Open Journal of Medicinal Chemistry, 2014, 4, 79-86
Published Online September 2014 in SciRes. http://www.scirp.org/journal/ojmc
http://dx.doi.org/10.4236/ojmc.2014.43006

Scientific Research

Design and Molecular Docking Study of Antimycin A_3 Analogues as Inhibitors of Anti-Apoptotic Bcl-2 of Breast Cancer

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Received 9 July 2014; revised 11 August 2014; accepted 10 September 2014

Abstract

In this paper, we report the design and moleculardocking study of analogues of antimycin A_3 as inhibitors of anti-apoptotic Bcl-2 of breast cancer. Twenty designed compounds and the original antimycin A_3 were docked based on their interaction with breast tumor receptor binding target Bcl-2. The docking resulted in the five top-ranked compounds, namely, compounds 11, 14, 15, 16, and 20, which have a lower ΔG binding energy, better affinity and stronger hydrogen bonding interactions to the active site of Bcl-2 than antimycin A_3. Among those five top-ranked compounds, analogue compounds 11 and 14, which have an 18-membered tetralactone core and 18-membered tetraol core, respectively, exhibited the strongest hydrogen bond interaction, formed high stability conformation, and demonstrated the greatest inhibitory activity on the catalytic site of Bcl-2.

Keywords

Design, Docking, Antimycin A_3, Analogue, Bcl-2, Breast Cancer

1. Introduction

Breast cancer is the most prevalent cancer for women both in the developed and the developing world. Approximately 30% of the women diagnosed with early-stage disease in turn progress to metastatic breast cancer,

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How to cite this paper: Arsianti, A., *et al*. (2014) Design and Molecular Docking Study of Antimycin A_3 Analogues as Inhibitors of Anti-Apoptotic Bcl-2 of Breast Cancer. *Open Journal of Medicinal Chemistry*, **4**, 79-86.
http://dx.doi.org/10.4236/ojmc.2014.43006

for which treatments with anti-breast cancer therapeutic agents are needed. Although many current anti-breast cancer therapies can alter tumor growth, in most cases the effect is not long-lasting. Cancer drug resistance is thought to reduce seriously the effectiveness of current anti-breast cancer therapies, which caused around 50% of all treated patients relapsed [1]-[3]. This indicates need for new agents, which are safer, more effective, and potentially able to extend the survival of breast cancer patients.

Antimycin A_3, a mixture of the two nine-membered dilactones A_{3a} and A_{3b} isolated from *Streptomyces sp*., is an active agent that inhibits the electron transfer activity of ubiquinol-cytochrome *c* oxidoreductase and prevents the growth of human cancer cells (**Figure 1**). Antimycin A_3 was also found to induce apoptosis of cancer cells by selectively killing the cancer cells that expressed high levels of anti-apoptotic Bcl-2 with IC_{50} of 50 μM on Hela cells [4]-[6]. While Bcl-2 is known to be over-expressed in 70% of breast cancer cells [7], it is reasonable to expect antimycin A_3 to induce apoptosis in those cells. Thus, it is also quite reasonable to expect its analogue to have a similar or higher anti-breast cancer activities. In this work, antimycin A_3 analogues (**Figure 2**), are designed and subsequently simulated based on their interactions with receptor binding target Bcl-2 by a computational molecular docking approach. The top-ranked compounds showing stronger interaction, better affinity, as well as a greater inhibitory activity than antimycin A_3 against breast tumor receptor binding target Bcl-2, may become lead compounds in our next synthesis project.

Studies on the structure-activity relationship of antimycin A_3 by Miyoshi *et al.* in 1995 revealed that the nine-membered dilactone core in antimycin A_3 was less effective for anticancer activity than 3-formamidosali-cylyl moiety [8]. Pettit *et al.* (2007) reported that respirantin which has an 18-membered polylactone core instead of nine-membered dilactone core in antimycin A_3, showed stronger cytotoxicity than antimycin A_3 on mouse leukimia P-388 cells and breast MCF-7 cells [9] (**Figure 1**). It has also been reported that the presence of hydroxyl groups in bioactive compounds significantly increase their biological activities due to the enhancement of its solubility in water, which is one of the important factors influencing the efficacy of drugs [10]. These facts suggested that, it is quite possible to design novel antimycin A_3 analogues by replacing the nine-membered di-lactone core in antimycin A_3 with either an 18-membered polylactone core or polyhydro-xylated 18-membered polylactone core that contributes to the improvement of its anticancer activity.

In a recent study, we succeeded in synthesizing of novel polyhydroxylated 18-membered analogue of antimy-cin A_3 (compound **14**), which showed a potent anticancer activity against breast MDA-MB-231 cells [11]. It re-vealed that the polyhydroxylated 18-membered core was very important for anti-breast cancer activity. There-fore, in this work, the nine-membered dilactone core of antimycin A_3 was replaced by an 18-membered tetralac-tone core in analogues **10**, **11** and **12**, and was replaced by polyhydroxylated 18-membered tetralactone core in **13** - **20**. To study how the stereochemistry can affect the binding capability, we designed four hydroxyl groups with bottom facial stereochemistry on the 18-membered core in **13** - **16**, and, in contrast to those analogues, with the top facial stereochemistry in **17** - **20**. Subsequently, in order to increase the anticancer activity, we introduce two parts of 3-formamidosalicylyl moiety in **11**, **14**, and **18**. To explore how the simple substitutions on 3-formamidosalicylyl moiety can influence the binding capability on receptor target Bcl-2, we substitute the existing hydroxyl group in formamidosalicylyl moiety with benzyloxy group in **10**, **13**, and **17**. Whereas **12**, **15**, and **19** were designed by replacing the hydroxyl group in 3-formamidosalicylyl moiety with methoxy group. Furthermore, 3-formamidosalicylyl moiety was modified into 3-N-methylformamido-2-methoxy-benzoyl moiety in **16** and **20**. In this work, we also investigated the interaction of some benzoic acid ring segments

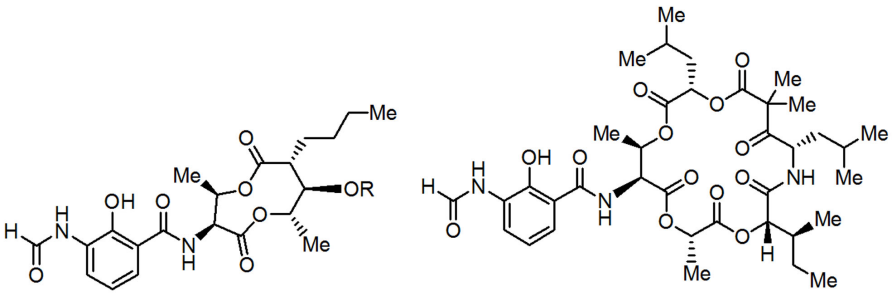


Figure 1. Structure of antimycin A_3 and respirantin.

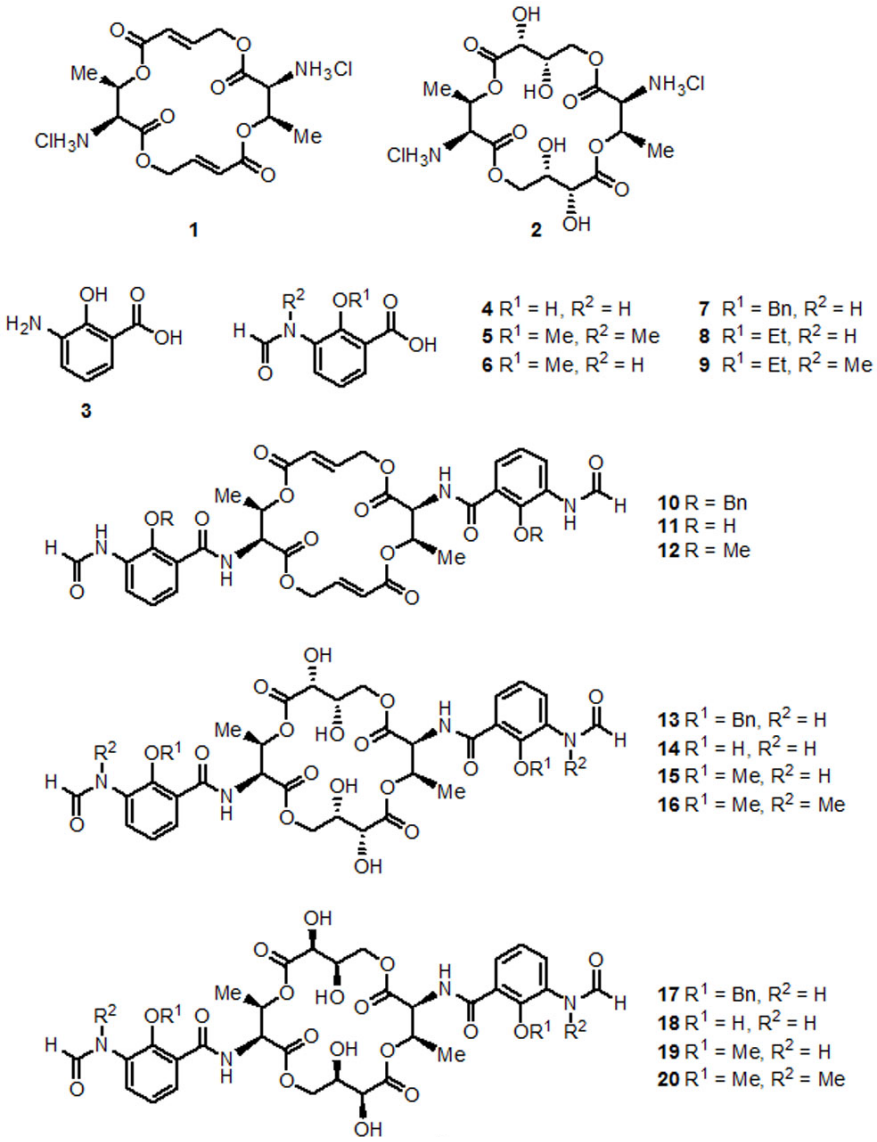


Figure 2. Structure of designed compound.

(compound **3** - **9**), the 18-membered tetralactone (**1**), and 18-membered tetraol (**2**) on receptor binding target Bcl-2 of breast cancer.

2. Methodology

In this research, we simulated some analogue compounds based on their interactions with Bcl-2 breast cancer, using computer software applications (*Molecular* method) [12] to determine the best compounds [13]. Analysis and screening were based on Gibbs Free energy (ΔG) values, affinity, conformation of the structure, and hydrogen bonding interaction between compounds and the target proteins [14].

2.1. Sequence Alignment and Homology Modelling

Target protein sequences were selected and downloaded from NCBI (http://www.ncbi.nlm.nih.gov/protein/133893254?report=fasta). The multiple sequence alignment method was based on the Clustal W2 program (www.ebi.ac.uk/Tools/clus talw2/index.html). Homology modeling was performed using the Swiss Model which can be accessed through

http://www.swissmodel.expasy.org/SWISS-MODEL.html. Swiss model showed that Bcl-2 has structurally homologous to a target protein with template PDB code 1g5mA (target region 3-204, 88.00 % of sequence identity.

2.2. Structural Analysis of Target Protein

Validation of 3D structure from homology modeling was performed using the Protein Geometry program and superimposed using superpose program in MOE 2009.10 software. Based on superimposed the RMSD was calculated to find out structural similarity between template model mutated with 3D structure from homology modeling. Identification of catalytic site of protein target using site finder program in MOE 2009.10 software.

2.3. Optimization and Minimization of 3D Structure

Optimization and minimization of three-dimensional structure of the enzyme were conducted using the software of MOE 2009.10 with addition of hydrogen atoms. Protonation was employed with protonating 3D programs. Furthermore, partial charges and force field were employed with MMFF94x. Solvation of enzymes was performed in the form of a gas phase with a fixed charge, RMS gradient of 0.05 kcal/A^0mol, and other parameters using the standard in MOE 2009.10 software.

2.4. Preparation of Compounds

Some antimycin A_3 analogues were designed using ACD Labs software. With this software, The analogues were built into three-dimensional structures. The three-dimensional shape was obtained by storing the analogue in the 3D viewer in ACD Labs. Furthermore, the output format was changed into Molfile MDL Mol format using the software Vegazz to confirm for the docking process. Compounds were in the wash with compute program, adjustments were made with the compound partial charge and partial charge optimization using MMFF94 xforcefield. The conformation structure energy of compounds was minimized using the RMS gradient energy with 0.001 kcal/A˚mol. Other parameters were in accordance with the default setting in the software.

2.5. Molecular Docking

The docking process was begun with the docking preparation that was employed using a docking program from MOE 2009.10 software. Docking simulations were performed with the Compute-Simulation dock program. The placement method was conducted using a triangle matcher with 1,000,000 repetition energy readings for each position and other parameters were in accordance with the default settings in the MOE software. Furthermore, scoring functions used London DG, refinement of the configuration repetition force field with 1000 populations. The first repetition was done for 100 times and the second setting was conducted only for one of the best result.

3. Results and Discussion

The twenty designed compounds, including the analogue compounds, 18-membered polylactones, and simple benzoic acid ring segments, were simulated using molecular docking on target protein of Bcl-2 breast cancer. The results are displayed in **Table 1**. The top-ranked compounds were selected based on low ΔG binding energy, high pK_i affinity, and number of hydrogen acceptor/hydrogen donors (hydrogen bonding interaction) to the catalytic site of Bcl-2 target protein.

As shown in **Table 1**, compared to antimycin A_3 and respirantin, the 18-membered tetraol (**2**) exhibited higher binding energy, affinity, and hydrogen bond interaction on Bcl-2 breast cancer cells, indicating that tetraol **2** has a stronger inhibitory activity against receptor target Bcl-2. In contrast, all the series of benzoic acid ring segments, **3** - **9**, showed a less number of hydrogen bonds than antimycin A_3 and respirantin. Suggesting that the benzoic acid ring segment itself has low interaction to protein target of Bcl-2 breast cancer. The docking of the analogue compound **10** - **20**, produced the five top-ranked compounds, namely, compounds **11**, **14**, **15**, **16**, and **20**, which showed lower ΔG binding energy value and a higher number of hydrogen bonding interaction than the others compounds. The ΔG values of compounds **11**, **14**, **15**, **16** and **20** are −16.4486, −15.9491, −15.0703, −17.1838 and −17.1553 kcal/mol, respectively, which are better than antimycin A_3 and respirantin, with a ΔG value of −11.4295 kcal/mol.These results showed that, compared to antimycin A_3, those five top-ranked compounds will form a more stable complex with Bcl-2, as well as, be better able to inhibit and reduce the activity of Bcl-2. The *pKi* value of the five top-ranked compounds are higher than antimycin A_3, indicating that

Table 1. The properties of twenty designed compounds and antimycin A_3 on the catalytic site of Bcl-2.

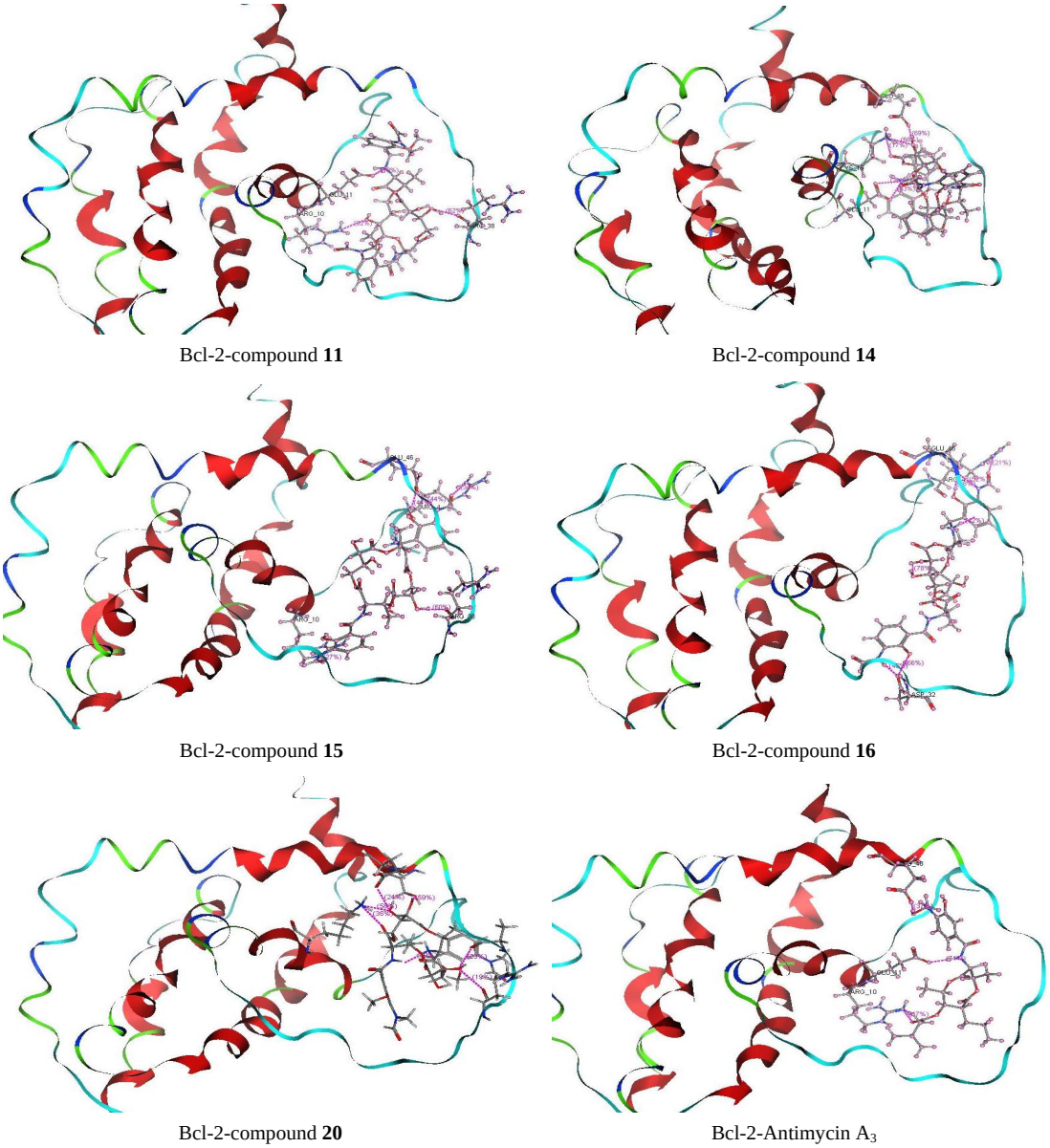
Compound	ΔG (Kcal/mol)	*pKi* (μM)	H acceptor/H donor interaction
1	−13.9579	9.916	4
2	−16.4607	9.595	6
3	−10.0695	7.403	2
4	−11.4296	7.916	4
5	−9.4166	6.930	2
6	−8.9445	8.539	5
7	−9.0453	7.165	2
8	−8.2019	5.280	2
9	−8.4125	4.539	2
10	−14.1850	10.482	5
11	−16.4486	13.351	6
12	−15.0360	9.113	4
13	−156846	11.566	4
14	−15.9491	11.823	8
15	−15.0703	11.718	8
16	−17.1838	11.985	8
17	−16.3661	11.114	5
18	−15.8967	11.149	5
19	−15.7977	10.378	3
20	−17.1553	10.699	7
Antimycin A_3	−11.4295	7.537	5
Respirantin	−11.0109	7.432	5

The blue color represents the top-ranked compound.

they have a higher affinity and interact effectively with the target Bcl-2. Moreover, all of those five top-ranked compounds have a number of hydrogen acceptor/hydrogen donor interactions more than antimycin A_3, which demonstrated greater inhibitory activities on receptor target Bcl-2.

The catalytic site of the Bcl-2 breast cancer cells are Arg10, Glu11, Met14, Trp28, Asp29, Ala30, Gly31, Asp32, Val34, Glu46, Asn37, Asp168, and Ala171. If a compound interacts with the catalytic site of the protein target, it will reduce the activity of the target protein, and change the protein conformation. Generally, the interaction of the compound with the complex protein target is the hydrogen bond. The quantities of hydrogen bond interactions of the compound with the catalytic site of the target protein indicate its ability to inhibit the protein target. **Figure 3** displays the ligand complex interaction of the five top-ranked compounds (**11**, **14**, **15**, **16**, and **20**) and antimycin A_3 with the receptor target Bcl-2. As shown, all the five of top-ranked compounds could change the conformation of the receptor target cavity, and were able to enter the binding site of the receptor target Bcl-2. In addition, compared to antimycin A_3, those five top-ranked compounds showed more hydrogen binding interaction against Bcl-2. Hydrogen bond interactions between amino acid residues of Bcl-2 breast cancer with **11**, **14**, **15**, **16**, **20** and antimycin A_3 are summarized in **Table 2**.

As shown in **Table 2**, all the analogue compounds **11**, **14**, **15**, **16** and **20** have a higher number of hydrogen bonds to the protein target Bcl-2 than that of the original antimycin A_3. Compared to respirantin, compound 11, 14 and 20 have a higher number of hydrogen bonds to the Bcl-2. Both compound **11** and **14** which form four



Bcl-2-compound **11** | Bcl-2-compound **14**

Bcl-2-compound **15** | Bcl-2-compound **16**

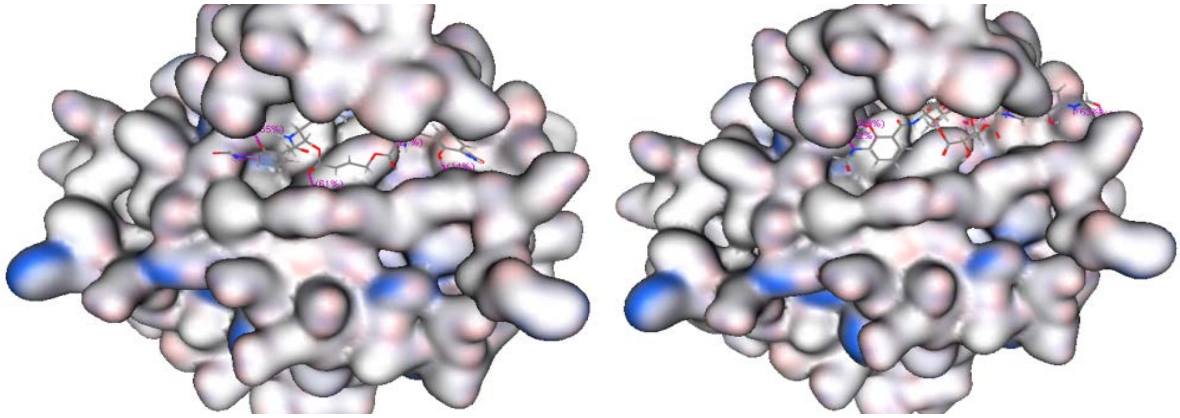
Bcl-2-compound **20** | Bcl-2-Antimycin A_3

Figure 3. Interaction of compound **11**, **14**, **15**, **16**, **20**, and antimycin A_3 to the catalytic site of Bcl-2.

Table 2. Hydrogen bond interactions of antimycin A_3, respirantin, compound **11**, **14**, **15**, **16**, and **20** with Bcl-2.

Compound	Interaction with the protein target Bcl-2
Antimycin A_3	Ser47, Glu48, Arg10, Arg10, Lys15
Respirantin	Arg10, Arg10, Ala41, Thr39
11	Thr5, Glu11, Asp32, Asp168, Arg10
14	Glu11, Asp32, Asp32, Glu43, Glu46, Arg4, Lys15, Lys15
15	Glu11, Arg38, Glu40, Glu48, Arg10, Asn37, Thr5
16	Glu11, Arg38, Ser47, Glu48, Lys15, Lys15, Ser47
20	Glu11, Asn37, Arg38, Arg10, Arg10, Lys15

The red color represents the catalytic site of Bcl-2.



(a) (b)

Figure 4. Conformation of compound **11** (a) and compound **14** (b) on catalyticsite of Bcl-2 breast cancer.

hydrogen bonds to the active site of Bcl-2, exhibited the strongest inhibitory activity. Compound **11** binds to the Bcl-2 catalytic site at the Glu11, Asp32, Asp168 and Arg10 residue whereas, **14** binds to the Bcl-2 catalytic site at the Glu11, Asp32 (two hydrogen bonds) and Glu46 residues. The three dimensional conformation of the two best analogues **11** and **14** on the catalytic site Bcl-2 are given in **Figure 4**. The docking results in **Figure 4** revealed **11** which bears an 18-membered tetralactone core and two parts of 3-formamidosalicylyl moiety as a ligand, has more binding interaction, a more stable conformation and a stronger inhibitory activity on the catalytic site of Bcl-2 than antimycin A_3. Similar to **11**, compound **14** bearing the18-membered core with four hydroxyl groups at the bottom facial stereochemistry as a ligand, also showed stable conformation and strongly inhibited the activity of the Bcl-2 catalytic site. Consistent with the previous *in-vitro* assay [11], the docking result of synthesized analogue **14** confirmed that introducing two parts of 3-formamidosalicylyl moiety and replacing the nine-membered dilactone core of antimycin A_3 with the 18-membered tetraol core in **14** could remarkably increase its anti-breast cancer activity. Moreover, replacing the nine-membered dilactone core of antimycin A_3 with the 18-membered tetralactone core in analogue **11**, could also greatly improve its inhibitory activity against the receptor target Bcl-2 of breast cancer. Thus, compound **11** and **14** are promising candidates for new anti-breast cancer agents, and should be considered as the lead compounds in the next synthesis project.

4. Conclusion

In conclusion, we have simulated twenty designed compounds by molecular docking approach. Among them, the analogues 11 and 14 which have an 18-membered tetralactonecore and 18-membered tetraol core, respectively, demonstrated stronger inhibitory activity and greater interaction with amino acid residues in the catalytic site of Bcl-2 breast cancer compared to the original antimycin A_3.

Acknowledgements

We wish to express our gratitute to Directorate of Research and Public Service University of Indonesia for the Research Grant, and to the Graduate School of Materials Science, Nara Institute of Science and Technology (NAIST), Japan, for International Research Collaboration Program (NAIST Global Initiative Program).

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