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Article

Anticancer Activities of Seven Peronemins (A2, A3, B1, B2, B3, C1, and D1) from *Peronema canescens* Jack: A Prediction Studies

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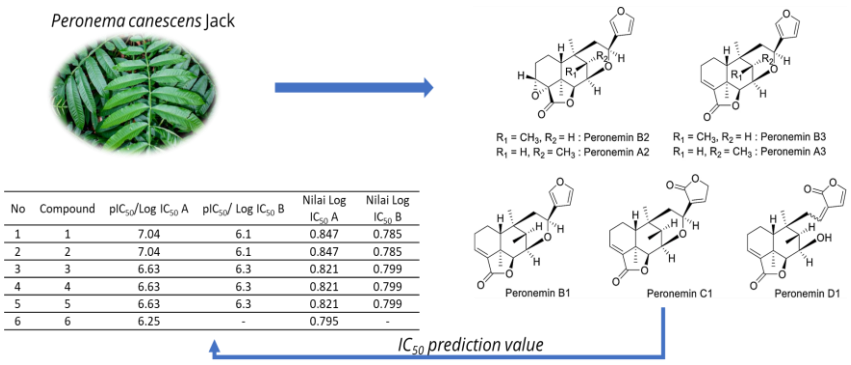
Abstract

Cancer is one of the leading causes of human death. In 2019, it was reported that cancer was the second (22%) cause of death due to non-communicable diseases in the world's population. Research for alternative anticancer drugs is still being done, including anticancer from plants. One of the plants that have the potential to be developed as an anticancer alternative is the sungkai plant. Sungkai leaves contain many bioactive compounds, one of which is the clerodane-type diterpenoids, peronemins, A2 **(1)**, A3 **(2)**, B1 **(3)**, B2 **(4)**, B3 **(5)**, C1 **(6)**, and D1 **(7)**. The aim of this study was to initial screen the potential of seven Peronemins compounds in Sungkai leaves extract as anticancer candidates. Initial screening was carried out by predicting in-silico anticancer activity of the seven compounds. Dihydrofolate reductase inhibitor (DHFR inhibitor) is one of the anticancer activity screening approaches. DHFR Inhibitor activity from perenomins derivatives with pIC_{50} values of 0.785 (A2), respectively; 0.785 (A3); 0.799 (B2); 0.799 (B3); 0.799 (C1 and D1). In addition, from compounds **1,2,3,4,5** peronemin derivatives have potential anticancer activity through interaction with the target protein Voltage-gated potassium channel subunit while compounds **6, 7** also have biological activity potential anticancer on target protein Dihydrofolate reductase.

Keywords: *anticancer, peronemins, Peronema canescens Jack*

Graphical Abstract



No	Compound	pIC_{50}/Log IC_{50} A	pIC_{50}/ Log IC_{50} B	Nilai Log IC_{50} A	Nilai Log IC_{50} B
1	1	7.04	6.1	0.847	0.785
2	2	7.04	6.1	0.847	0.785
3	3	6.63	6.3	0.821	0.799
4	4	6.63	6.3	0.821	0.799
5	5	6.63	6.3	0.821	0.799
6	6	6.25	-	0.795	-

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Introduction

Cancer is one of the main causes of human death. In 2015 it was reported that cancer was the second (22%) cause of death due to non-communicable diseases in the world's population. Breast cancer rates are known to grow faster in Asia than in the West. It was reported by WHO that nearly 1.38 million cases of breast cancer were diagnosed in 2008 [1], with a prevalence rate of 23% of all cancer cases in the world. In addition, it is known that 209,000 new cases were found, especially in Southeast Asia [2]. According to the International Agency on Research in Cancer, breast cancer has become the most common malignant tumor among Indonesian women [3]. Oral cancer, on the other hand is one of the most frequently detected cancers in the world. In several South-Central Asian countries, the mortality rate caused by this cancer has become an important public health problem. Globally, this disease is usually detected after a late medical diagnosis and causes a high mortality rate. Squamous cell carcinoma is the most common malignancy of the oral cavity. Oral cancer cases are estimated to be around 275,000 for oral and 130,300 for pharyngeal cancer per year, excluding the nasopharynx. Two thirds of these incidents occur in developed countries [4]. Various types of cancer therapies and complementary agents have been developed for their treatment.

Efforts to find alternatives to treat cancer are still being carried out, but there are still very few alternative drug candidates for the disease. One of the plants that has the potential to be explored and developed as raw material for anticancer drugs is the Sungkai plant (Peronema cannescens Jack) [5]. Sungkai Acetone Extract has seven peronemins compounds (A2, A3, B1, B2, B3, C1 and D1). The results of the isolation of the seven compounds have not been tested for their activities. This study is an initial screening of the anticancer activity of the peronemin compound of sungkai extract, using an in-silico approach.

One of the widely used areas of computational chemistry is the Quantitative Structure-Activity Relationship (QSAR). QSAR can be used to study the relationship between molecular structure and its biological activity expressed quantitatively. The field of drug design is the most widely used in this area. The QSAR method is able to reduce costs and risks in the pharmaceutical industry. The basic assumption of QSAR/HKSA is that there is a quantitative relationship between microscopic (molecular structure) and macroscopic/empirical (biological activity) properties of a molecule. The term structure is not only limited to understanding the spatial arrangement and the relationships between atoms in a molecule, but also includes the physical and chemical properties inherent in the arrangement.

To study the interaction of a drug molecule with its receptor and to study the potential of a molecule as a drug by examining the electronic structure or quantum chemical aspects of the molecule, computational chemistry methods are used. For this reason, it is necessary to have an initial simulation in the design of new drug discovery, this initial simulation was carried out using the pIC50 predictor with the help of the pChembl website in describing target proteins (receptors) related to the structure of peronemin derivative compounds of the sungkai plant, especially sungkai leaves using the QSAR machine learning method. The results of the QSAR Machie learning quantitatively describe the pIC50 value of the compound and a qualitative description of the target protein. The pIC50 value is the same as Log IC50 Peronemine A2 (1) and B2 (2).

Experimental Section

Compound Structure

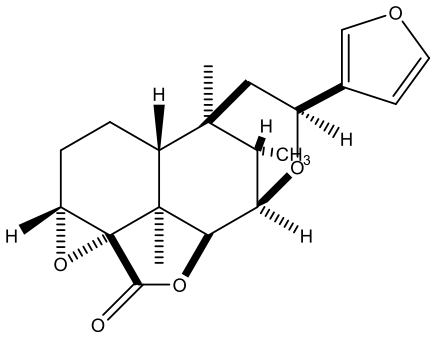
A total of seven peronemin compounds used as research materials were made into two-dimensional (2D) structures using the Hyperchem® 7.0 program, then the structures were equipped with hydrogen atoms to obtain a complete structure as well as its three-dimensional (3D) shape. The structure of the 3D shape is formed by a molecular model (model build) to obtain a structure that is close to the most stable state. The next step is geometry optimization, which is to find the most stable molecular structure. Furthermore, compound SMILES will be generated to be used in the prediction of pIC_{50}.

Predictor Calculation

A single point calculation was performed using the pChEMBL® program package on an optimized structure to obtain the electronic parameter (σ) in the form of the net charge of the atom (q) contained in the molecule, as well as selecting the R2test value from 0.8 to 1.00 with a pIC50 value ranging from 0, 6-0.9 for enzymes/precursors involved in anticancer activity.

Results and Discussions

Sungkai is a novel compound belonging to the clerodane-type diterpenoids, pronemins. Peronemins is reported to have seven types of peronemins compounds, A2(1), A3(2), B1(3), B2(4), B3(5), C1(6), and D1(7) (Figure 1).



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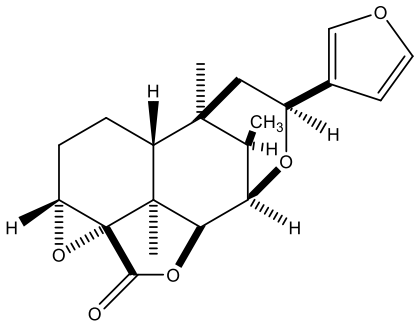

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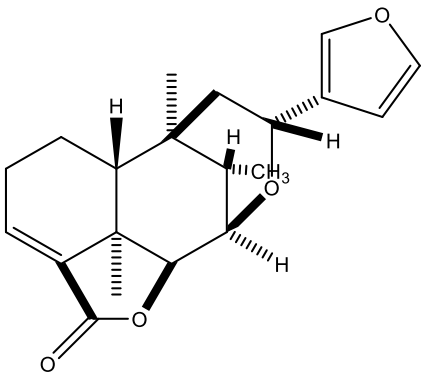

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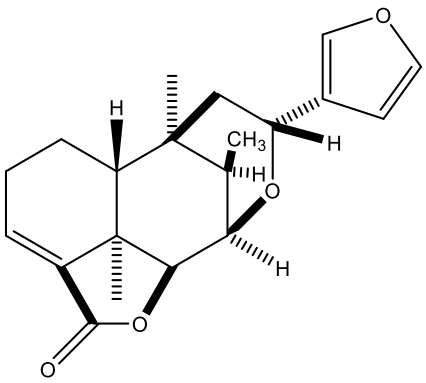

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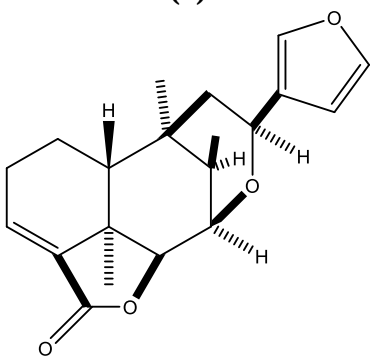

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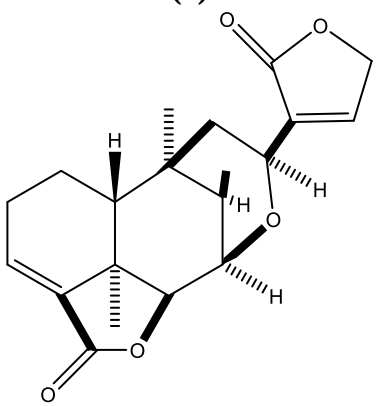

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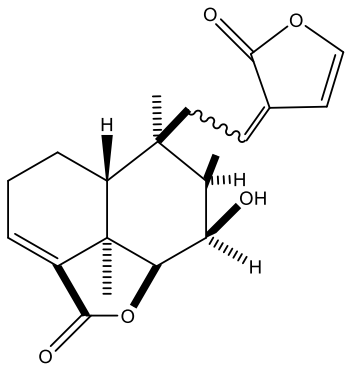

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Figure 1. Peronemins, A2(1), A3(2), B1(3), B2(4), B3(5), C1(6), dan D1(7).

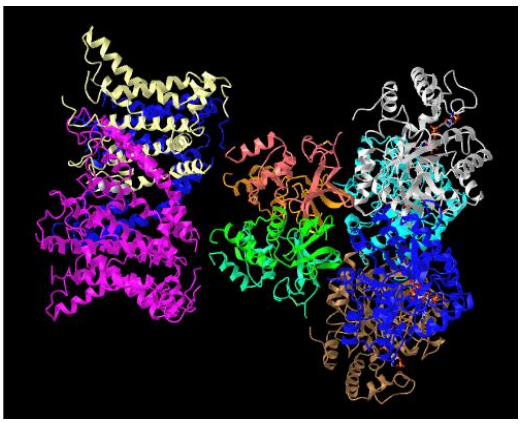

Figure 2. Protein target voltage-gated potassium channel subunit Kv1.3.

Figure 3. Protein target **Dihydrofolate reductase**.

Based on the pCHEMBL Machine Learning analysis, the pIC_{50} value of peronemin derivative compounds contained 2 target proteins that were active as receptors for anticancer drugs from peronemine derivatives. The 2 target proteins are Voltage-gated potassium channel subunit Kv1.3 (Figure 2) and Dihydrofolate reductase (Figure 3). Voltage-gated potassium channel Kv1.3 is an integral membrane protein, which is selectively permeable for potassium

ions and is activated upon a change of membrane potential. Kv1.3 channel is expressed in human T and B lymphocytes, macrophages, fibroblast, platelets, macrophages, osteoclasts, microglia, oligodendrocytes, brain (e.g., olfactory bulb, hippocampus, and cerebral cortex), lung, islets, thymus, spleen, lymph nodes, and testis [6]. Kv1.3 channel is also expressed in the inner mitochondrial membrane (mito Kv1.3) of normal human T lymphocytes and cancer cells, such as human leukemic T cell line Jurkat, prostate cancer PC-3 cells and breast cancer MCF-7 cells [6,7].

Recently published data demonstrated that Kv1.3 channel is also expressed in the nuclei of cancer cells, such as Jurkat T cells, breast cancer MCF-7, lung cancer A549 and gastric cancer SNU-484 cells as well as in human brain tissues [8]. Moreover, Kv1.3 channel was also discovered in the cis-Golgi apparatus membrane in rat cancer astrocytoma C6 cells as well as in non-cancerous CTX TNA2 astrocyte cell line and in rat primary astrocytes [9]. Activity of Kv1.3 channel plays an important role in cell proliferation and apoptosis [10–13]. The channel activity is inhibited by many chemically unrelated compounds: heavy-metal cations, smallmolecule organic compounds and venom-isolated oligopeptides.

The most potent specific inhibitors inhibit the channel at subnanomolar concentrations. Inhibition of Kv1.3 channel by specific inhibitors may be beneficial in therapy of T-lymphocyte-mediated autoimmune diseases (e.g., sclerosis multiplex, type I diabetes mellitus, rheumatoid arthritis, psoriasis), chronic renal failure, asthma, obesity, type II diabetes mellitus, cognitive disabilities, and some cancer disorders [9].

A good pIC_{50} value has a range of 6-8. The smaller the IC_{50} value of a compound in relation to biological activity, the potential for biological activity increases, meaning that the reactivity of a compound increases when associated with drug candidates (Table 1 – Table 4).

Table 1. Prediction of pIC_{50} based on machine learning (pchembl) QSAR of peronemin compounds A2 and B2.

Protein Target	pIC_{50}	∑ Dataset	R^2 test	Target Report Card	Drug/Clinical Candidates
Peronemins A2 and B2					
Tissue factor pathway inhibitor	7.69	3382	0.83	CHEMBL3713062	Anticoagulation potential (blood clotting)
Serine/threonine-protein kinase WEE1	7.13	578	0.91	CHEMBL5491	Adavosertib (8.28)
Voltage-gated potassium channel subunit Kv1.3	7.04	801	0.85	CHEMBL4633	Cancer therapy potential (+)
Angiotensin II type 2 (AT-2) receptor	6.93	690	0.83	CHEMBL257	Anti-inflammatory potential
Serine/threonine-protein kinase/endoribonuclease IRE1	6.47	670	0.87	CHEMBL1163101	Endoplasmic reticulum (ES) stress (heart drug potential)
Phosphodiesterase 7A	6.43	621	0.8	CHEMBL3012	Potential chronic obstructive pulmonary disease, erectile dysfunction, pulmonary arterial hypertension, benign prostatic hyperplasia, acute

					decompensated heart disease, psoriasis, arthritis, psoriatic arthritis, atopic dermatitis, neonatal apnea
Calcitonin gene-related peptide type 1 receptor	6.31	744	0.83	CHEMBL3798	Olcegepant (10.70), Telcagepant (8.70)
Apoptosis regulator Bcl-2	6.17	888	0.87	CHEMBL4860	Venetoclax (8.13), Navitoclax (8.70)
Phosphodiesterase 5A	6.15	1987	0.83	CHEMBL1827	Vardenafil Hydrochloride (9.15), Sildenafil Citrate (8.66), Gisadenafil (8.91), Avanafil (8.15), Pf-00489791(9.30), Tadalafil (8.92)
Dihydrofolate reductase	6.1	1442	0.83	CHEMBL2425	Potential anticancer (+), antimalarial, antifungal, antibacterial

Table 2. Prediction of pIC_{50} based on machine learning (pchembl) QSAR of peronemin compounds A3, B3 and B1.

Protein Target	pIC_{50}	∑Dataset	R^2 test	Target Report Card	Drug/Clinical Candidates
Peronemin compounds A3, B3 and B1					
Calcitonin gene-related peptide type 1 receptor	9.65	744	0.83	CHEMBL3798	Olcegepant (10.70), telcagepant (8.70)
Tissue factor pathway inhibitor	8.28	3382	0.83	CHEMBL3713062	Anticoagulant potential (blood clotting)
Voltage-gated potassium channel subunit Kv1.3	6.63	801	0.85	CHEMBL4633	Cancer therapy potential (+)
Phosphodiesterase 5A	6.51	1987	0.83	CHEMBL1827	Vardenafil Hydrochloride (9.15), Sildenafil Citrate (8.66), Gisadenafil (8.91), Avanafil (8.15), Pf-00489791(9.30), Tadalafil (8.92)
Phosphodiesterase 7A	6.34	621	0.8	CHEMBL3012	Anti-inflammatory potential
Dihydrofolate reductase	6.3	1442	0.83	CHEMBL2425	Potential anticancer (+), antimalarial, antifungal, antibacterial
Angiotensin II type 2 (AT-2) receptor	6.25	690	0.83	CHEMBL257	Anti-inflammatory potential

Table 3. Prediction of pIC_{50} based on machine learning (pchembl) QSAR of peronemin C1 compound.

Protein Target	pIC_{50}	∑ Dataset	R^2 test	Target Report Card	Drug/Clinical Candidates
Calcitonin gene-related peptide type 1 receptor	9.43	744	0.83	CHEMBL3798	Olcegepant (10.70), Telcagepant (8.70)
Angiotensin II type 2 (AT-2) receptor	6.79	690	0.83	CHEMBL257	Anti-inflammatory
Serine/threonine-protein kinase RAF	6.31	1248	0.83	CHEMBL1906	Regorafenib (8.82)
Phosphodiesterase 7A	6.31	621	0.8	CHEMBL3012	Anti-inflammatory
Dihydrofolate reductase	6.25	1442	0.83	CHEMBL2425	Potential anticancer (+), antimalarial, antifungal, antibacterial
Apoptosis regulator Bcl-2	6.2	888	0.87	CHEMBL4860	Venetoclax (8.13), Navitoclax (8.70)
Peroxisome proliferator-activated receptor delta	6.03	638	0.88	CHEMBL3979	GW501516 (9.00)

Table 4. Prediction of pIC_{50} based on machine learning (pCHEMBL) QSAR Peronemin D1 Compound.

Protein Target	pIC_{50}	∑ Dataset	R_2 test	Target Report Card	Drug/Clinical Candidates
Calcitonin gene-related peptide type 1 receptor	7.66	744	0.83	CHEMBL3798	Olcegepant (10.70), Telcagepant (8.70)
Apoptosis regulator Bcl-2	6.38	888	0.87	CHEMBL4860	Venetoclax (8.13), Navitoclax (8.70)
Phosphodiesterase 7A	6.34	621	0.8	CHEMBL3012	Anti-inflammatory potential
Serine/threonine-protein kinase WEE1	6.07	578	0.91	CHEMBL5491	Adavosertib (8.28)
Estradiol 17-beta-dehydrogenase 2	6.06	627	0.84	CHEMBL2789	Antiosteoporosis Potential
Angiotensin II type 2 (AT-2) receptor	6.04	690	0.83	CHEMBL257	Anti-Inflammatory Potential
Serine/threonine-protein kinase RAF	6.03	1248	0.83	CHEMBL1906	Regorafenib (8.82)

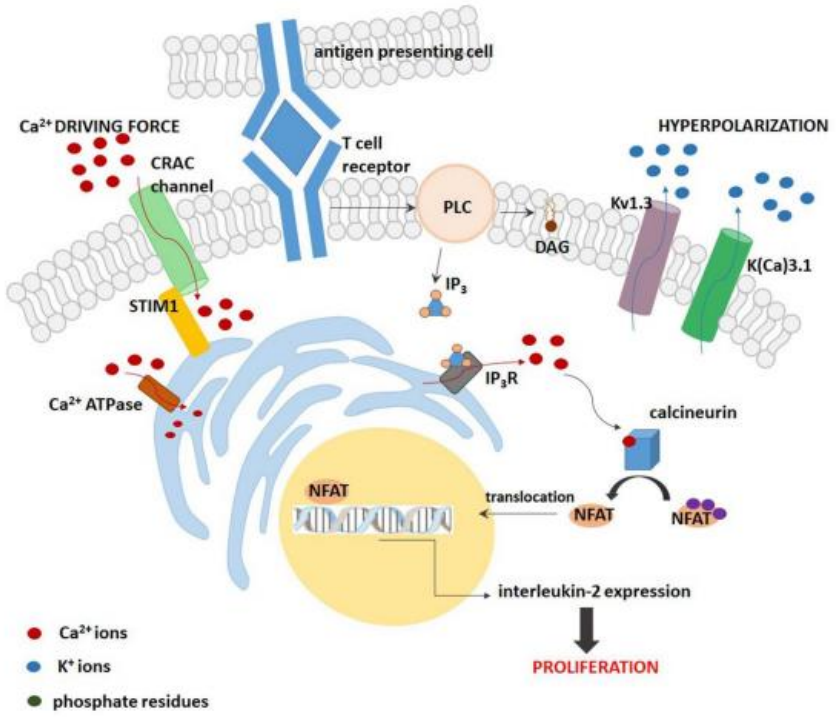

Figure 4. A scheme of the "membrane potential model" for the contribution of Kv1.3 and K(Ca) channels to proliferation of T lymphocytes [14].

Table 5. IC_{50} prediction value of peronemine derivative compounds.

No	Compound	pIC_{50}/Log IC_{50} A	pIC_{50}/ Log IC_{50} B	Nilai Log IC_{50} A	Nilai Log IC_{50} B
1	1	7.04	6.1	0.847	0.785
2	2	7.04	6.1	0.847	0.785
3	3	6.63	6.3	0.821	0.799
4	4	6.63	6.3	0.821	0.799
5	5	6.63	6.3	0.821	0.799
6	6	6.25	-	0.795	-

A = Voltage-gated potassium channel subunit Kv1.3
B = Dihydrofolate reductase

Compounds 1,2,3,4,5 peronemin derivatives have potential for biological activity, namely anticancer potential on the target protein Voltage-gated potassium channel subunit Kv1.3 and Dihydrofolate reductase, while compound 6 also has potential biological activity for anticancer on target protein Dihydrofolate reductase (Figure 4). This is evidenced by the IC_{50} value of each compound in Table 5. The smaller the IC_{50} value, the greater the potential for biological activity, which means that it is possible to find new drug compounds, but compound 7 does not appear to have potential anticancer activity, it is possible to change the structure of peromemin derivatives. due to the loss of one of the bonds in one of the bonds that bind the furan ring, this difference is shown in compounds 6 and 7 (Figure 5).

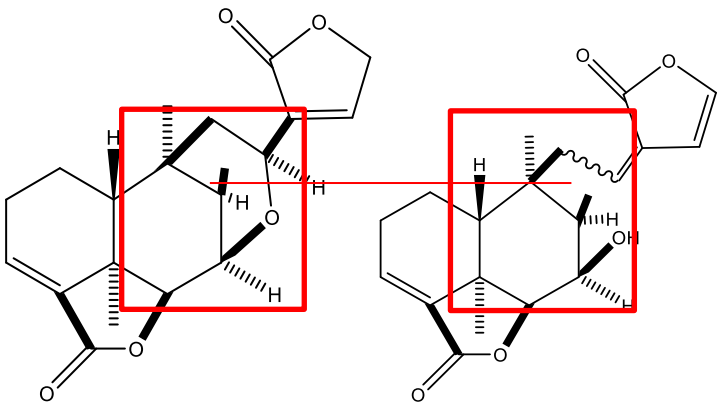

Figure 5. Differences in bonds in the Furan ring of compound 6 and compound 7

The decrease in the inductive effect of the structural change is possible because the oxygen atom has a greater electronegativity than carbon based on the Pauling scale this is possible the flow of electrons through the sigma bond (σ) decreases when viewed from the biological activity of anticancer potential.

In contrast to the Kv1.3 channel mechanism, DHFR enzyme has complex interactions so that it is potential for antibacterial, antimalarial, and anticancer activities. DHFR catalyzes the reduction of dihydrofolate to tetrahydrofolate using NADPH, and it is involved in the synthesis of raw material for cell proliferation, in both prokaryotic and eukaryotic cells. DHFR inhibitors are commonly used for fighting malaria and other protozoal infections, as well as for treating fungal, bacterial, and mycobacterial infections [15].

Conclusions

DHFR Inhibitor activity from perenomins derivatives with pIC50 values of 0.785 (A2), respectively; 0.785 (A3); 0.799 (B2); 0.799 (B3); 0.799 (C1 and D1). In addition, from compounds 1,2,3,4,5 peronemin derivatives have potential anticancer activity through interaction with the target protein Voltage-gated potassium channel subunit while compounds 6, 7 also have biological activity potential anticancer on target protein Dihydrofolate reductase.

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