Pharmacophore Optimization Of Berberine As HER2 Inhibitor

Mohammad Rizki Fadhil Pratama[*], Guntur Satrio Pratomo

PHARMACY DEPARTMENT, FACULTY OF HEALTH SCIENCE, MUHAMMADIYAH UNIVERSITY OF
PALANGKARAYA, PALANGKA RAYA, INDONESIA 73111
[*]EMAIL: m.rizkifadhil@umpalangkaraya.ac.id

ABSTRAK

Penelitian sebelumya menunjukkan bahwa berberin, alkaloid yang terdapat pada beberapa tumbuhan seperti akar kuning (*Arcangelisia flava*) menunjukkan potensi inhibisi terhadap *Human epidermal receptor-2* (HER2). Modifikasi *pharmacophore* pada struktur kimia berberin diprediksi dapat meningkatkan afinitas derivat berberin terhadap HER2. Tujuan penelitian ini adalah menemukan *pharmacophore* utama pada berberin yang memiliki pengaruh paling penting terhadap afinitas berberin pada HER2.

Metode yang digunakan adalah *molecular docking* hasil modifikasi *pharmacophore* terhadap HER2.

Hasil *docking* menunjukkan atom O pada posisi nomor 23 memiliki pengaruh penting terhadap afinitas berberin pada HER2, dimana modifikasi atom O menurunkan afinitas berberin. Afinitas tertinggi ditunjukkan oleh derivat berberin-6 dengan energi bebas ikatan dan konstanta disosiasi secara berturut-turut sebesar -10,80 kcal/mol dan 12,17 nM. Berbeda dengan derivat berberin lainnya, derivat berberin-6 tidak berinteraksi dengan asam amino treonin pada posisi 862. Hal tersebut memberikan prediksi bahwa interaksi pada asam amino tersebut berpotensi menurunkan afinitas berberin terhadap sisi aktif HER2. Lebih jauh derivat berberin-6 dapat dikembangkan menjadi inhibitor HER2 dan berpotensi untuk dikembangkan pada terapi kanker payudara HER2-positif.

Kata kunci: berberin, HER2, *pharmacophore*

ABSTRACT

Previous research has shown that berberine, an alkaloid found in several plants such as akar kuning (*Arcangelisia flava*) shows the potential for inhibition of Human epidermal receptor-2 (HER2). Pharmacophore modification on the berberine was predicted could increase the affinity of berberine derivatives against HER2. The present study aims to determine the main pharmacophore of berberine with the highest influence towards berberine affinity against HER2.

Molecular docking was performed on several modified pharmacophore of berberine against HER2.

The docking results show that O atom at position number 23 has the most important influence on the berberine affinity towards HER2, where modification of O atom resulting in a decrease of berberine affinity. The highest affinity showed by derivate berberine-6 with the free energy of binding score and dissociation constant -10.80 kcal/mol and 12.17 nM, respectively. In contrast with other berberine derivatives, the berberine-6 derivate does not interact with the amino acid threonine at position 862. It provides a prediction that interaction of that amino acid potentially decrease the berberine activity towards active site of HER2. Further, derivate berberine-6 could be developed into HER2 inhibitor and should be potential to be developed as HER2-positive breast cancer therapy.

Keywords: berberine, HER2, pharmacophore

INTRODUCTION

The anticancer properties of akar kuning (*Arcangelisia flava*) has been studied in various types of cancer cells, one of them in breast cancer (Sun *et al.*, 2009). The activity is due to various secondary metabolites of akar kuning, especially on the stem (Kunii *et al.*, 1985; Subiandono & Heriyanto, 2009). Our previous study showed that berberine, an alkaloid found in the stem part of akar kuning, has the highest affinity for HER2 receptor compared to other secondary metabolites (Pratama, 2016). Inhibition of HER2 receptor itself is known to be one of the main therapeutic approaches to breast cancer, especially HER2-positive breast cancer (Incorvati *et al.*, 2013).

Despite has the highest affinity, berberine affinity is still lower than several known HER2 inhibitors including TAK-285, an investigational drug with potent HER2 inhibition properties (Pratama, 2016). Optimization of berberine affinity as HER2 inhibitor can be done with several ways, one of them by pharmacophore modification (Kaserer *et al.*, 2015). The discovery of major pharmacophore with the greatest influence on the affinity of a ligand is performed by removing each pharmacophore from a ligand one by one (Kroemer, 2007). The modification result was reexamined by molecular docking method to see changes in the affinity of modified ligands (Meng *et al.*, 2011).

This study aims to find the best-modified pharmacophore results from berberine as HER2 inhibitor. The modification results were retested and compared with a known HER2 inhibitor to determine the potency of berberine derivatives in HER2-positive breast cancer therapy.
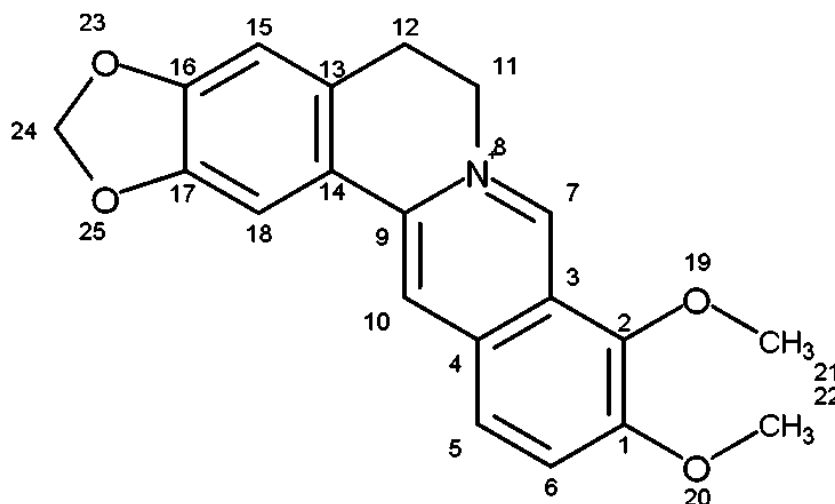

Figure 1. 2D structure of berberine

MATERIALS AND METHODS

The ligand used in this study were berberine and derivatives namely berberine-1 (O-19 to C-19), berberine-2 (O-20 to C-20), berberine-3 (N-8 to C-8), berberine-4 (O-23 to C-23), and

berberine-5 (O-25 to C-25). Structures of berberine and derivatives were sketched using GaussView 3.08 software from Gaussian, Inc (Cosconati *et al.,* 2010). The molecular structure of HER2 was obtained from the website of Protein Data Bank (PDB) www.rscb.org. HER2 structure used in this study contains tyrosine kinase domain from HER2 in complexed with known HER2 inhibitor TAK-285 (PDB ID 3PP0) (Aertgeerts *et al*, 2011). Tyrosine kinase domain was chosen since this site have already known development for HER2 inhibitor development (Pratama, 2015).

All ligand structures were geometry optimized by the molecular mechanic method with AMBER force field using Gaussian 03W software from Gaussian, Inc. Geometry optimization provided an ideal conformation of following ligands that approaching conformation of the compound in nature (Cosconati *et al.,* 2010). The format of optimized structures changed from .log to .pdb using OpenBabel 2.3.2 software (O'Boyle *et al.*, 2011). Molecular docking performed using AutoDock 4.2.6 software from The Scripps Research Institute (Morris *et al.*, 2009).

Docking process validation was performed by redocking using TAK-285 as the co-crystal ligand of HER2 in 3PP0 receptor active site with pose selecti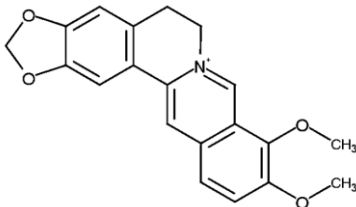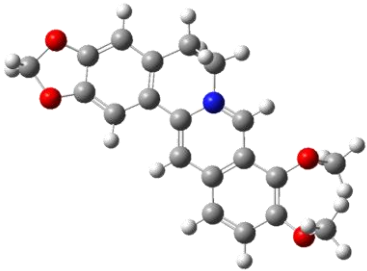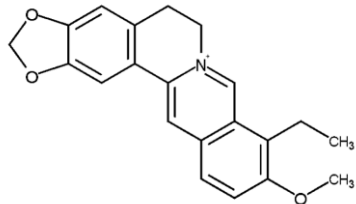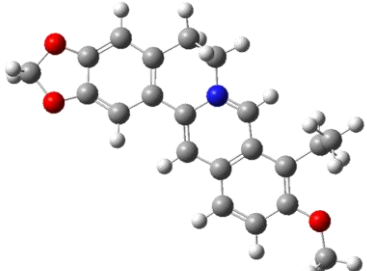on method (Kontoyianni *et al.*, 2004). The parameters of validation observed were root-mean-square deviation (RMSD) of co-crystal ligand at selected binding si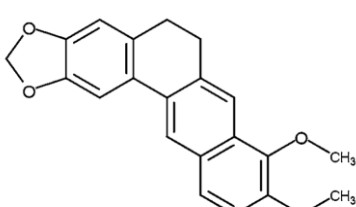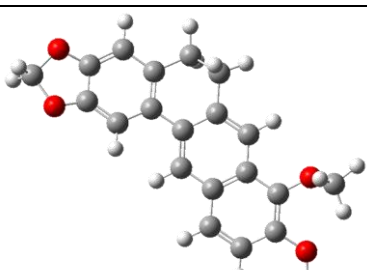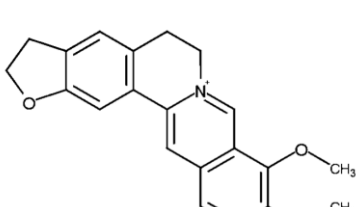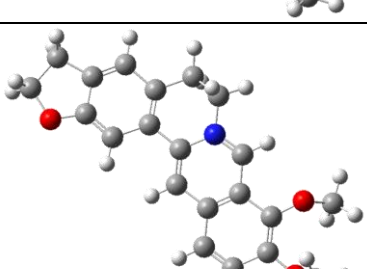te. Docking process is preferred to predict results from experimental poses with RMSD score no more than 2Å. Smaller RMSD score indicates the closer position of the redocking result against crystallography result (Bissantz *et al.*, 2000).
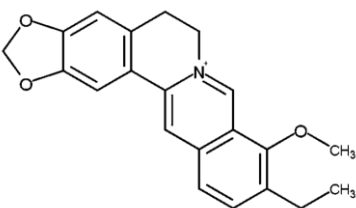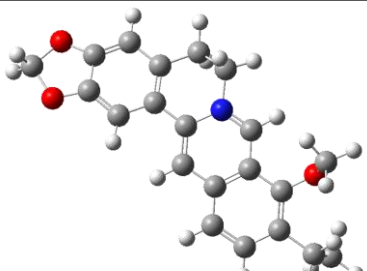
Molecular docking for all test ligands performed in same way as validation process using similar grid box size and position (Pratama, 2015). The main parameter observed were the free energy of binding (ΔG), the dissociation constant (K_i), amino acids residues, and numbers of hydrogen bonds. Ligand affinity to the receptor was determined by ΔG and K_i scores. More negative ΔG and lower K_i indicated higher ligand affinity toward receptor's binding site (Kim & Skolnick, 2008). The ligand with amino acids residues and numbers of hydrogen bonds which closest to co-crystal ligand indicate similarity type of interaction, in this case, describes similarity of activities (Cosconati *et al.,* 2010).

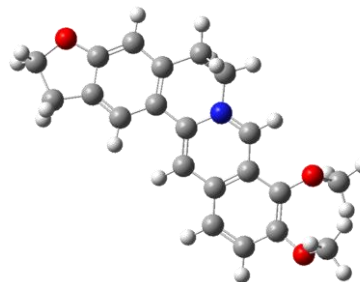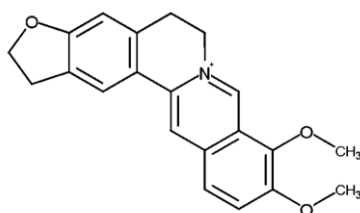## RESULTS AND DISCUSSION

Test ligands were sketched and performed geometry optimization with AMBER force field. All ligands structures were shown in Table 1.

Table 1. 3D structure of ligands

Compounds	2D structure	3D structure
Berberine (BBR-0)		
Berberine-1 (BBR-1)		
Berberine-2 (BBR-2)		
Berberine-3 (BBR-3)		
Berberine-4 (BBR-4)		

Berberine-5
(BBR-5)

Docking validation was done with redocking method using AutoDock 4.2.6. Validation was performed on the entire binding site of TAK-285 as a co-crystal ligand of the selected receptor (Cosconati *et al.,* 2010). Redocking results from this study were provided almost at the stacked position with crystallography results (Figure 2) with RMSD value 0.807 Å, indicated that receptor 3PP0 was valid for molecular docking purpose (Bissantz *et al.*, 2000). Other parameters observed in validation was ΔG, K_i, amino acids residues, and numbers of hydrogen bonds of the co-crystal ligand as shown in Table 2.
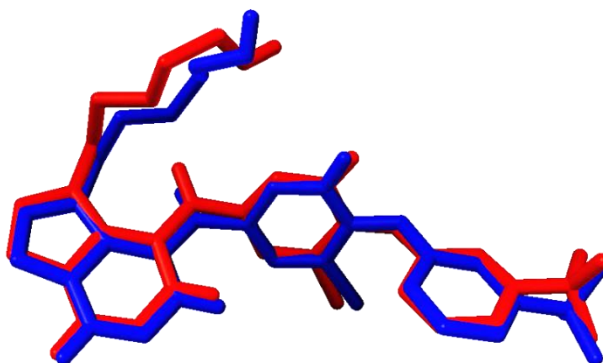
Figure 2. Results of validation from TAK-285; root-mean-square deviation = 0.807 Å (Red: TAK-285 redocking results; blue: TAK-285 crystallography result)

Table 2. Validation results of HER2 receptor PDB ID 3PP0 with TAK-285

Receptor	Ligand	RMSD (Å)	ΔG (kcal/mol)	K_i, (μM)	Amino acids residues	Numbers of hydrogen bonds
HER2	TAK-285	0.807	-10.62	0.01652	726-Leu, 734-Val, 751-Ala, 753-Lys, 770-Glu, 774-Met, 783-Ser, 785-Leu, 796-Leu, 798-Thr, 799-Gln, 800-Leu, 801-Met, 850-Asn, 852-Leu, 862-Thr, 863-Asp	1

Docking was performed using AutoDock 4.2.6 at the active site of HER2 receptor as used on validation process. A number of runs have been set on 100 genetic algorithms to improve the accuracy of docking results (Morris *et al.*, 2009). For each test ligand, one pose with most negative ΔG and lowest K_i scores was selected as representatives of test ligands (Cosconati *et al.,* 2010). The docking result data of five ligands to HER2 were compared each other as shown in Table 3.
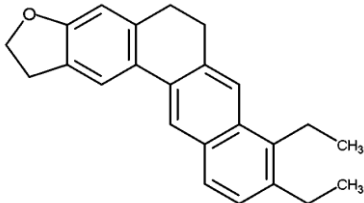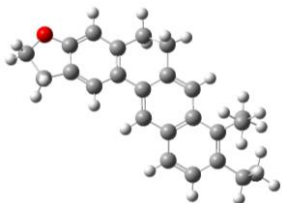
Table 3. Docking results of berberine derivatives at HER2 receptor

Parameters	BBR-0	BBR-1	BBR-2	BBR-3	BBR-4	BBR-5
ΔG (kcal/mol)	-9.34	-9.54	-9.46	-9.47	-9.14	-9.70
K_i, (µM)	0.14181	0.10243	0.11659	0.11400	0.20059	0.07757
Amino acids residues	726-Leu	726-Leu	726-Leu	726-Leu	726-Leu	726-Leu
	734-Val	734-Val	734-Val	734-Val	734-Val	734-Val
	751-Ala	751-Ala	751-Ala	751-Ala	751-Ala	751-Ala
	753-Lys	753-Lys	753-Lys	753-Lys	753-Lys	753-Lys
	785-Leu	785-Leu	785-Leu	785-Leu	785-Leu	785-Leu
	796-Leu	796-Leu	796-Leu	796-Leu	796-Leu	796-Leu
	798-Thr	798-Thr	798-Thr	798-Thr	798-Thr	798-Thr
	805-Cys	-	-	-	-	805-Cys
	849-Arg	849-Arg	-	849-Arg	849-Arg	849-Arg
	-	-	-	852-Leu	-	-
	862-Thr	862-Thr	862-Thr	862-Thr	862-Thr	862-Thr
	863-Asp	863-Asp	863-Asp	863-Asp	863-Asp	863-Asp
	-	-	864-Phe	-	864-Phe	864-Phe
Numbers of hydrogen bonds	0	0	0	1	1	0

All ligands provided a negative score of ΔG, indicated that interaction between HER2 receptor active site and all ligands will occur spontaneously (Kontoyianni *et al.*, 2004). Compared to other ligands, berberine-4 had the less negative ΔG and highest K_i scores, indicating deletion of pharmacophore at position 23 (O-23 to C-23) was resulting in loss of affinity of berberine. In contrast to berberine-4, other ligands had more negative ΔG and lower K_i scores than berberine, indicating pharmacophore at that position was not preferable as HER2 inhibitor. Regarding docking results, another test ligand namely berberine-6 was sketched contain the only pharmacophore at position 23, as shown in Table 4.

Table 4. 3D structure of berberine-6

Compounds	2D structure	3D structure
Berberine-6 (BBR-6)		

Another docking process then performed again using newly-designed berberine-6 toward HER2. Docking result then compared to TAK-285 as known HER2 inhibitor to analyze similarities and differences between docking results of two compounds as shown in Table 5.

Table 5. Comparison of docking results between TAK-285 and BBR-6

Parameters	TAK-285	BBR-6
ΔG (kcal/mol)	-10.62	-10.80
K_i, (µM)	0.01652	0.01207
Amino acids residues	726-Leu	726-Leu
	734-Val	734-Val
	751-Ala	751-Ala
	753-Lys	753-Lys
	770-Glu	-
	774-Met	-
	783-Ser	-
	785-Leu	785-Leu
	796-Leu	796-Leu
	798-Thr	798-Thr
	799-Gln	-
	800-Leu	-
	801-Met	-
	850-Asn	-
	852-Leu	852-Leu
	862-Thr	-
	863-Asp	863-Asp
	-	864-Phe
Numbers of hydrogen bonds	1	0

More observation conducted to reveal the interaction between berberine-6 and HER2 receptor active site by overlay the docking result of berberine-6 with the redocking result of TAK-285 as shown in Figure 3. Berberine-6 was docked into very similar position and orientation again TAK-285 (Figure 3a), while the interactions between berberine-6 and amino acids residues of HER2 active site also occur tightly without any obstacle (Figure 3b).
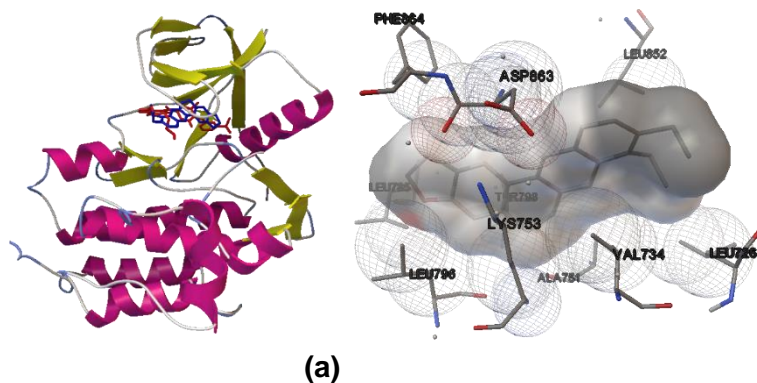

(a)   (b)

Figure 3. Comparison between docking results of TAK-285 (red) and BBR-6 (blue) (a); amino acids residues from docking results of BBR-6 toward HER-2 (b)

Whether berberine-6 had the same activity with TAK-285 or not was still unclear. However, since comparison results indicated almost every amino acids residues which interacted with berberine-6 also interacted with TAK-285 (9 out of 10), there was big possibility that berberine-6 also inhibits HER2 like TAK-285. Since HER2 inhibition was a key primary target for HER2-positive breast cancer therapy, this study results indicated that berberine-6 had the potency to be developed as HER2 inhibitor.

CONCLUSION

This study was successfully described the most important pharmacophore of berberine as HER2 inhibitor, which occurs at position number 23. Deletion of other pharmacophores resulting in an increase of berberine affinity towards HER2 active site with ΔG and K_i scores -10.80 kcal/mol and 12.07 nM, respectively. This result also opens up opportunities to further development of berberine as a potent HER2 inhibitor by optimizing the interactions mainly with another pharmacophore at position number 23. Thus, this study clearly indicates a promising potential of berberine derivatives to be developed as HER2 inhibitor for HER2-positive breast cancer therapy.

REFERENCES

Aertgeerts K, Skene R, Yano J, Sang BC, Zuo H, Snell G, Jennings A, Iwamoto K, Habuka N, Hirokawa A, Ishikawa T, Tanaka T, Miki H, Ohta Y, & Sogabe S. 2011. Structural analysis of the mechanism of inhibition and allosteric activation of the kinase domain of HER2 protein. *J Biol Chem*. 286(21):18756-18765.

Bissantz C, Folkers G, & Rognan D. 2000. Protein-based virtual screening of chemical databases 1. Evaluation of different docking/scoring combinations. *J Med Chem*. 43(25):4759-4767.

Cosconati S, Forli S, Perryman AL, Harris R, Goodsell DS, & Olson AJ. 2010. Virtual screening with autodock: Theory and Practice. *Expert Opin Drug Discov*. 5(6):597-607.

Incorvati JA, Shah S, Mu Y, & Lu J. 2013. Targeted therapy for HER2-positive breast cancer. *J Hematol Oncol*. 6(38):1-9.

Kaserer T, Beck KR, Akram M, Odermatt A, & Schuster D. 2015. Review: Pharmacophore models and pharmacophore-based virtual screening: concepts and applications exemplified on hydroxysteroid dehydrogenases. *Molecules*. 20(12):22799-22832.

Kim R & Skolnick J. 2008. Assesment of programs for ligand binding affinity prediction. *J Comput Chem*. 29(8):1316-1331.

Kontoyianni M, McClellan LM, & Sokol GS. 2004. Evaluation of docking performance: comparative data on docking algorithms. *J Med Chem*. 47(3):558-565.

Kroemer RT. 2007. Structure-based drug design: docking and scoring. *Curr Protein Pept Sci*. 8(4):312-328.

Kunii T, Kagei K, Kawakami Y, Nagai Y, Nezu Y, & Sato T. 1985. Indonesian medicinal plants. I. New furanoditerpenes from Arcangelisia flava Merr. (1). *Chem Pharm Bull*. 33(2):479-487.

Meng XY, Zhang HX, Mezei M, & Cui M. 2011. Molecular docking: a powerful approach for structure-based drug discovery. *Curr Comput Aided Drug Des*. 7(2):146-157.

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, & Olson AJ. 2009. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem*. 30(16):2785-2791.

O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, & Hutchison GR. 2011. Open Babel: An open chemical toolbox. *J Cheminform*. 3:33.

Pratama MRF. 2015. 'Molecular docking of anticancer agents: artemisinin and derivatives as HER2 inhibitor' in the Mahdiyah D. *Proceeding of 1st Sari Mulia International Conference on Health and Sciences 2015*, Banjarmasin, South Borneo, Indonesia, December 18th-19th 2015. Sari Mulia Institute of Health Science. 155-168.

Pratama MRF. 2016. Akar kuning (Arcangelisia flava) sebagai inhibitor EGFR: Kajian in silico. *Farmagazine*. 3(1):7-18.

Subiandono E & Heriyanto NM. 2009. Kajian tumbuhan obat akar kuning (Arcangelisia flava Merr) di kelompok hutan Gelawan, Kabupaten Kampar, Riau. *Buletin Plasma Nutfah*. 15:43-44.

Sun Y, Xun K, Wang Y, & Chen X. 2009. A systematic review of the anticancer properties of berberine, a natural product from chinese herbs. *Anticancer Drugs*. 20(9):757-769.