Molecular Docking of Antimycin A₃ Analogs and Its Aromatic Segments as Inhibitors of Apoptosis Protein Marker Bcl-xl and Mcl-1

Ade Arsianti*, Fadilah Fadilah, Linda Erlina, Rafika Indah Paramita

Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia, Jalan Salemba Raya 6 Jakarta 10430, Indonesia.
Email: arsi_ade2002@yahoo.com

Received: 27 February 2017, Revised and Accepted: 27 April 2017

INTRODUCTION

Apoptosis is a key for a cellular process that causes the death of damaged cells [1]. Malfunction of apoptosis can trigger cancer development and poor response to conventional chemotherapy [2]. Cellular proteins from the B-cell lymphoma 2 (BCL-2) family are important for apoptosis [3,4]. Understanding their interactions is vital for anticancer drug design [5]. Proteins from the BCL-2 family can be either pro-apoptotic or pro-survival proteins. Pro-apoptotic proteins such as BCL-2, BCL-xl, and MCL-1 share homology in three to four conserved BH peptide domains, namely BH1, BH2, BH3, and BH4 [6,7]. Pro-apoptotic proteins such as BAX, BAK, BIM, BAD, and BID share homology only in the BH3 domain [8].

The BH3 region is responsible for mediating the interactions with anti-apoptotic proteins and is related to the ability of a protein to promote programed cell death [9]. The structures of BCL-2 and BCL-xl are composed of eight α-helices with a hydrophobic groove on the protein surface containing all four (BH1, BH2, BH3, and BH4) conserved domains [10,11]. MCL-1 shows structural similarity with BCL-2 and BCL-xl except for the absence of BH4 domain at the N-terminal [11]. After receiving the appropriate signals, pro-apoptotic proteins bind to anti-apoptotic proteins via BH3 domains on their surfaces. When cytochrome C is released from the mitochondrial inner membrane space, the apoptosis process is initiated [12]. Overexpression of anti-apoptotic BCL-2 family proteins prevents the release of cytochrome C from mitochondria and is responsible for many types of human cancers such as breast and prostate cancer [13,14].

Breast cancer is the most commonly diagnosed cancer among women worldwide. Approximately 30% of the women diagnosed with the early-stage disease in turn progress to metastatic breast cancer, for which treatment with anti-breast cancer therapeutic agents is needed. Although many current anti-breast cancer therapies can induce apoptosis of those cells.

Antimycin A₃, a nine-membered dilactone which 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 [15]. Antimycin A₃ was also found to induce apoptosis of cancer cells by selectively killing the cancer cells that expressed high levels of anti-apoptotic Bcl-2 and Bcl-xl [18-20]. Bcl-2 is known to be over-expressed in 70% of breast cancer patients [21]. Therefore, it is reasonable to expect antimycin A₃ and its analogs to induce apoptosis of those cells.

Antimycin A₃ analogs have been utilized to find the potential anticancer from antimycin A₃ analogs and its aromatic segments. Antimycin A₃ analogs and its aromatic segments were modeled into three-dimensional (3D) structures using Marvin Sketch. Based on Protein Data Bank, 3ZLN for Bcl-xl, and 5IEZ for Mcl-1 were selected as apoptosis protein marker from BCL-2 family. Geometry optimization and minimization of energy 3D structure of antimycin A₃ analogs and segments using AutoDock software. Docking process and amino acid residue analysis were executed using AutoDock software. The best docking score was shown by the lowest binding energy and also checked with Lipinski rule of five.

Results: In silico molecular docking showed antimycin A₃ analogs, amide 5 and aromatic segment 14 have the best interaction and activity for Bcl-xl receptor inhibition. Moreover, amide 5 and segment 15 showed the best interaction and activity for Mcl-1 receptor inhibition.

Conclusion: Our results clearly demonstrate that amide 5, segment 14, and segment 15 of antimycin A₃ analog have a strong inhibitory activity against Bcl-xl and Mcl-1, and should be further developed as a promising candidate for the new anti-apoptosis agents.

Keywords: Molecular docking, Antimycin A₃ analog, Apoptosis, Bcl-xl, Mcl-1, Breast cancer.

© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/) DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i8.18165

ABSTRACT

Objective: Apoptosis is an important cellular process that causes the death of damaged cells. Its malfunction can lead to cancer development and poor response to conventional chemotherapy. Cellular proteins from the B-cell lymphoma 2 (BCL-2) family are crucial for apoptosis. Breast cancer is the most commonly diagnosed cancer among women worldwide. The aim of this work was to design using in silico docking antimycin A₃, antimycin analogs, and its aromatic segments as inhibitors of Bcl-xl and Mcl-1.

Methods: In silico molecular docking approach has been utilized to find the potential anticancer from antimycin A₃ analogs and its aromatic segments. Antimycin A₃ analogs and its aromatic segments were modeled into three-dimensional (3D) structures using Marvin Sketch. Based on Protein Data Bank, 3ZLN for Bcl-xl, and 5IEZ for Mcl-1 were selected as apoptosis protein marker from BCL-2 family. Geometry optimization and minimization of energy 3D structure of antimycin A₃ analogs and segments (ligands) using the AutoDock software. Docking process and amino acid residue analysis were executed using AutoDock software. The best docking score was shown by the lowest binding energy and also checked with Lipinski rule of five.

Results: In silico molecular docking showed antimycin A₃ analogs, amide 5 and aromatic segment 14 have the best interaction and activity for Bcl-xl receptor inhibition. Moreover, amide 5 and segment 15 showed the best interaction and activity for Mcl-1 receptor inhibition.

Conclusion: Our results clearly demonstrate that amide 5, segment 14, and segment 15 of antimycin A₃ analog have a strong inhibitory activity against Bcl-xl and Mcl-1, and should be further developed as a promising candidate for the new anti-apoptosis agents.

Keywords: Molecular docking, Antimycin A₃ analog, Apoptosis, Bcl-xl, Mcl-1, Breast cancer.

© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/) DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i8.18165
the molecular docking study of antimycin A_3 analog and its aromatic segments (Figs. 1 and 2) which have simple opened-chain structures but are expected to have higher cytotoxic activities than the original antimycin A_3 (Fig. 3).

Many researchers have accomplished the molecular docking study of active compounds with protein or drug target. Agarwal et al. in 2014 conducted in silico molecular docking analysis to access the antibacterial effect of thiazides on peptide deformylases [24]. In 2016, Dirar et al. reported molecular docking study of three phytochemicals isolated from Tarchonanthus camphoratus L. [25]. As our contribution to develop in silico molecular docking as a screening method, we reported in silico screening of antimycin A_3 analogs as inhibitors of anti-apoptotic Bcl-2 of breast cancer in 2014 [26], and as caspases inhibitors of apoptosis in colorectal cancer in 2016 [27]. Recently, in 2017, we carried out the screening of 15 gallic acid derivatives as inhibitors of malarial dihydrofolate reductase by in silico docking [28].

In this work, we designed the structure of antimycin A_3 analogs by opening the nine-membered dilactone ring system of antimycin A_3 and introducing the hydroxyl groups on the side chain of the ester group (Table 1), as well as replacing 3-formamidosalicylic moiety in antimycin A_3 with 3-formamido-2-methoxybenzoyl moiety in the analogs (Table 2).

Tzung et al. reported 2-methoxy-antimycin A_3 which was synthesized by methylation of the hydroxyl group on 3-formamidosalicylic moiety of antimycin A_3 is inactive as an inhibitor of cellular respiration but still retain its cytotoxicity against cancer cells that expressed high levels of anti-apoptotic Bcl-2 and Bcl-xl [29]. Thus, the introduction of 3-formamido-2-methoxybenzoyl moiety and a hydroxyl group on the side chain of the ester group in the analogs are expected to significantly improve its cytotoxic activities as well as its selectivity as an apoptotic trigger in breast cancer cells compared to that of the original antimycin A_3.

**Table 1: R1-R3 modification of antimycin A_3 analogues**

| Compound  | R1     | R2     | R3     |
|-----------|--------|--------|--------|
| Amide 3   | −CH_2-C_6H_5 | H      |        |
| Amide 4   | −CH_2-C_6H_5 | H      |        |
| Amide 5   | −CH_2-C_6H_5 | H      | CH=CH_2|
| Amide 6   | −CH_3      | H      | CH=CH_2|
| Amide 9   | −CH_3      | −CH_3 | CH=CH_2|
| Analogue 1| H        | H      |        |
| Analogue 2| H        | H      |        |
| Analogue 7| −CH_3     | H      |        |
| Analogue 8| −CH_3     | H      |        |
| Analogue 10| −CH_3 | −CH_3 |        |
| Analogue 11| −CH_3   | −CH_3 |        |

**Table 2: R4-R5 modification of aromatic segments**

| Compound  | R4     | R5     |
|-----------|--------|--------|
| Segment 13| H      | H      |
| Segment 14| H      | CH_3   |
| Segment 15| CH_3   | CH_3   |

![Fig. 1: Designed structure of antimycin A_3 analogs](image1)

![Fig. 2: Designed structure of aromatic segments](image2)

![Fig. 3: Structure of antimycin A_3](image3)
METHODS

Design and preparation of antimycin A₃ analogs and its aromatic segments compounds

Antimycin A₃ analogs and its aromatic segments compound modeled into three-dimensional (3D) structures. The modeling was performed using MarvinSketch and openbabel for converting the type of file from mol into.pdb.

Preparation 3D structure of proteins

The 3D structure of protein Bcl-xl and MCL-1 was downloaded from Protein Data Bank (PDB) (http://www.rcsb.org/pdb/home/home.do). From PDB, 3ZLN (PDB ID for Bcl-xl) was selected with the resolution 2.29 Å and 181 aa length. PDB ID 5IEZ for protein Mcl-1 was selected with the resolution 2.6 Å and 159 aa length.

Preparation 3D structure of proteins Bcl-xl and Mcl-1 (PDB ID: 3ZLN and 5IEZ) using the AutoDock 4.2.3 software that runs on a single computer Intel® Core™ i5-2450M with processor 2.50 Ghz. Polar hydrogens and Gasteiger charge were added.

In silico docking receptor on target antimycin A₃ analogs

The process of in silico docking begins with file preparation using a docking program in AutoDock 4.2.3 software. Antimycin A₃ analogs and its aromatic segments (then called ligand) and the receptor, polar hydrogen, and Gasteiger charge were added, and nonpolar hydrogen was merged. Ligand and macromolecule were saved in.pdbqt format file for later use in the preparation parameters. Grid box used for optimization and validation docking method was 40 × 40 × 40, 50 × 50 × 50, and 60 × 60 × 60 grid points with a grid spacing of 0.375 Å. Docking calculations executed by the algorithm parameters Lamarckian genetic algorithm with a population size of 150, 10,000,000 energy evaluation and repetition (engine runs) as much as 10 times. These parameters were saved in a format.dlg as a file to be used to carry out the process of docking. Docking process was performed using AutoDock 4.2.3 software.

Analysis of in silico docking

The results of in silico docking calculations can be seen in output.dlg format. Determination of ligand-receptor conformation results of docking is done by selecting ligand conformations which have lowest binding affinity energy of the group (cluster) with the largest number of population with a limit of a standard deviation of 1.5 Å. Energy binding and inhibition constants (IC₅₀) docking results can be seen in output.dlg format.dlg. The amino acid interaction was determined using Ligplus software. Visualization of docking pose in 3D was analyzed using PyMOL software.

RESULTS AND DISCUSSION

Molecular docking for in silico study

All of the designed compounds were acceptable as drug candidate based on Lipinski rule’s of five (molecular weight ≤500, hydrogen bond donor ≤5, hydrogen bond acceptor ≤10, and LogP <5).

Validation of Bcl-xl docking method

The optimum grid box size for Bcl-xl (PDB ID 3ZLN) is 40 × 40 × 40 points with the grid center is −19.38, −12.437, and 13.099 (Table 3). H0Y as cocrystal ligand was re-docked and the result of docking score is −14.81 Kcal/mol with RMSD 0.60.

H0Y as cocrystal ligand has interaction with 16 amino acids on binding site of receptor target (Bcl-xl) and has four hydrogen bonds with Arg139 (2.97 Å), Asn136 (2.86 Å), Ser106 (2.76 Å), and Leu108 (2.99 Å) (Fig. 4).

Docking analysis for Bcl-xl

Based on docking results for antimycin A₃ have docking score −7.74 Kcal/mol (Table 4). Antimycin A₃ has four hydrogen bonds with Ser106 (2.61 Å; 2.99 Å; 2.96 Å and 2.76 Å) and 13 amino acids interaction with binding site of Bcl-xl (Fig. 5). This hydrogen bond can improve the stability amino acid interaction between ligand and receptor.

Amide 5 and aromatic segment 14 have the best docking score with value of −9.20 and −6.04 Kcal/mol respectively (Table 4). Amide 5 has three hydrogen bonds with Ser106 (2.56 Å) and Arg102 (2.67 Å and 2.90 Å) (Fig. 6). Amide 6 and analog 2 have the same docking score with value of −8.45 Kcal/mol (Table 4), it showed that modification of R1 (-CH₂-C₅H₅) into another group (CH₃ and H) resulted in decreasing of activity. Amide 3, amide 4, and amide 5 have the same R1 (-CH₃-...
C₆H₅, but they are different in the R3 side chain. Compared to amide 3 and 4, amide 5 which has R3 (CH=CH₂) shows higher affinity. It can be concluded that the presence of R3 (CH=CH₂) in amide 5 is necessary to increase its affinity against Bcl-xl. Amide 9 and analog 10 have the same methyl (CH₃) group in R1 and R2, but different in R3 side chain. Amide 9 with (CH=CH₂) group as R3 side chain has docking score of −8.06 Kcal/mol, whereas analog 10 with the phenolic group as R3 side chain has docking score of −6.85 Kcal/mol. It revealed the presence of more hydrophobic R3 side chain will increase the activity (Table 4).

Aromatic segment 14 with (H) as R4 and (CH₃) as R5, has the best docking score compared to segment 13 and segment 15. Based on all segments docking score, we can analyze that the differences are not significant when R4 and R5 are H, CH₃ or combination of them. Segment 14 has two hydrogen bonds with Ser106 (3.03 Å) and Leu108 (2.89 Å) (Fig. 7).

Valiation of Mcl-1 docking method
The optimum grid box size for Mcl-1 (PDB ID 5IEZ) is 60 × 60 × 60 points with the grid center is 3.926, 17.33, and 65.765 (Table 5). 6AL as cocrystal ligand was re-docked and the result of docking score is −10.64 Kcal/mol with RMSD 1.01. 6AL as cocrystal ligand has interaction with 15 amino acids on binding site of receptor target (Mcl-1) (Fig. 8).

Docking analysis for Mcl-1
Based on docking results, antimycin A₃ with the docking score of −7.60 Kcal/mol, have 14 amino acids interaction with binding site of Mcl-1 (Fig. 9). Whereas, amide 5 with docking score of −7.05 (Table 4), has one hydrogen bond with Met250 (2.78 Å) and 14 amino acids interaction (Fig. 10). Segment 15 with docking score of −4.93 Kcal/mol has one hydrogen bond with Arg263 (3.24 Å) and 7 amino acids interaction with binding site of Mcl-1 (Fig. 11).

Amide 3 and amide 5 have the same groups on R1 (−CH₂-C₆H₅) and R2 (H), but different in R3 side chain. Amide 5 the replacement for R3 position will make an impact for activity (increase or decrease). Amide 5 with (CH=CH₂) group as R3 showed the best interaction, this group is more hydrophobic than alkyl-diol group in amide 3 and amide 4, it revealed that (CH=CH₂) group could be strengthen the interaction of amide 5 with target receptors Mcl-1.

Amide 9 and analouge 11 have the same docking score (−6.81 Kcal/mol) (Table 4), which they have the same R1 and R2 (CH₃), the replacement on R3 positions with (CH=CH₂) and phenolic groups are not significantly changing the activity.

| Compounds | Bcl-xl | Mcl-1 |
|-----------|--------|-------|
|           | Docking score (Kcal/mol) | IC (µM) | Docking score (Kcal/mol) | IC (µM) |
| Amide 3   | −8.37  | 0.733 | −6.66  | 13.04 |
| Amide 4   | −7.89  | 1.64  | −4.96  | 233.23 |
| Amide 5   | −9.20  | 0.181 | −7.05  | 6.81  |
| Amide 6   | −8.45  | 0.640 | −6.20  | 28.33 |
| Amide 9   | −8.06  | 1.24  | −6.81  | 10.27 |
| Analogue 1 | −7.94 | 1.51  | −4.18  | 862.18 |
| Analogue 2 | −8.45 | 0.638 | −4.15  | 906.84 |
| Analogue 7 | −7.04 | 6.87  | −4.88  | 264.99 |
| Analogue 8 | −6.18 | 29.29 | −5.31  | 128.92 |
| Analogue 10 | −6.85 | 9.57  | −5.33  | 123.83 |
| Analogue 11 | −6.38 | 21.12 | −6.81  | 10.2  |
| Segment 13 | −5.88 | 49.37 | −4.57  | 449.61 |
| Segment 14 | −6.04 | 37.63 | −4.78  | 311.71 |
| Segment 15 | −5.84 | 52.77 | −4.93  | 242.68 |
| Antimycin A3 | −7.74 | 2.14  | −7.60  | 2.67  |

Segment 15 has the best docking score compared to segment 13 and 14, with R4 (CH₃) and R5 (CH₃). Based on all segments docking score, we can analyze that methyl groups on R4 and R5 or one of them, can increase the affinity between ligand and receptor.
Table 5. Docking score

Based on docking results on Table 4, IC was analyzed. For Bcl-xl, amide 5 and segment 14 have a good IC value of 0.181 and 37.63 μM, respectively. For Mcl-1, amide 5 and segment 15 have a good IC value of 6.81 and 242.68 μM, respectively. Antimycin A has IC value of 2.14 μM on Bcl-xl and 2.67 μM on Mcl-1.

Furthermore, a combination of amide 5 and segment 14 and combination of amide 5 and segment 15 become a potential for Bcl-xl and Mcl-1 inhibitors.

CONCLUSION

Benzyl groups on R1 position and (CH=CH) on R3 position in amide 5 have an important role for its inhibitory activity on receptor Bcl-xl and Mcl-1. Methyl groups on R4 and R5 position in segment 14 and segment 15 have an important role in increasing ligand affinity against Bcl-xl and Mcl-1 receptor. Our results clearly demonstrate that amide 5, segment 14, and segment 15 of antimycin A analogs should be continued to in vitro assay as a promising candidate for the new anti-apoptosis agents.

ACKNOWLEDGMENTS

We wish to express our gratitude to UNESCO for The World Academy of Sciences research grant.

REFERENCES

1. Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 1988;335(6189):440-2.
2. Campos L, Rouault JP, Sabido O, Oriol P, Roubi N, Vasselon C, et al. High expression of bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. Blood 1993;81(11):3091-6.
3. Waksman G, editor. Proteomics and Protein-Protein Interactions: Biology, Chemistry, Bioinformatics, and Drug Design. New York, US: Springer; 2005.
4. Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 2005;435(7042):677-81.
5. Kann MG. Protein interactions and disease: Computational approaches to uncover the etiology of diseases. Brief Bioinform 2007;8(5):333-46.
6. Huang DC, Strasser A. BH3-Only proteins: essential initiators of apoptotic cell death. Cell 2000;103(6):839-42.
7. Kelekar A, Thompson CB. Bcl-2 family proteins: The role of the BH3 domain in apoptosis. Trends Cell Biol 1998;8(8):324-30.
8. Mitchell C, Yacoub A, Hossein H, Martin AP, Bareford D, Eulitt PJ, et al. Inhibition of MCL-1 in breast cancer cells promotes cell death in vitro and in vivo and in vitro inhibition of MCL-1 in breast cancer cells promotes cell death in vitro and in vivo. Cancer Inform 2010;9:169-77.
9. Chittenden T, Flemington C, Houghton AB, Ebb RG, Gallo GJ, Elangovan B, et al. A conserved domain in Bak, distinct from BH1 and BH2, mediates cell death and protein binding functions. EMBO J 1995;14(22):5589-96.
10. Zhai D, Ke N, Zhang H, Ladror U, Joseph M, Eichinger A, et al. Characterization of the anti-apoptotic mechanism of Bcl-B. Biochem J 2003;376:229-36.
11. Michels J, Johnson PW, Packham G. Mcl-1. Int J Biochem Cell Biol 2005;37(2):267-71.
12. Petros AM, Olejniczak ET, Fesik SW. Structural biology of the Bcl-2 family of proteins. Biochim Biophys Acta 2004;1644(2-3):83-94.
13. Fernández Y, Gu B, Martínez A, Torregrosa A, Sierra A. Inhibition of apoptosis in human breast cancer cells: Role in tumor progression to the metastatic state. Int J Cancer 2002;101(4):317-26.
14. Lebedeva I, Rando R, Ojwang J, Cossum P, Stein CA. Bcl-xl in prostate cancer cells: Effects of overexpression and down-regulation on chemosensitivity. Cancer Res 2000;60(21):6052-60.
15. O’Shaughnessy J. Extending survival with chemotherapy in metastatic breast cancer. Oncologist 2005;10 Suppl 3:20-9.
16. Lage H. Drug resistance in breast cancer. Cancer Ther 2003;1:81-91.
17. Rivera E, Gomez H. Chemotherapy resistance in metastatic breast cancer: The evolving role of taxibiphenyl. Breast Cancer Res 2010;12 Suppl 2:S2.
18. Kim KM, Giedt CD, Basañeza G, O’Neill JW, Hill JJ, Han YH, et al. Biophysical characterization of recombinant human Bcl-2 and its interactions with an inhibitory ligand, antimycin A. Biochemistry 2001;40(16):4911-22.
19. Han YH. Antimycin A as a mitochondrial electron transport inhibitor prevents the growth of human lung cancer A549 cells. Oncol Rep 1994;20:104-8.
20. Park WH, Han YW, Kim SH, Kim SZ. An ROS generator, antimycin A, inhibits the growth of HeLa cells via apoptosis. J Cell Biochem
21. Liu W, Bulgaru A, Haigentz M, Stein CA, Perez-Soler R, Mani S. The BCL2-family of protein ligands as cancer drugs: The next generation of therapeutics. Curr Med Chem Anticancer Agents 2003;3(3):217-23.

22. Arsianti A, Tanimoto H, Morimoto T, Bahtiar A, Takeya T, Kakiuchi K. Synthesis and anticancer activity of polyhydroxylated 18-membered analogue of antimycin A3. Tetrahedron 2012;68(13):2884-91.

23. Arsianti A, Fadilah F, Kusmardi K, Tanimoto H, Kakiuchi K. Design, synthesis, and cytotoxicity evaluation of novel open-chain analogues of antimycin A3 as potential anti-colorectal cancer agents. Asian J Pharm Clin Res 2015;8(6):120-4.

24. Agarwal T, Gupta P, Asthana S, Khursheed A. In silico analysis to access the antibacterial effect of thiazides on PDFS: Molecular docking approach. Int J Pharm Pharm Sci 2014;6(6):387-91.

25. Dirar AI, Waddad AY, Mohamed MA, Mohamed MS, Osman WJ, Mohammed MS, et al. In silico pharmakokinetics and molecular docking of three leads isolated from Tarconanthus camphoratus. Int J Pharm Pharm Sci 2016;8(5):71-7.

26. Arsianti A, Fadilah F, Tanimoto H, Morimoto T, Kakiuchi K. Design and molecular docking study of antimycin A3 analogues as inhibitors of anti-apoptotic Bcl-2 of breast cancer. Open J Med Chem 2014;4(4):79-86.

27. Fadilah F, Arsianti A, Kusmardi K, Tedjo A. Molecular docking studies of opened-chain analogues of antimycin A3 as caspases inhibitors of apoptosis in colorectal cancer. Asian J Pharm Clin Res 2016;9(3):350-2.

28. Arsianti A, Astuty H, Fadilah F, Bahtiar A, Tanimoto H, Kakiuchi K. Design and screening of gallic acid derivatives as inhibitors of malarial dihydrofolate reductase by in silico docking. Asian J Pharm Clin Res 2017;10(2):330-4.

29. Tzung SP, Kim KM, Basañez G, Giedt CD, Simon J, Zimmerberg J, et al. Antimycin A mimics a cell-death-inducing Bcl-2 homology domain 3. Nat Cell Biol 2001;3(2):183-91.