problems, and seizures [3].

B⁰AT1, also known as SLC6A19, is a neutral amino acid transporter (AA0), which can be expressed in the intestine, especially in the jejunum [4]. Structurally SLC consists of 12 transmembrane helix, 10 helix formed a catalytic

structure, the 11th and 12th helix emerge dimerization on the transporter. Then in its natural form in the intestine B⁰AT1 binds to the ACE2 receptor to form a heterodimer. Absorbed neutral amino acids consist of phenylalanine (Phe), leucine (Leu), methionine (Met), isoleucine (Ile), valine (Val), glycine (Gln), asparagine (Asn), cysteine (Cys), alanine (Ala), serine (Ser), threonine (Thr), glycine (Gly), tyrosine (Tyr), lysine (Lys), proline (Pro), tryptophan (Trp), and histidine (His). B⁰AT1 structurally similar to Leu due to the usage of active Na⁺ transport as its mechanism [5,6]. Excessive absorption of AA0 on B⁰AT1 will cause malabsorption in the intestine. The presence of inhibition of B⁰AT1 shows a positive side for some cases of diseases such as restoring amino acid imbalances in phenylketonuria disease disorders [7].

Bok choy (*Brassica rapa* ssp. *chinensis*) is one of the most consumed vegetables in Asia, including Indonesia [8]. The cultivation and consumption of Bok choy increases worldwide [9]. In implementing the Phe diet in PKU

treatment, Bok choy is one of the foods that can be consumed without measuring its phenylalanine content in daily consumption due to the low content of phenylalanine (<75mg/100g). This does not affect the control of phenylalanine in the blood [10].

Bok choy contains beneficial compounds for health, such as carotenoids, which are useful as antioxidants. Carotenoid is seen in vitamin A and helps to improve the function of the immune system in the body. These carotenoids include alpha-carotene, beta-carotene, lutein, neoxanthin, violaxanthin, and zeaxanthin [10]. Other ingredients are terpenoids which are isopentenyl diphosphate (IPP), dimethylallyl diphosphate (DMAPP), and geranylgeranyl diphosphate (GGPP) [11].

The purpose of this study was to screen *Brassica rapa* ssp *chinensis* compounds that have the activity of B⁰AT1 inhibitors. In finding alternative treatment solutions for PKU patients, an in-silico screening of *Brassica rapa* ssp *chinensis* compounds was carried out, which has potential as a phenylalanine transporter B⁰AT1 inhibitor in the intestinal epithelium.

RESEARCH METHODS

Data Mining and Preparation of Protein and Ligand

The B⁰AT1 (PDB ID 6M18) structure was downloaded from RCSB PDB website (www.rcsb.org) with PDB format. The ligand structure of phenylalanine (CID 6140) was downloaded from the PubChem website (www.pubchem.ncbi.nlm.nih.gov) with SDF format, the same is done with other ligands such as alpha carotene (CID 4369188), beta-carotene (CID 46783370), lutein (CID 5281243), neoxanthin (CID 5281247), violaxanthin (CID 448438), zeaxanthin (CID 5280899), dimethylallyl pyrophosphate (CID 647), geranylgeranyl diphosphate (CID 447277), and isopentenyl pyrophosphate (CID 1195). These nine active compounds of Bok choy had chosen by KNApSACk database (<http://www.knapsackfamily.com/KNApSACk/>). B⁰AT1 as protein was prepared by utilizing Biovia Discovery Studio Visualizer ver 21.1.1 software to remove native ligand and water molecules. Meanwhile, ligands were prepared using PyRx 0.8 software. The protein and ligand preparation results then stored in PDB (.pdb) format.

Protein-Ligand Docking and Visualization

Molecular docking of protein and ligand was done using HEX 8.0.0 software [4, 12]. The docking results were stored in PDB (.pdb) format

and the binding energy was saved in TXT (.txt) format. Biovia Discovery Studio Visualizer ver 21.1.1 software was used to visualize the results of interactions between proteins and ligands (<https://www.3ds.com/products-services/>). The parameters that analyzed including the domain and bond energy, type of bond, and amino acid residues.

RESULTS AND DISCUSSIONS

Interaction between Compounds and B⁰AT1

The interaction visualized in Figure 1 shows that three terpenoid group compounds bind to C domain of B⁰AT1, namely dimethylallyl pyrophosphate, isopentenyl pyrophosphate, and geranylgeranyl diphosphate, while compounds that bind to A domain of B⁰AT1 which is a transmembrane region are alpha carotene, beta-carotene, lutein, neoxanthin, violaxanthin, and zeaxanthin, which are belong to the carotenoid group. The 3D interactions are shown in Figure 2. The interaction of phenylalanine with its transporter, B⁰AT1 shows Glu146 residue involved in hydrogen and electrostatic chemical bonds. The other residue Phe144 formed in the hydrogen bond. Hydrophobic bonds were formed at the between phenylalanine and B⁰AT1 has a binding energy of -158.5 kJ/mol. Phenylalanine and B⁰AT1 bonds occur in the helix structure and domain A in B⁰AT1 (Figure 2 and Table 1).

Alpha-carotene binds to B⁰AT1 in A domain and part of the helix structure of B⁰AT1. This interaction results in a binding energy of -316.1 kJ/mol, lower than the B⁰AT1 interaction with phenylalanine. Low energy bonds tend to bind stronger. Based on the amino acid residue, alpha carotene has the same amino acid residue with phenylalanine, namely Phe144. Other amino acid residues are Phe141, Phe467, Ala202 and Cys203. All residues formed in hydrophobic bonds.

The interactions between beta-carotene and B⁰AT1 produce residues of Leu43, Leu46, Ile239, Ile309, Tyr231, and Phe312 involved in hydrophobic bonds with binding energy -354.5 kJ/mol and the interaction happens on the A domain and the helix structure in B⁰AT1. Alpha carotene and beta-carotene are natural carotenoid compounds that act as precursors for vitamin A. Beta-carotene has more possibilities in the conversion process to cortisol (vitamin A) [13]. Another function of alpha-carotene and beta-carotene is similar as other carotenoid compounds, it can act as an antioxidant, anti-carcinogenic and can boost the immune system. Carotenoid compounds are able to bind to membrane lipids and proteins [14].

In contrast to other compounds that bind to the A domain such as phenylalanine, dimethylallyl pyrophosphate interacts to B⁰AT1 on the C

domain. This bond occurs in the B⁰AT1 helix structure and produces a binding energy of -176 kJ/mol. The other amino acid residues are Ala222, Met210, Cys211 involved with dimethylallyl pyrophosphate in hydrophobic interactions and they bind into helix structure of B⁰AT1.

The other bond, hydrogen bond involved the Arg214 and Glu217 residues. Another compound that is bound to the C domain and the helix structure of B⁰AT1 is isopentenyl pyrophosphate. Results of the amino acid residues namely Met210, Thr218 and Lys221 interact through hydrogen bonds and produce binding energy of -165.3 kJ/mol.

All of the resulting amino acid residues bound to the helix structure of B⁰AT1 protein except for Met210. Isopentenyl diphosphate is one of the main constituents of a compound that functions as an isoprenoid precursor, condensing it into its isomeric compound, dimethylallyl pyrophosphate (DMAPP) with the help of the IPP isomerase enzyme [15].

Geranylgeranyl diphosphate interacts with B⁰AT1 and produces amino acid residues namely Met42, Leu46, Ile309, Val232 and Tyr231 which are formed by hydrophobic bonds. Like dimethylallyl pyrophosphate, this compound binds on the C domain and helix structure of B⁰AT1. This interaction creates a binding energy of -219 kJ/mol. Geranylgeranyl diphosphate is an isoprenoid compound that acts as a precursor to various compounds essential for vital metabolism

(carotenoids, chlorophyll, gibberellins, etc.). This compound is formed from the plastic pathway GGPPP synthase (GGPPS). It is composed of five carbon chains (C₅), IPP (Isopentenyl diphosphate) and the allylil isomer DMAPP (Dimethylallyl Pyrophosphate) [16].

Lutein is a compound that interacts with B⁰AT1 in the A domain of B⁰AT1. This bond produces amino acid residues, namely Cys200, Ala202, Cys203, Leu464, Leu199, Phe141, Trp195 and Try209 through hydrophobic bonds. All amino acid residues lie in the helix structure, except for Tyr209. The binding energy is -318.1 kJ/mol emerged. Lutein has a structure consisting of a single carbon chain accompanied by carbon-carbon double bonds, there is also a methyl side group. A cyclic hexenyl structure with hydroxyl groups can be seen at both ends of the carbon backbone. Lutein and zeaxanthin differ in this area due to the location of the methyl group on the long carbon chain and the double bond in the hexenyl ring [17].

Neoxanthin B⁰AT1 complex produced two amino acid residues, Cys 200 and Phe141. The two residues have hydrophobic interactions and produce 315.2 kJ / mol binding energy. Neoxanthin interacts to the A domain and helix structure of B⁰AT1. Neoxanthin carotenoids derivatives (other than lutein and violaxanthin) in the leaves of plants and its absorption capacity is close to violaxanthin [18].

Figure 1. Three dimensional virtual molecular docking between B⁰AT1 with nine *Brassica rapa* compounds, A) phenylalanine; B) alpha-carotene; C) beta-carotene; D) dimethylallyl pyrophosphate; E) geranylgeranyl diphosphate; F) isopentenyl pyrophosphate; G) lutein; H) neoxanthin; I) violaxanthin; and J) zeaxanthin. Picture in the red box shows an enlarged image of the compound that is bound to the B⁰AT1 structure.

Figure 2. The 3D interaction between B⁰AT1 and compounds: A) phenylalanine; B) alpha-carotene; C) beta-carotene; D) dimethylallyl pyrophosphate; E) geranylgeranyl diphosphate; F) isopentenyl pyrophosphate; G) lutein; H) neoxanthin; I) violaxanthin; and J) zeaxanthin.

Table 1. The interaction result of neutral amino acid transporter B⁰AT1 with phenylalanine and nine compounds of *Brassica rapa* ssp. chinensis.

Complexes (Binding energy (kJ/mol))	Interactions	Binding Region/Domain	Category	Type
Phenylalanine (-158,5)	A:PHE1:HT2 - A:GLU146:OE1	Topological	Hydrogen Bond; Electrostatic	Salt Bridge; Attractive Charge
	A:PHE144:HA - A:PHE1:OXT	Topological	Hydrogen Bond	Carbon Hydrogen Bond
	A:PHE1 - A:PRO414	Transmembrane	Hydrophobic	Pi-Alkyl
	A:PHE1 - A:LEU415	Transmembrane	Hydrophobic	Pi-Alkyl
Alpha Carotene (-316,1)	A:ALA202 - :LIG1:C26	Transmembrane	Hydrophobic	Alkyl
	A:CYS203 - :LIG1	Transmembrane	Hydrophobic	Alkyl
	A:PHE141 - :LIG1:C34	Transmembrane	Hydrophobic	Pi-Alkyl
	A:PHE144 - :LIG1:C6	Topological	Hydrophobic	Pi-Alkyl
	A:PHE467 - :LIG1:C17	Transmembrane	Hydrophobic	Pi-Alkyl
	A:LEU43 - :LIG1	Transmembrane	Hydrophobic	Alkyl
	A:LEU46 - :LIG1	Transmembrane	Hydrophobic	Alkyl
Beta-carotene (-354,5)	A:ILE239 - :LIG1	Transmembrane	Hydrophobic	Alkyl
	A:ILE309 - :LIG1	Transmembrane	Hydrophobic	Alkyl
	:LIG1:C12 - A:LEU43	Transmembrane	Hydrophobic	Alkyl
	A:TYR231 - :LIG1:C24	Transmembrane	Hydrophobic	Pi-Orbitals
	A:PHE312 - :LIG1:C34	Transmembrane	Hydrophobic	Pi-Orbitals
	C:ARG214:HH11 - :LIG1:O3	Topological	Hydrogen Bond	Conventional Hydrogen Bond
	:LIG1:H11 - C:GLU217:O	Topological	Hydrogen Bond	Conventional Hydrogen Bond
Dimethylallyl Pyrophosphate (-176)	C:ALA222 - :LIG1:C4	Transmembrane	Hydrophobic	Alkyl

Complexes (Binding energy (kJ/mol))	Interactions	Binding Region/Domain	Category	Type
	:LIG1:C3 - C:MET210	Transmembrane	Hydrophobic	Alkyl
	:LIG1:C3 - C:CYS211	Transmembrane	Hydrophobic	Alkyl
	:LIG1:C - C:MET42	Transmembrane	Hydrophobic	Alkyl
	:LIG1:C - C:LEU46	Transmembrane	Hydrophobic	Alkyl
	:LIG1:C - C:LEU43	Transmembrane	Hydrophobic	Alkyl
	:LIG1:C - C:ILE309	Transmembrane	Hydrophobic	Alkyl
	:LIG1:C - C:VAL232	Transmembrane	Hydrophobic	Alkyl
	C:TYR231 - :LIG1:C	Transmembrane	Hydrophobic	Pi-Alkyl
	:LIG1:H10 - C:MET210:O	Transmembrane	Hydrogen Bond	Conventional Hydrogen Bond
Isopentenyl Pyrophosphate (-165,3)	C:THR218:HB - :LIG1:O6	Topological	Hydrogen Bond	Carbon Hydrogen Bond
	C:LYS221:HE2 - :LIG1:O	Topological	Hydrogen Bond	Carbon Hydrogen Bond
	A:CYS200 - :LIG1	Transmembrane	Hydrophobic	Alkyl
Lutein (-318,1)	A:ALA202 - :LIG1	Transmembrane	Hydrophobic	Alkyl
	A:CYS203 - :LIG1	Transmembrane	Hydrophobic	Alkyl
	:LIG1:C16 - A:LEU464	Transmembrane	Hydrophobic	Alkyl
	:LIG1:C23 - A:LEU199	Transmembrane	Hydrophobic	Alkyl
	:LIG1:C34 - A:CYS200	Transmembrane	Hydrophobic	Alkyl
	A:PHE141 - :LIG1:C34	Transmembrane	Hydrophobic	Pi-Alkyl
	A:TRP195 - :LIG1:C23	Transmembrane	Hydrophobic	Pi-Alkyl
	A:TYR209 - :LIG1	Transmembrane	Hydrophobic	Pi-Alkyl
Neoxanthin (-315,2)	A:PHE141 - :LIG1	Transmembrane	Hydrophobic	Pi-Alkyl
	A:CYS203 - :LIG1	Transmembrane	Hydrophobic	Alkyl
Violaxanthin (-300,4)	A:MET210 - :LIG1	Transmembrane	Hydrophobic	Alkyl
	:LIG1 - A:LEU199	Transmembrane	Hydrophobic	Alkyl
	:LIG1:C34 - A:CYS200	Transmembrane	Hydrophobic	Alkyl
	A:TRP138 - :LIG1:C12	Transmembrane	Hydrophobic	Pi-Alkyl
	A:PHE141 - :LIG1:C34	Transmembrane	Hydrophobic	Pi-Alkyl
	A:TRP195 - :LIG1	Transmembrane	Hydrophobic	Pi-Alkyl
	A:TRP196 - :LIG1:C12	Transmembrane	Hydrophobic	Pi-Alkyl
	A:TYR209 - :LIG1:C13	Transmembrane	Hydrophobic	Pi-Alkyl
Zeaxanthin (-337,7)	:LIG1:C12 - A:MET210	Transmembrane	Hydrophobic	Alkyl
	:LIG1 - A:LEU199	Transmembrane	Hydrophobic	Alkyl
	A:TRP138 - :LIG1:C25	Transmembrane	Hydrophobic	Pi-Alkyl
	A:PHE141 - :LIG1	Transmembrane	Hydrophobic	Pi-Alkyl
	A:TRP195 - :LIG1:C19	Transmembrane	Hydrophobic	Pi-Alkyl
	A:TRP196 - :LIG1:C19	Transmembrane	Hydrophobic	Pi-Alkyl

Violaxanthin interacts with B⁰AT1 in the A domain and helix structure and produces binding energy -300.4 kJ/mol. The active sites of Violaxanthin - B⁰AT1 were Cys203, Met210, Leu199, Cys200, Trp138, Phe141, Trp195, Trp196, and Tyr209 through hydrophobic interactions. Violaxanthin is a major type of epoxycarotenoid biosynthesis present in higher

plants and is usually found in due to its important role in the xanthophyll cycle [8]. Violaxanthin has an antioxidant capacity that is stronger than β -carotene and lutein [19]. Zeaxanthin binds to B⁰AT1 and produces amino acid residues, namely Met210, Leu199, Trp138, Phe141, Trp195, and Trp196 involved in hydrophobic interactions. The resulting bond energy is -337.7 kJ/mol. This

interaction has binding on domain A and the helix structure of B⁰AT1.

Zeaxanthin is a type of non-provitamin A carotenoid that has an oxygen molecule. This compound consists of 40 hydroxylated carbons which are the same as lutein. Therefore, both of them are included in the xanthophyll group. These compounds are useful as a deterrent from oxidative damage to proteins, lipids, or DNA by using other antioxidant mechanisms [20].

Screening the potential of nine compounds as an inhibitor of B⁰AT1

Based on the analysis of molecular docking results, it was shown that all the nine compounds screened had lower binding energy values compared to phenylalanine against B⁰AT1 with the majority forming hydrophobic bonds. There are no unfavorable bonds in all the bonds between the compounds and B⁰AT1. Six compounds, alpha-carotene, beta-carotene, lutein, neoxanthin, violaxanthin, and zeaxanthin bind B⁰AT1 in domain A the same as phenylalanine. Almost all of these six carotenoid compounds bind to amino acid residues on transmembrane region. The structure of B⁰AT1 has two main region, topological and transmembrane. Topological domain, is a domain where the regulation of gene expression occurs. This behavior arises to limit the interaction of cis sequence regulator and its targeted gene [21]. The study of Spitz exhibits that topological domain can be matched with the domain regulator [22]. The transmembrane region located in B⁰AT1 is where the bound protein and amino acid residual [21]. It means that interaction between compounds into transmembrane region can disturb the interaction of amino acid and the transporter, B⁰AT1. Meanwhile, three terpenoid compounds, namely dimethylallyl pyrophosphate, geranylgeranyl diphosphate, and isopentenyl pyrophosphate bind to the C domain of B⁰AT1.

These compounds also exhibit the top three high energy binding values. In addition, none of the residues were the same as the phenylalanine residue in B⁰AT1. This indicates that the three compounds have the lowest probability of being inhibitors of phenylalanine in binding to B⁰AT1.

CONCLUSION

This study indicated that six of nine Bok choy compounds, alpha-carotene, beta-carotene, lutein, neoxanthin, violaxanthin, and zeaxanthin bind B⁰AT1 in domain A the same as phenylalanine. Furthermore, alpha-carotene has same active sites with phenylalanine, which was Phe144, indicating alpha-carotene might has similar function with phenylalanine. Nevertheless, Bok choy is recommended to consume as a low phenylalanine diet food.

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