

## Prediction of Binding Mode of Secondary Metabolites in *Apium graveolens* to Bcl-2

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### Abstract

Developing new cytotoxic agent which has minimal effect against normal cell is required to reduce the side effects obtained from the existing chemotherapy agents. Celery (*Apium graveolens*) showed several pharmacology activities, including anti-cancer. This study was conducted to predict and visualize the binding mode of ten secondary metabolites in *A. graveolens*, i.e., apigenin, caffeic acid, kaempferol, limonene, shikimic acid, chlorogenic acid, ascorbic acid, quercetin, eugenol and ferulic acid against Bcl-2. Navitoclax was employed as reference. Molecular docking simulation was performed using AutoDoc Vina v.1.5.6. The results showed that the interaction energy ( $E_i$ ) ranged from -4.20 to -6.01 kcal/mol, whereas inhibition constant ( $K_i$ ) were 40.15 to 842.29  $\mu$ M. Kaempferol showed the best affinity to Bcl-2 ( $E_i$ =-6.01 kcal/mol;  $K_i$ =24,21  $\mu$ M). Hydrogen bonds were bond between kampferol and Lys20, Ser102, and Arg103, amino acid residues in Bcl-2. In conclusion,

**Keywords:** anti-cancer, *Apium graveolens*, Bcl-2, navitoclax

### Introduction

A cancer cell is a mutated abnormal cell, which proliferate progressively and become predominantly malignant. Variety of chemotherapy agents act to inhibit cell proliferation and malignancy.<sup>1</sup> Developing new cytotoxic agent which has minimal effect against normal cell is required to reduce the side effects obtained from the existing chemotherapy agents. Thus, developing new chemotherapy agents from herbal medicine is a potential alternative in order to reduce the

side effects.<sup>2</sup>

Secondary metabolites which exist in celery plants, such as apigenin, caffeine, kaempferol, limonene, cymic acid, chlorogenic acid, ascorbic acid, quercetin, eugenol and ferulic acid exhibited antitumor and cytotoxic activity.<sup>3</sup> Moreover, apigenin inhibited human lung cancer (NCI-H460) cells proliferation and induced apoptotic by increasing Bax and caspase-3 expression and decreasing Bcl-2

expression.<sup>4</sup>

Bcl-2 is a family of Bcl-2 protein regulator, which inhibits apoptotic activity.<sup>5</sup> Bcl-2 is a homodimer form, consists of monomer A and B. The expression of Bcl-2 will increase in lung cancer patient due to the inhibition of apoptotic activity.<sup>6</sup> The high level of Bcl-2 will protect the cells through the early death of apoptotic. Thus, Bcl-2 is an anti-apoptotic protein, which is classified as an oncogene.<sup>7</sup>

The purpose of this study was to predict and visualize the binding mode of ten secondary metabolites in *A. graveolens*, i.e., apigenin, caffeic acid, kaempferol, limonene, shikimic acid, chlorogenic acid, ascorbic acid, quercetin, eugenol and ferulic acid against Bcl-2.

## Methods

### *Instruments*

The hardware used for molecular calculations, molecular modeling and docking included personal computer with Windows 8.1 Ultimate 64-bit operating systems equipped with Intel Core i5-5200U @ 2.2GHz processors,

Ubuntu Linux 14.04 LTS operating system, 1000 GB hard disk capacity, and 4 GB DDR3 RAM memory. The softwares were:

1. Swiss-PdbViewer v.4.1.0 (Glaxo Smith Kline R&D) downloaded from <https://spdbv.vital.it.ch/>
2. ChemOffice 2004 free trial, downloaded from [www.cambridgesoft.com](http://www.cambridgesoft.com)
3. HyperChem Professional 7.03 by Hypercube Incorporation downloaded from <http://www.hyper.com>
4. RCSB-Ligand Explorer Viewer v.4.2.0 application by Research Collaboratory for Structural Bioinformatics, which is a data online from <http://www.pdb.org/pdb/explore>
5. AutoDock Vina in MGLTools v.1.5.6 (by Molecular Graphic Laboratory). The Scripps Research Institute downloaded from <http://mgtools.scripps.edu/>
6. Open Babel v.2.3.2 downloaded from <http://openbabel.org>
7. PyMOL v.1.6.X.

### *Materials*

3D structure of Bcl-2 complexed with navitoclax (indol), resolution 2.07 Å (PDB

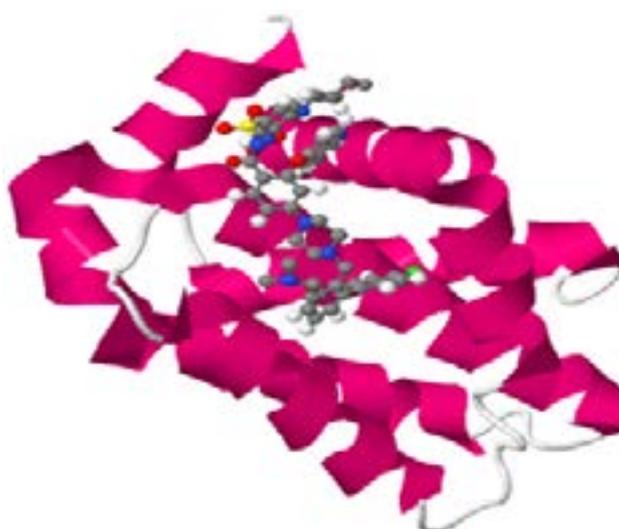


Figure 1. The 3D Structure of Bcl-2 Active site adapted from [www.pdb.org](http://www.pdb.org) with 4MAN code. Visualization using JS mole.

**Table 1. Molecular docking simulation**

Ligands	Parameter of analysis				$\pi - \pi$ inter- action
	Ei (SD) kcal/mol	Ki (SD) $\mu$ M	Hydrogen bonds	Amino acid residues	
Apigenin	-5.83 (0.17)	56.045 (17.87)	Asp99 Arg106	Tyr19, Lys20, Gln23, Arg24, Gly98, Asp99, Asp100, Phe101, Ser102, Arg106, Asp108, Glu149.	-
Kaempferol	-6.01 (0.56)	96.56 (21.85)	Lys20 Ser102 Arg103	Tyr19, Lys20, Gln23, Arg24, Gly96, Asp99, Asp100, Phe101, Ser102, Arg103, Arg106, Asp106, Arg107, Phe109, Ala110, Ser113, Glu149, Gly152.	-
Chlorogenic acid	-5.55 (0.12)	88.47 (21.05)	Arg24 Ser102	Lys20, Gln23, Arg24, Ser102, Phe109, Met112, Ser113, Ser114, Leu116, Glu149, Gly152, Val153, Met154, Lys155, Val156, Glu157	-
Caffeine acid	-5.55 (0.12)	88.47 (21.05)	Arg24 Ser113	Arg24, Phe109, Met112, Ser113, Ser114, Leu116, Glu149, Val153, Val156, Glu157	-
Ferrulic acid	-4.71(0.14)	358.75 (47.089)	Arg24 Ser102	Lys20, Leu21, Ser22, Gln23, Arg24, Gly98, Phe101, Ser102, Arg103, Tyr105, Phe109, Gly152, Val153, Val156	-
Eugenol	-4.20 ( 0.03)	842.29 (54.86)	Asp99	Lys20, Gln23, Asp99, Ser102, Tyr105, Phe109, Glu149	-
Ascorbic acid	-4.92 $\pm$ 0.140	252.68 (29.52)	Lys20 Arg24 Asp99	Lys20, Gln23, Arg24, Gly98, Asp99, Phe101, Ser102, Phe109, Glu149, Gly152, Val153, Val156	-
Limonene	-4.44 (0.19)	578.81 (124.28)	-	Met112, Ser113, Ser114, Leu116, Gly152, Val153, Val156, Glu157	-
Shikimic acid	-4.80 (0.00)	304.08 (0.00)	Gln23 Ser102	Tyr16, Lys20, Gln23, Arg24,	-
Quercetin	-6.00 (0.00)	40.15 (0.00)	Lys20 Asp99 Ser102	Lys20, Gln23, Arg24, Asp99, Ser102, Arg103, Tyr105, Arg106, Phe109, Ala110, Ser113, Glu149	-
Navitoclax indol	-11.24 $\pm$ 0.08	0.01 (0.00)	Asp100	Ala97, Asp100, Phe101, Tyr105, Met112, Val 130, Leu134, Gly142, Val145, Ala146, Phe150, Tyr199	-

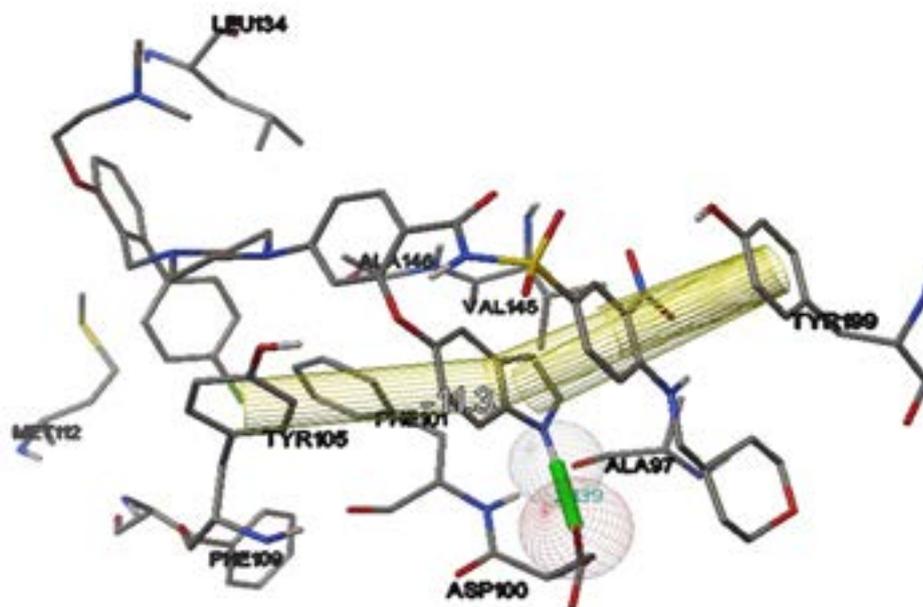


Figure 2. The binding mode of navitoclax with Bcl-2

code:4MAN www.pdb.org).<sup>10</sup> The 2D and 3D structures of all ligands were built using the ChemOffice 2004.

#### Procedure

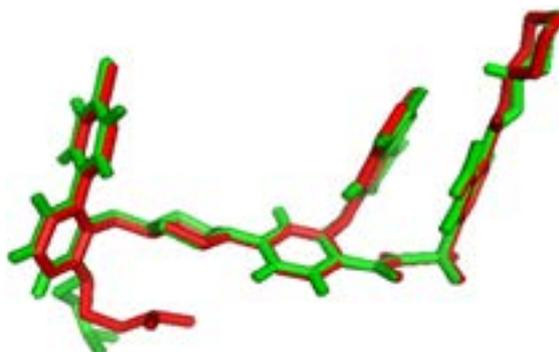
1. Bcl-2 preparation:
  - a. Active site analysis using Ligand Explorer Viewer
  - b. Bcl-2 bond reduction from homodimer to monomer using Swiss-PdbViewer v.4.1.0
2. Ligands preparation:
  - b. Geometry optimization with HyperChem Professional 7.03.
  - c. Analysis of molecular properties.
3. Program validation.
  - a. Structure superimposed between navitoclax indol model with crystal.
  - b. Re-docking navitoclax indol crystal to the Bcl-2 active site using Autodock Vina.
  - c. Data analysis.

4. Docking all ligands to Bcl-2 using AutoDock Vina

#### Results and Discussion

Ligand-protein interaction showed 12 amino acid residues (Leu 134, Met 112, Val 130, Val 145, Gly 142, Tyr 105, Tyr 199, Phe 150, Phe 101, Asp 100, Ala 146, and Ala 97), and one hydrogen bond (Asp 100) (Table 1).

Six ligands were optimized using the Austin mode I (AM1) method. This method is used for relatively small molecular weight compounds with less than 500 daltons. In addition, the AM1 method can optimize van der Waals interactions of a compound. The Poly-Ribiere (Conjugate Gradient) algorithm was chosen for the better results, shorter time and more accurate optimization result than other forms of algorithm. The shape of the Polak-Ribiere algorithm was the default setting of the HyperChem Professional 7.03.



**Figure 3. Superimposed of navitoclax model ■ and crystal ■ , visualized using PyMOL.**

The maximum number of cycles indicated the maximum number of rounds required by a compound to achieve the most stable conformation with minimum global energy or potential energy. The arrangement of geometrical optimization condition utilized the 0.1 kcal/Åmol of root mean square gradient (RMSG) value, which indicated an increasing amount of energy detected due to stable conformation changes in the compound.<sup>11</sup> The optimization results of the six compounds exhibited the difference value of 0.00000, which indicated all optimized compounds have reached the most stable conditions with the lowest energy levels, where the conformer potential energy equals to the minimum global energy (the minimum point on the potential energy curve). The volume of Bcl-2 active site was 2156.54 Å<sup>3</sup>, thus all ligands were predicted could occupy the active site, since all of the ligands have smaller volume.

Furthermore, molecular properties of the ligands were analyzed with quantitative structure-activity relationship (QSAR) using Portable HyperChem Professional 7.03. Analysis of the molecular properties included the basic parameters of analysis, such as molecular mass, molecular volume,

coefficient partition (log P), geometric optimization energy, and electrostatic potential.

The Lipinski's Rule of Five is a method for evaluating drug-likeness, where the rule specifies drug molecules must have a relative molecular mass less than 500 dalton, log P value less than five, a coefficient partition value between -2 to 5 and have ten maximum hydrogen bonds donor. Drugs with molecular weight over 500 daltons are more difficult to penetrate through lipid bilayers and tend to undergo first pass metabolism.<sup>12</sup>

In this study, all ligands qualified for Lipinski's Rule of Five. The higher of the log P, the more hydrophobic or lipophilic the compounds, hence it will be more easily to penetrate the cell membrane.<sup>13</sup> The most hydrophilic compound was caffeic acid, while, the most hydrophobic was limonene.

Figure 3 showed that the superimposed of navitoclax model and crystal. Based on the RMSD value (1.086 Å) obtained, the program was valid.

Redocking of navitoclax crystal into its origin location in Bcl-2 resulted in  $E_i = -11.24$

$\pm 0.08$  kcal/mol,  $K_i=0.006 \pm 0, 001$   $\mu$ M. The re-docking results had 66.67% similarity compared to the origin.

#### Molecular docking simulation

The ligands which easily interact with Bcl-2 in order were kaempferol, quercetin, apigenin, caffeine acid, chlorogenic acid, ascorbic acid, shikimic acid, ferulic acid, limonene, and eugenol. Kaempferol showed the best interaction with Bcl-2 active site ( $K_i=96.56$   $\mu$ M,  $EI= -6,01$  kcal/mol). Moreover, kaempferol formed three hydrogen bonds with Lys20, Ser102, and Arg103, which was similar to navitoclax. All ligands were predicted could interact to Bcl-2 due to their binding mode similarity with navitoclax.

#### Conclusion

Secondary metabolites in *A.graviolen* were predicted could interact with amino acid residues in Bcl-2 binding pocket. Their binding modes were similar to navitoclax. Kaempferol showed the best interaction.

#### Acknowledgement

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#### Funding

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#### Conflict of Interest

None declared

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