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Original Article

Virtual prediction of potential immunogenic epitope of candoxin protein from Malayan krait (*Bungarus candidus*) venom

[Predicción virtual del epítopo inmunogénico potencial de la proteína candoxina del veneno de krait malayo (*Bungarus candidus*)]

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Abstract

Context: Malayan krait (*Bungarus candidus*) is a snake that is considered highly venomous snake and widely distributed across Southeast Asia. Envenomation by this snake is characterized by facial weakness, paralysis, respiratory muscle weakness, and in most cases, it renders the victim dead. Unfortunately, there is only one antivenom for neutralizing venom that is only available from the Thai Red Cross Society.

Aims: To predict the epitopes from candoxin protein of *B. candidus* venom that could be a candidate for vaccine-based antivenom.

Methods: In this study, IEDB and SYFPHEITHI databases were utilized to predict candoxin epitope sequences and determine their immunogenicity, conservancy, and population coverage. Next, the epitopes were modeled, and the binding interactions between epitopes and MHC-II were analyzed. The epitope that binds into the active site of human and murine MHC-II proceeded to the next step. Then, the allergenic properties of the chosen epitope were assessed to ensure its safety. Lastly, the physicochemical characteristics prediction and molecular dynamics simulation were conducted to verify the epitope's stability when produced *in vivo*.

Results: The results showed that epitope 47-CFKESWREARGTRIE-61 has the best binding interaction when compared to others. This epitope was confirmed that did not show potential allergenic properties. The physicochemical properties and molecular dynamics simulation demonstrated that this epitope was stable.

Conclusions: The results of this study will be useful in developing a novel antivenom for *Bungarus candidus* using a vaccine-based method.

Keywords: animal toxin; antivenom; neurotoxin; vaccine.

Resumen

Contexto: La krait malaya (*Bungarus candidus*) es una serpiente que se considera altamente venenosa y está ampliamente distribuida en el sudeste asiático. El envenenamiento por esta serpiente se caracteriza por debilidad facial, parálisis, debilidad de los músculos respiratorios y, en la mayoría de los casos, provoca la muerte de la víctima. Desafortunadamente, solo hay un antiveneno para neutralizar el veneno que solo está disponible en la Sociedad de la Cruz Roja Tailandesa.

Objetivos: Predecir los epítopos de la proteína candoxina del veneno de *B. candidus* que podrían ser candidatos a antiveneno vacunal.

Métodos: En este estudio, se utilizaron las bases de datos IEDB y SYFPHEITHI para predecir las secuencias de epítopos de candoxina y determinar su inmunogenicidad, conservación y cobertura poblacional. A continuación, se modelaron los epítopos y se analizaron las interacciones de unión entre los epítopos y el MHC-II. El epítopo que se une al sitio activo del MHC-II humano y murino pasó al siguiente paso. Luego, se evaluaron las propiedades alergénicas del epítopo elegido para garantizar su seguridad. Por último, se realizó la predicción de las características fisicoquímicas y la simulación de la dinámica molecular para verificar la estabilidad del epítopo cuando se produce *in vivo*.

Resultados: Los resultados mostraron que el epítopo 47-CFKESWREARGTRIE-61 tiene la mejor interacción de unión en comparación con otros. Se confirmó que este epítopo no presentaba propiedades alergénicas potenciales. La simulación de propiedades fisicoquímicas y dinámica molecular demostró que este epítopo era estable.

Conclusiones: Los resultados de este estudio serán útiles para desarrollar un nuevo antiveneno para *Bungarus candidus* utilizando un método basado en vacunas.

Palabras Clave: antiveneno; neurotoxina; toxina animal; vacuna.

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INTRODUCTION

Malayan krait (*Bungarus candidus*) is a snake that belongs to Category I Medically Important snakes according to WHO because its habitat is in the range of human settlement and causes many life-threatening bites (Warrell, 2010). This snake can be found naturally in Southeast Asia countries, such as Cambodia, Laos, Indonesia, Malaysia, Singapore, Thailand, and Vietnam (Uetz et al., 2022). *Bungarus candidus* has a potent neurotoxic venom, and the envenomation may lead to death by paralyzing the respiratory organ (Adiwinata and Nelwan, 2015; Khow et al., 2003). While this snake has a wide range of distributions, it is unfortunate that there is only one monovalent antivenom available for this snake (Ahmad et al., 2014). The limited availability of antivenom is mainly caused by the production method. To date, the majority of antivenom were produced traditionally using modified methods from the late 19th century (Calmette, 1894; 1896; Gutiérrez, 2019). Traditional antivenom production may face difficulties in snake collection, maintenance, and venom extraction. In addition, there is a low commercial value of the antivenom due to the lack of interest on the part of pharmaceutical companies to produce it (Ramos and Ho, 2015). Moreover, traditional antivenom safety and efficacy were challenged due to the presence of non-neutralizing horse serum (De Silva et al., 2016; Ko and Chung, 2013). Therefore, a replacement of traditional antivenom is needed to overcome these problems.

The advance in modern biotechnology and bioinformatics gave rise to antivenom alternatives, such as vaccine-based antivenom. Several studies (Cao et al., 2016; Machado De Avila et al., 2011; Wagstaff et al., 2006) showed the potential value of vaccine-based antivenom for Viperid snakes using DNA vaccine. These studies concluded that vaccine-based antivenom for Viperids could prevent the animal models from developing hemorrhagic effects from the venom (Cao et al., 2016; Machado De Avila et al., 2011; Wagstaff et al., 2006). Ashraf et al. (2014) and Kurniawan et al. (2020) predicted that epitopes from krait (Genus: *Bungarus*) phospholipase A_2 (PLA_2) protein could induce immune responses against the neurotoxic activity of PLA_2. However, the main component of *B. candidus* venom was the three-finger toxins (3FTx) protein family such as α-bungarotoxin, α-β-bungarotoxin-2, α-neurotoxin, and candoxin (Nirthanan et al., 2002; Rusdi et al., 2019; Rusmili et al., 2014).

To neutralize the neurotoxic activities of *B. candidus* venom, a specific, safe, and fast-producing antivenom is needed. By using bioinformatics analysis, the specificity and safety of the vaccine-based antivenom can be predicted; moreover, the time and the cost of developing antivenom might be reduced. In this study, the bioinformatics approaches were employed to predict candoxin protein epitopes as an initial step to develop a vaccine-based antivenom for *B. candidus*. The result showed that one of the predicted epitopes had favorable binding interactions with major histocompatibility complex class II (MHC-II). Furthermore, the physicochemical characteristics and molecular dynamics analysis verified that the epitope was stable. These results would give accurate preliminary data for developing vaccine-based antivenom for *B. candidus*. This study aims to predict candoxin venom protein's epitopes and understand their interactions with murine and human MHC-II.

MATERIAL AND METHODS

Prediction of candoxin epitopes

The candoxin protein sequence was downloaded from NCBI (https://www.ncbi.nlm.nih.gov/protein) with accession number P81783.2 (Nirthanan et al., 2002). The prediction of candoxin epitopes for murine MHC-II was conducted using SYFPHEITHI database at: http://www.syfpeithi.de/bin/MHCServer.dll/EpitopePrediction.htm (Schuler et al., 2007). This database utilized the occurrence of amino acids in natural ligands for scoring (Schuler et al., 2007). The epitopes with scores between 1-15 were considered suitable for further analysis. The prediction of the epitopes of candoxin that human MHC-II can recognize was harnessed using IEDB (http://tools.iedb.org/mhcii/) with a consensus method (Wang et al., 2008; 2010). HLA reference sets provided by IEDB were used to predict candoxin epitopes for Human MHC-II (Greenbaum et al., 2011). Epitopes with a percentile rank lower than five were included in the subsequent analysis (Shah and Raghava, 2006). The B cell epitopes were also necessary for antibody-producing, so the B cell epitopes were also predicted using IEDB (http://tools.iedb.org/bcell/) with Bepipred Linear Epitope Prediction 2.0 method (Jespersen et al., 2017).

Prediction of epitopes conservancy and immunogenicity

The IEDB (http://tools.iedb.org/CD4episcore/) (Dhanda et al., 2018) was utilized to predict the immunogenicity of the epitopes. The epitope conservancy was calculated using IEDB at http://tools.iedb.org/conservancy/ (Bui et al., 2007).

Table 1. Vina search space used in this study.

MHC-II	Vina search space	Dimensions (Å)
Murine H2-Ak	X: 14.7455, Y: 76.8158, Z: 46.1697	X: 53.0125, Y: 42.0993, Z: 47.5793
HLA-DQA1*01:02/DQB1*06:02	X: -23.5078, Y: -68.5545, Z: 145. 9409	X: 43.3407, Y: 33.5898, Z: 45. 7314
HLA-DRB1*07:01	X: 54.7443, Y: -14.672, Z: 42.3426	X: 60.5904, Y: 37.4749, Z: 40.4071

The conserved epitopes had IEDB scores higher than 70% (Kurniawan et al., 2020). The epitopes with an immunogenicity score higher than 70% were considered immunogenic (Dhanda et al., 2018).

Epitope structure modeling and molecular docking

The murine H2-Ak and Human Leukocyte Antigen (HLA) DQA1*01:02/DQB1*06:02 3D structures were downloaded from PDB (https://www.rcsb.org) with accession numbers of 1IAK (Fremont et al., 1998) and 6DIG (Jiang et al., 2019), respectively. Swiss-Model (https://swissmodel.expasy.org/) was used to model the 3D structure of HLA-DRB1*07:01 (Waterhouse et al., 2018). Epitopes that met the criteria were then modeled using PEP-FOLD3 (https://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#forms::PEP-FOLD3) (Lamiable et al., 2016). The 3D structure of epitopes was retrieved and processed for molecular docking. PyRx v0.8 (Schrodinger, 2022) was used to minimize the energy of the epitopes prior to molecular docking. Autodock Vina (Dallakyan and Olson, 2015) on PyRx v.0.8 was used to conduct specific docking between MHC-II and epitopes. The Vina Search Spaces and dimensions are displayed in Table 1. The interactions between epitopes and the MHC-II proteins were then visualized using Discovery Studio 2019 (BIOVIA, 2019). The epitope that had been successfully bound into the active site cleft of MHC-II and exhibited low binding energy were proceeded to the subsequent analysis.

Allergenic properties of epitope and population coverage

Prediction of epitope's allergenic properties was conducted to ensure the safety of humans. The allergenic properties of epitope were predicted by Algpred (https://webs.iiitd.edu.in/raghava/algpred/submission.html) using Mapping of IgE epitopes and PID, MEME/MAST motif, and Blast search on allergen representative peptides (ARPs) (Shah et al., 2018). The calculation of population coverage was done by IEDB (http://tools.iedb.org/population/) (Bui et al., 2006) to predict the percentage of the population with a specific HLA genotype for recognizing the epitopes. The population coverage analysis was restricted to four countries with *B. candidus* in their natural habitat.

Estimation of physicochemical characteristics

Physicochemical characteristics of the selected epitope were estimated with ProtParam at ExPASy web server (https://web.expasy.org/protparam/) (Duvaud et al., 2021) to ensure the viability of the epitope *in vitro*. The characteristics that were considered in the analysis were as follows, molecular weight, theoretical pI, half-life of epitope in cells, instability index, aliphatic index, and grand average of hydropathicity (GRAVY).

Molecular dynamics simulation

A molecular dynamics simulation was conducted to predict the stability of the selected epitope over specific time periods. YASARA (Krieger and Vriend, 2014) was used to run the molecular dynamics simulation with 50 nanosecond periods. The cellular environment settings were set to pH 7.4 with a 0.9% concentration of NaCl ion. The temperature and water density for simulation were set to 310°K and 0.997 g/L, respectively. After that, the epitope's root-mean-square deviation (RMSD) backbone was analyzed. If the RMSD value of the epitope did not exceed 3 Å, the epitope was considered stable (Wibowo et al., 2021).

Data analysis

The candoxin protein sequence was retrieved from NCBI with accession number P81783.2. After that, the potential epitopes were predicted by using IEDB and SYFPHEITHI web server. The epitope conservancy and immunogenicity analysis were conducted using the IEDB server. Then, the epitopes that had met the criteria were modeled using the PEPFOLD3 server. Three MHC-II 3D structures that were used for receptors were downloaded from RCSB PDB. The molecular docking analysis between epitopes and three MHC-II was conducted using Autodock Vina at PyRx v0.8 and visualized using Discovery Studio 2019. Next, the epitope's allergenic properties, population coverage, and physicochemical characteristics were predicted using AlgPred, IEDB, and ExPASy web servers. Finally, molecular dynamics simulation analysis was carried out by using YASARA, and the data was visualized using Microsoft Excel.

RESULTS

The predicted epitopes of candoxin protein

A total of 47 candoxin epitopes for murine H2-Ak and 10 candoxin epitopes for human MHC-II were predicted and met the scoring criteria. All epitopes have 15 amino acid sequences, with the core peptides located in the middle of the sequence. Among those epitopes, only five epitopes exhibited shared sequences between murine and human MHC-II. The predicted epitopes showed an immunogenicity range of 91.55-98.24% and had 100% conservancy (Table 2). The B cell epitope prediction returned with three potential epitopes that showed various lengths, as shown in Table 3. However, this study found that only one B cell epitope (FKESWREARGTRI) displayed identical sequences with five epitopes from MHC-II. Then, these epitopes were modeled, and further analyses were made (Fig. 1).

Interaction of helper T cell epitopes with murine H2-Ak and human MHC-II

The docking simulation showed the binding position and interactions between MHC-II and epitopes. Among five epitopes, 47-CFKESWREARGTRIE-61 was the only epitope that bound with murine H2-Ak, HLA-DQA1*01:02/DQB1*06:02, and HLA-DRB1*07:01 in their binding site cleft (Fig. 2) and presented a binding affinity of -10.8, -10.2, and -11.6 kcal/mol, respectively (Table 4).

The docking between 47-CFKESWREARGTRIE-61 and murine H2-Ak formed six conventional hydrogen bonds at the amino acid sequences N:CYS1:HT1-A:TYR9:O, N:CYS1:HS-B:GLU74:OE2, N:ARG10:HT2-A:GLN57:OE1, N:ARG10:HT1-A:GLN57:OE1, N:ARG10:HT3-A:GLN57:OE1, and N:ARG10:HT3-A:GLN57:OE1. The distance of hydrogen bonds were 2.58, 2.93, 2.33, 2.51, 2.58, and 2.95 Å, respectively. Only one hydrophobic bond was formed and located at N:CYS1-B:VAL78 with a distance of 4.50 Å (Table 4, Fig. 2). Nine interactions were found between 47-CFKESWREARGTRIE-61 and HLA DQA1*01:02/DQB1*06:02. Four of them were conventional hydrogen bonds at N:CYS1:HT1-B:ASN82:OD1, N:GLU4:O-B:GLU74:OE1, N:ARG7:HN2-B:GLU74:OE1, and B:THR77:HG1-N:GLU4:O that had distances of 2.88, 3.01, 2.99, and 2.13 Å, respectively. Two carbon-hydrogen bonds were found at N:SER5:HC1-A:ASP58:O (2.83 Å), and A:GLY61:HA1-N:SER5:O (3.09 Å). The three other interactions were non-hydrogen bonds that consisted of two hydrophobic N:CYS1-A:CYS11 (4.38 Å), N:CYS1-B:VAL78 (3.28 Å), and one Pi-sulfur N:CYS1:S-A:HIS27 (4.71 Å) (Table 4, Fig. 2). Hydrogen bonds were the only interactions that found between 47-CFKESWREARGTRIE-61 and HLA-DRB1*07:01. The total of six interactions was divided into four conventional hydrogen bonds N:SER5:HO1-B:ASP70:OD2 (2.32 Å) N:SER5:HO2-B:ASP70:OD2 (2.36 Å) N:ARG7:HN2-A:ASN62:OD1 (2.29 Å) B:GLN74:NE2-N:GLU8:O (2.72 Å), and two carbon-hydrogen bonds N:SER5:HC-B:ASP70:OD2 (2.35 Å) N:ARG7:HC2-A:ASN62:OD1 (2.20 Å) (Table 4, Fig. 2). The detailed interaction types and residues involved in the binding between MHC-II and other epitopes were given in supplementary material (Annex 1). Based on the binding affinity and the interaction with MHC-II, the 47-CFKESWREARGTRIE-61 was proceeded to further analyses.

Allergenic prediction of the epitope

The prediction method by mapping of IgE epitope showed that 47-CFKESWREARGTRIE-61 did not contain experimentally proven IgE epitope. In addition, MAST and Blast analysis also predicted that there was no potential allergen for the epitope (Table 5). The allergenic prediction from AlgPred resulted in the non-allergenic potential of the epitope.

Population coverage of the epitope

The population coverage for epitope was restricted to four countries (Indonesia, Malaysia, Singapore, and Thailand) that encompass the naturally occurring habitat of *Bungarus candidus*. The average population coverage for those countries was 30.92%. Thailand was the top country that provided 45.99% coverage population for its population. The lowest population coverage country was Malaysia, with only 10.92% (Table 6).

Physicochemical characteristics of the epitope

The results from physicochemical characteristics prediction showed that the selected epitope had a molecular weight of 1868.10 Da, and this epitope was considered stable because it demonstrated an instability index of 30.13. The aliphatic index of this epitope was 32.67 and exhibited a GRAVY value of -1.273. The *in vivo* lifespan of this epitope was 1.2 hours in mammalian reticulocyte cells and more than 20 hours in yeast cells (Table 7).

Molecular dynamics of the epitope

The RMSD backbone generated from molecular dynamics was used to capture the alterations of amino acid residues within the epitope. Within 50 nanoseconds of simulation, the average RMSD backbone for the epitope was 1.658 Å with a peak of 3.068 Å at 29.975 ns, and the minimum RMSD backbone value was 0.364 at 0 ns. The RMSD backbone value at the half-time of the simulation was 1.433 Å and at the end of the simulation presented a value of 2.236 Å (Fig. 3).

Table 2. Predicted T Cell epitope from IEDB and SYFPHEITHI databases.

Amino acid sequence	Immunogenicity (%)	Conservancy (%)	SYFPHEITHI score	IEDB percentile rank	HLA Alleles
46-YC**FKESWREAR**GTRI-60	91.55	100	10	3.70	HLA-DRB1*07:01
47-C**FKESWREAR**GTRIE-61	94.97	100	2	3.60	
50-E**SWREARGTR**IERGC-64	98.24	100	2	3.10	HLA-DQA1*01:02/DQB1*06:02
52-WREAR**GTRIERGCA**A-66	97.61	100	2	2.40	
53-REAR**GTRIERGCA**AT-67	97.84	100	8	3.20	

The peptide core of the epitopes are indicated in bold.

Table 3. Predicted B cell epitopes from IEDB database.

No.	Start	End	Peptide	Length
1	26	45	ICNFDTCRAGELKVCASGEK	20
2	48	60	FKESWREARGTRI*	13
3	69	76	PKGSVYGL	8

*B cell epitope that matched with murine and human T cell epitope

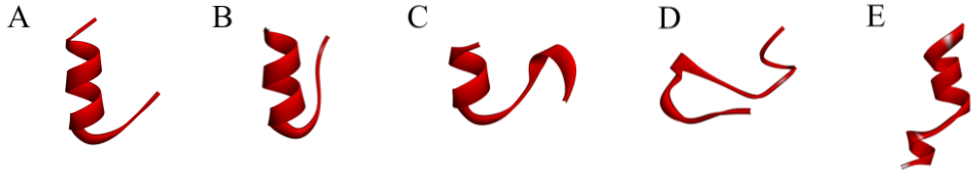

Figure 1. 3D model of the candoxin epitopes.

(A) 46-YCFKESWREARGTRI-60, **(B)** 47-CFKESWREARGTRIE-61, **(C)** 50-ESWREARGTRIERGC-64, **(D)** 52-WREARGTRIERGCAA-66, and **(E)** 53-REARGTRIERGCAAT-67.

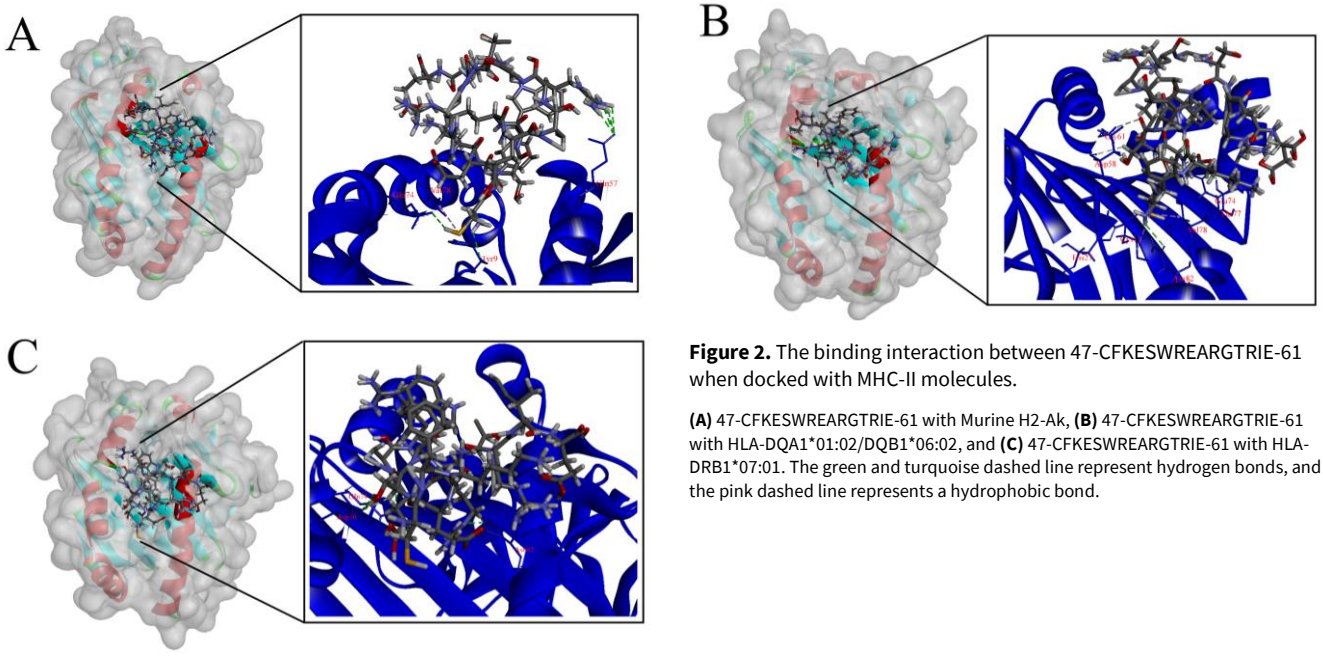

Figure 2. The binding interaction between 47-CFKESWREARGTRIE-61 when docked with MHC-II molecules.

(A) 47-CFKESWREARGTRIE-61 with Murine H2-Ak, **(B)** 47-CFKESWREARGTRIE-61 with HLA-DQA1*01:02/DQB1*06:02, and **(C)** 47-CFKESWREARGTRIE-61 with HLA-DRB1*07:01. The green and turquoise dashed line represent hydrogen bonds, and the pink dashed line represents a hydrophobic bond.

DISCUSSION

The most crucial part of producing vaccine-based antivenom is the ability of the epitope to produce protective antibodies against venom's toxic properties (Li et al., 2014). From the results, one epitope (47-CFKESWREARGTRIE-61) showed potential as a vaccine-based antivenom. The potential of this epitope was proven by this study using databases and a molecular docking prediction. This epitope has acceptable scores in web databases (Table 2), meaning its probability of binding into MHC-II active site was high (Ortega-Tirado et al., 2020). Further analyses were conducted using molecular docking, and this epitope was proven to bind to the MHC-II binding site (Table 4; Fig. 2). The interactions between the epitope and MHC-II were mediated by both hydrogen and hydrophobic bonds. The hydrogen bonds that are involved in the interactions improve the binding stability. In addition, hydrophobic bonds strengthen the interactions between epitope and MHC-II molecules (Chen et al., 2016). Epitope presentation by the MHC-II molecule will activate the helper T cells. Furthermore, the activated helper T cells induce B cells differentiation into plasma cells, resulting in antibody production (Sethu et al., 2012). Moreover, the presence of B cells epitope sequences in 47-CFKESWREARGTRIE-61 peptide increases the probability of inducing antibodies since B cells will differentiate into plasma cells and then secrete antibodies upon recognizing the epitope (Sanchez-Trincado et al., 2017). Apart from the immunogenicity, the allergenic of the epitope needs to be evaluated to ensure that there are no reactogenic responses in humans (Ahmad et al., 2021). The results from this study showed that epitope 47-CFKESWREARGTRIE-61 does not have the potential to be an allergen as no IgE epitope is found.

The population coverage was used to predict the percentage of individuals that could respond to the 47-CFKESWREARGTRIE-61 epitope based on the genotypic frequencies of HLA class II (Ortega-Tirado et al., 2020). The population coverage for this epitope was limited only to 30.92% of the four countries' populations (Table 6). This study only uses two HLA alleles, namely DQA1*01:02/DQB1*06:02 and HLA-DRB1*07:01, whereas these countries have different alleles of HLA class II for the majority of the population (Jinam et al., 2010; Pradana et al., 2020; Satapornpong et al., 2020). Hence, additional HLA alleles are required in future studies to extend this epitope's population coverage.

Table 4. Summary of the binding interactions between 47-CFKESWREARGTRIE-61 and MHC-II.

MHC-II	Epitope	Binding affinity (Kcal/mol)	Interactions	Amino acids
Murine H2-Ak	47-CFKESWREARGTRIE-61	-10.8	Hydrogen bond	N:CYS1:HT1 - A:TYR9:O
				N:CYS1:HS - B:GLU74:OE2
				N:ARG10:HT2 - A:GLN57:OE1
				N:ARG10:HT1 - A:GLN57:OE1
				N:ARG10:HT3 - A:GLN57:OE1
				N:ARG10:HT3 -A:GLN57:OE1
			Hydrophobic	N:CYS1 - B:VAL78
HLA-DQA1*01:02 /DQB1*06:02	47-CFKESWREARGTRIE-61	-10.2	Hydrogen bond	N:CYS1:HT1 - B:ASN82:OD1
				N:GLU4:O - B:GLU74:OE1
				N:ARG7:HN2 - B:GLU74:OE1
				B:THR77:HG1 - N:GLU4:O
				N:SER5:HC1 - A:ASP58:O
				A:GLY61:HA1 - N:SER5:O
			Hydrophobic	N:CYS1 - A:CYS11
				N:CYS1 - B:VAL78
HLA-DRB1*07:01	47-CFKESWREARGTRIE-61	-11.6	Hydrogen bond	N:SER5:HO1 - B:ASP70:OD2
				N:SER5:HO2 - B:ASP70:OD2
				N:ARG7:HN2 - A:ASN62:OD1
				B:GLN74:NE2 - N:GLU8:O
				N:SER5:HC - B:ASP70:OD2
				N:ARG7:HC2 - A:ASN62:OD1
			Hydrophobic	-

Table 5. Allergenic prediction of the 47-CFKESWREARGTRIE-61 epitope.

Amino acid sequence	Mapping IgE epitope	MAST result	BLAST result
47-CFKESWREARGTRIE-61	Does not contain experimentally proven IgE epitope	Non-Allergen	Non-Allergen

Table 6. The population coverage for the 47-CFKESWREARGTRIE-61.

Countries	Population coverage (%)
Indonesia	41.86
Malaysia	10.92
Singapore	24.91
Thailand	45.99
Average	30.92

Table 7. Physicochemical characteristics of 47-CFKESWREARGTRIE-61.

Characters	Value
Molecular weight	1868.10
Theoretical pI	8.22
Estimated half-life in mammalian cells	1.2 hours
Estimated half-life in yeast	>20 hours
Estimated half-life in *E. coli*	>10 hours
Instability index	30.13
Aliphatic index	32.67
Grand average of hydropathicity	-1.273

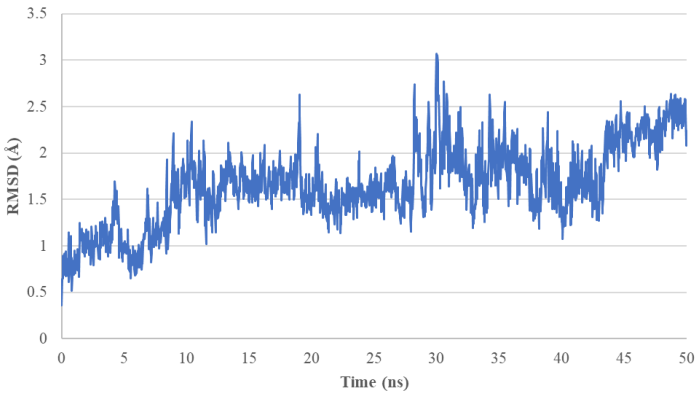

Figure 3. The RMSD backbone value of the 47-CFKESWREARGTRIE-61 epitope.

In silico prediction of physicochemical properties was utilized to determine its viability when expressed using various cell types. The results showed that the most viable expression of the epitope was in yeast cells. The stability index also showed that the epitope was considered stable when expressed *in vitro*. Based on the aliphatic index and the GRAVY value, this epitope is also considered to be thermostable and hydrophilic (Table 7). Furthermore, molecular dynamics analysis showed that the RMSD backbone of the epitope was considered stable (Fig. 3). Overall, these results indicated that this epitope could be a suitable candidate for vaccine-based antivenom.

Several studies have conducted bioinformatic methods to construct epitope-based antivenom; for example, Wagstaff et al. (2006) designed the epitope from the cDNA library of *Echis ocellatus* snake venom metalloproteinase (SVMP) venom; Castro et al. (2015) predicted 3FTx and PLA_2 B Cell epitopes of *Micrurus corallinus* venom; and Cao et al. (2016) calculated immunogenic linear B Cell epitope for *Deinagkistrodon acutus*. All of these studies have confirmed the antibody induction and the neutralization of the venom

activities using *in vivo* studies (Cao et al., 2016; Castro et al., 2015; Wagstaff et al., 2006). Using similar bioinformatics approaches, this preliminary study was believed feasible to be tested *in vivo*.

Bioinformatics approaches in this study provide the epitope candidate for vaccine-based antivenom. Based on the binding interactions and the immunogenicity prediction, this epitope is believed that capable of inducing immune responses toward *B. candidus* neurotoxicity. With the stable properties, this epitope may be feasible if produced using cells *in vitro*. Therefore, further *in vitro* and *in vivo* studies are needed to confirm the ability of this epitope to induce antibodies and neutralize the neurotoxicity activity of *B. candidus* venom.

CONCLUSION

In the current study, one candoxin epitope was identified. This epitope could be considered to become a candidate for the novel *B. candidus* antivenom design. Based on bioinformatics approaches, this epitope was predicted to induce antibody production by binding with murine and human MHC-II. In addition, this study also predicted that this epitope is safe and stable when produced *in vitro*.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Grahadi R	Fatchiyah F	Kurniawan N
Concepts or ideas	x	x	x
Design	x	x	x
Definition of intellectual content	x	x	x
Literature search	x	x	x
Experimental studies	x		
Data acquisition	x	x	x
Data analysis	x	x	x
Statistical analysis	x	x	x
Manuscript preparation	x		
Manuscript editing	x		
Manuscript review	x	x	x

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Annex 1. The complete list of binding interactions between epitopes and MHC-II.

MHC-II	Epitope	Binding affinity (Kcal/mol)	Interactions	Amino acids
Murine H2-Ak	46-YCFKESWREARGTRI-60	-10.5	Hydrogen bond	N:ALA10:HN1 - B:THR77:O N:ALA10:HN2 - B:THR77:O A:GLN61:NE2 - N:ILE15:O
			Hydrophobic	N:CYS2 - B:ARG70
HLA-DQA1*01:02 /DQB1*06:02	46-YCFKESWREARGTRI-60	-11.2	Hydrogen bond	N:TYR1:HT2 - A:ASP58:O N:LYS4:HN1 - A:ASP58:OD2
			Hydrophobic	N:CYS2 - B:VAL78
HLA-DRB1*07:01	46-YCFKESWREARGTRI-60	-10.3	Hydrogen bond	N:ARG11:HO - A:PHE54:O N:GLY12:HN2 - A:PHE54:O N:GLY12:HO - A:ALA52:O N:ARG14:HN - A:GLY49:O A:THR41:N - N:GLU9:O
			Hydrophobic	-
Murine H2-Ak	50-ESWREARGTRIERGC-64	-13.5	Hydrogen bond	N:GLU1:O - A:VAL139:O N:ARG4:HN - N:GLU12:O N:ARG4:HN1 - A:ASN140:OD1 N:GLU5:HN - N:GLU1:O N:ALA6:HN1 - N:SER2:O N:ALA6:HN1 - N:TRP3:O N:ALA6:HN2 - N:SER2:O N:ALA6:HN2 - N:TRP3:O N:ARG7:HN - N:TRP3:O N:ARG7:HN - N:ARG4:O N:GLY8:HN1 - N:ARG4:O N:GLY8:HN3 - N:ARG4:O N:THR9:HN1 - N:ARG4:O A:ARG141:N - N:GLU1:O N:SER2:HC - N:GLU5:O
			Hydrophobic	-
HLA-DQA1*01:02 /DQB1*06:02	50-ESWREARGTRIERGC-64	-11.2	Hydrogen bond	N:ARG4:HN - N:GLU12:O N:GLU5:HN - N:GLU1:O N:GLU5:HN - N:SER2:O N:ALA6:HN1 - N:SER2:O N:ALA6:HN1 - N:TRP3:O N:ALA6:HN2 - N:SER2:O N:ALA6:HN2 - N:TRP3:O N:ARG7:HN - N:TRP3:O N:ARG7:HN - N:ARG4:O N:GLY8:HN1 - N:ARG4:O N:GLY8:HN3 - N:ARG4:O N:THR9:HN1 - N:ARG4:O N:SER2:HC - N:GLU5:O
			Hydrophobic	-

Annex 1. The complete list of binding interactions between epitopes and MHC-II (continued...)

MHC-II	Epitope	Binding affinity (Kcal/mol)	Interactions	Amino acids
HLA-DRB1*07:01	50-ESWREARGTRIERGC-64	-10.9	Hydrogen bond	N:SER2:HN - A:GLU55:OE2 N:TRP3:HN - A:GLU55:OE2 N:ARG4:HN1 - A:GLU55:OE1 N:ARG13:HT1 - B:ASP70:OD2 N:GLY14:O - B:ASP70:OD1 N:GLU1:HC - A:GLU55:OE1 N:GLY14:HC2 - B:THR77:OG1
			Hydrophobic	-
Murine H2-Ak	52-WREARGTRIERGCAA-66	-11.5	Hydrogen bond	N:ARG5:O - A:GLN61:OE1 N:ILE9:HO - B:THR77:O A:GLN57:NE2 - N:GLY6:O A:GLN61:NE2 - N:ARG5:O A:GLN61:NE2 - N:THR7:O N:ALA15:HC - A:ASN62:OD1 N:CYS13:S - A:PHE54
			Hydrophobic	-
HLA-DQA1*01:02 /DQB1*06:02	52-WREARGTRIERGCAA-66	-11.3	Hydrogen bond	N:TRP1:HN - B:GLU69:OE1 N:ARG8:HN2 - B:THR77:O N:ARG11:HO - A:ASP58:OD2 N:GLY12:HC1 - A:GLN60:O
			Hydrophobic	-
HLA-DRB1*07:01	52-WREARGTRIERGCAA-66	-10.8	Hydrogen bond	N:ARG2:O - N:THR7:O N:ARG5:HN - N:TRP1:O N:ARG5:HN - N:ARG2:O N:GLY6:HN - N:ARG2:O N:THR7:HN - N:ARG2:O N:ARG2:HC - N:THR7:O N:ARG2:HC1 - N:THR7:O
			Hydrophobic	-
Murine H2-Ak	53-REARGTRIERGCAAT-67	-11.4	Hydrogen bond	N:THR15:HO1 - A:ALA3:O
			Hydrophobic	-
HLA-DQA1*01:02 /DQB1*06:02	53-REARGTRIERGCAAT-67	-12.1	Hydrogen bond	N:ARG10:HT1 - A:ASP58:O N:ARG10:HT4 - A:ASP58:O N:ARG7:HC1 - A:GLN60:OE1
			Hydrophobic	-
HLA-DRB1*07:01	53-REARGTRIERGCAAT-67	-11.1	Hydrogen bond	N:ARG1:HT - A:SER53:OG N:ARG10:HT1 - A:GLU55:O N:ARG1:HC2 - B:HIS81:NE2
			Hydrophobic	-