

Document Viewer

Turnitin Originality Report

Processed on: 01-Mar-2019 15:44 WIB

ID: 1085910501

Word Count: 3980

Submitted: 1

Evaluation of New Drug By
Jurnal Farm

Similarity Index	Similarity by Source
9%	Internet Sources: 9%
	Publications: 0%
	Student Papers: 0%

[include quoted](#)
[include bibliography](#)
[excluding matches < 8%](#)
[download](#)

[print](#)
mode:

9% match (Internet from 10-Jan-2019)

<https://seekdl.org/conferences/paper/details/7468.html>

See discussions, stats, and author profiles for this publication at:

<https://www.researchgate.net/publication/303566463> [Evaluation Of New Drug Candidature of Usnic Acid Derivatives As Selective Bcl-2 Inhibitors: Computational Designing And Molecular Docking](#) Conference Paper · March 2016 DOI: 10.15224/978-1-63248-091-0-64 CITATIONS 0 3 authors: Restuan Lubis Universitas Muslim Indonesia 1 PUBLICATION 0 CITATIONS SEE PROFILE READS 110 Ahmad Najib Universitas Muslim Indonesia 39 PUBLICATIONS 29 CITATIONS SEE PROFILE Zainal Abidin Universitas Muslim Indonesia 4 PUBLICATIONS 5 CITATIONS SEE PROFILE Some of the authors of this publication are also working on these related projects: Determination of Phenolic and Flavonoid Contents of Ethanolic Extract of Kanunang Leaves (Cordia myxa L.) View project Development of Herbal Material for Cosmetic View project All content following this page was uploaded by Ahmad Najib on 27 May 2016. The user has requested enhancement of the downloaded file.

[Evaluation Of New Drug Candidature of Usnic Acid Derivatives As Selective Bcl-2 Inhibitors: Computational Designing And Molecular Docking Restuan Lubis Restuan Lubis, Ahmad Najiba, and Zainal Abidinb Abstract— Breast cancer is malignant tumor that begun in the cells of breast. Abnormal or failed apoptosis consequences a malignant tumor can grow up then attack tissues around or metathesis to other tissue in the body. Apoptosis controlled by the Bcl-2 as anti-apoptotic. This situation happens on breast cancer MCF-7 cells. Therefore, at this study needed, molecular modification as a strategy in the design and development of drug analogs with better bioavailability, higher intrinsic activity and less toxicity. To be used the molecular docking method with Molegro Virtual Docker software and ADME-T properties of these compounds are calculated using ACD/ILab, Molinspiration, and preADMET calculator. The preparation of ligand and protein, and then need validation of](#)

research method that uses DRO ligand with the result RMSD (Root Mean Square Deviation) 0.180, and Moldock score -98,696 Kcal/Mol, prepare of ligand test ligand consideration, furthermore docking to the ligands. This process refers about interaction between ligand and protein which supervised then can be used as a new basic composition compound. New compound docked and inspected to know the reaction between ligand and protein. The Moldock score of the best five new compounds are -115.152, -106.454, -106.050, -105.185, and - 104.964 Kcal/Mol. That percentage show us about these candidature compounds have better high binding energy percentage than the native DRO ligand. Interestingly, there are have good bioavailability and toxicity better than DRO was have bad and failed cathegory by ADMET approach. Keywords— Molecular docking, usnic acid, bcl-2, breast cancer.

I. Introduction Cancer is one of the worldwide major causes of death. Based on the data compiled from Indonesian Ministry of Health in 2015 said that the breast cancer is the biggest cause of cancer deaths in each year with prevalence about 61.682. Breast cancer is a malignant tumor that starts from a group of cancer cells which can grow and invade surrounding tissues or metastasize to other tissues in the body as a result of abnormal apoptosis. Apoptosis or death programmed cell is the control mechanism of the elimination the dead cells in multicellular organisms [1]. The increase of cancer rates is not accompanied by prevention and treatment. It is shown from the growing of the amount of breast cancer patients. Hence, effort is needed to get through with this issue, which is to discover the new drugs. Previously new drug discovery was generally done by trial and error. It costed much and it took very long time. This prompted scientist in the development of new drug discovery in recent decades by utilizing advanced technology development in the field of medicinal chemistry Restuan Lubis Restuan Lubis, Ahmad Najiba, and Zainal Abidinb Pharmacy Faculty, Muslim University of Indonesia Indonesia with computational chemistry methods to learn about drug interactions with the receptor molecule or compound called molecular docking [2][3]. The death cell in the process of apoptosis has been given signal and mediated by several genes that encode proteins for digestion enzyme called caspase. Apoptotic events appearing after the disruption function of mitochondrial membrane barrier with the relinquish of cytochrome c through caspase-3 pathway in normal cells. Activation of caspase-3 is influenced by the Bcl-2 family protein. Protein is made up of anti-apoptotic such Bcl-2 and Bcl-X. Overexpression of Bcl-2 anti-apoptotic forestall the activation of caspase-3 at this stage to the release of cytochrome c, so that apoptosis does not occur. This is what happens in breast cancer cells MCF-7 [4]. Usnic acid (UA), a dibenzofuran originally hermit from lichens [5], In humans, it can act as antimittotic [6], antineoplastic [7,8], antibacterial [9], and antimycotic [10] agent. However, the potential benefits of UA therapeutic application are limited by its unfavorable physicochemical properties, particularly its very poor water solubility [11, 12]. In the study, as an addition to the molecular docking study for derivatives usnic acid and also this was followed by ADMET prediction and drug likeliness as well as drug score analysis of the docked compounds to evaluate the usage of some usnic acid as selective inhibitors of Bcl-2 Protein in MCF-7. II. Materials and Methods In this research, we simulated some derivatives usnic acid compounds based on their interactions with Bcl-2 breast cancer, using computer software applications (Molecular method [13] to determine the best compounds [14]. A. Hardware and software Molegro Virtual Docker 5.0, Chem Sketch 12.00, Marvin Beans Suite Version 15.9.7, preADMET, Molinspiration, ACD / I-Prediction Lab and YASARA 15.9, Intel ® Core i7-2670 QM TM® CPU @ 2.20GHz, 8GB RAM. B. Software Methodology In the present molecular docking study, software Molegro Virtual Docker (MVD) v 5.0, MVD tools was utilized to generate grid, calculate dock score and evaluate conformers.

Molecular docking was performed using MolDock docking engine of software. The scoring function used by MolDock is derived from the Piecewise Linear Potential (PLP) scoring functions. The active binding site region was defined as a spherical region which encompasses all protein within 15.0 Å of bound crystallographic ligand atom with selected coordinates of X, Y and Z axes, respectively. Default settings were used for all the calculations. Docking was performed using a grid resolution of 0.30 Å and for each of the 10 independent runs; a maximum number of 1500 iterations were executed on a single population of 50 individuals. The active binding site was considered as a rigid molecule, whereas the ligands were treated as being flexible, i.e. all non-ring torsions were allowed [15].

C. Methods

Preparation of Target Protein: The selection of protein for docking studies is based upon several factors i.e. structure should be determined by X-ray diffraction, and resolution should be between 2.0-2.5 Å, it should contain a co-crystallized ligand. The crystal structure of Bcl-2 Protein in complex with DRO (PDB code: 2W3L) was selected as the protein target model in this virtual screening study [16]. Water molecules were removed from the protein molecule using Molegro Virtual Docker version 5.0.

Preparation of Ligand: The ligand molecule structures of usnic acid (2,6-diacetyl-7,9-dihydroxy-8,9-dimethyldibenzofuran-1,3-dione) (Fig.1), and its derivatives were made design form Marvin Beans Suite 15.9.7 (Tab. 1) and The three dimensional models of all the compounds were generated using ChemSketch 12.00 Software. Each ligand structure was optimized by ChemSketch 12.00.

Validation Methods: Before screening the ligands, the docking protocol was validated by redocking DRO ligand into its binding pocket within the Bcl-2 crystal structure to obtain the docked pose and RMSD (Root Mean Square Deviation) by YASARA Program. The result showed that the optimized native DRO almost exactly superimposed with the experimental crystal structure of DRO (Fig. 2). Thus, the protocol is good in reproducing the X-ray crystal structure and can be applied for further docking experiments.

Protein-Ligand Docking: The docking of the target protein with the ligand was performed using the Molegro Virtual Docker. Docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site. The binding site was defined as a spherical region which encompasses all protein atoms within 15.0 Å of bound crystallographic ligand atom (dimensions X (38.8691 Å), Y (26.8816 Å), Z (-12.5203 Å) axes, respectively). Default settings were used for all the calculations. The best conformation was chosen with the lowest docked energy, after the docking search was completed. The interactions of complex protein-ligand conformations, including hydrogen bonds and the bond lengths were analyzed (Fig. 3).

Figure 1. Structure of Usnic Acid

TABLE I. THE SUBSTITUENTS OF USNIC ACID

No	R	1	CH ₃	1	H	2	C ₃ H ₇	2	Cl	3	Siklo-C ₅ H ₉	3	Cl ₂	4	Siklo-C ₆ H ₁₁	4	CF ₃	5	CH ₂ -C ₆ H ₅	5	NO ₂	6	(CH ₂) ₂ - C ₆ H ₅	6	Br	7	Siklo-C ₄ H ₉	7	I	8	CH ₂ -Siklo-C ₃ H ₅	8	NO ₂	9	C ₄ H ₉	9	CH ₃	10	C ₂ H ₅	10	N(CH ₃)	11	CHCl ₂	11	OCH ₃	12	CF ₃	12	F	13	CH ₂ -CF ₃	13	OH	14	CH ₂ -SCH ₃	14	OCH(CH ₃)	15	C ₆ H ₅	16	H	17	CH ₂ -OCH ₃	18	CH ₂ -SO ₂ CH ₃	19	C ₅ H ₉
----	---	---	-----------------	---	---	---	-------------------------------	---	----	---	-------------------------------------	---	-----------------	---	--------------------------------------	---	-----------------	---	--	---	-----------------	---	---	---	----	---	-------------------------------------	---	---	---	--	---	-----------------	---	-------------------------------	---	-----------------	----	-------------------------------	----	---------------------	----	-------------------	----	------------------	----	-----------------	----	---	----	----------------------------------	----	----	----	-----------------------------------	----	-----------------------	----	-------------------------------	----	---	----	-----------------------------------	----	--	----	-------------------------------

Figure 2. The superimposition of the docking structure of DRO experimental (red) with the X-ray structure (blue) within the active binding site region of 2W3L

III. Result and Discussion

Preparation of proteins and ligands used to prepare before the docking process. The purpose of this process is to prepare the ligand and protein docking process. Repair and remove water of the target protein used in the process is a protein docking results of X-Ray. The result of this preparation is a protein without a ligand and a ligand. Preparation of protein and ligand is done directly using software Molegro Virtual Docker. Optimization performed to correct existing deficiencies of protein data downloaded, among others improvement cargo and residues as well as the addition of hydrogen. Optimization performed using Molegro program with facilities protein

preparation serves to repair residues proteins imperfect such as Arg12, Lys17, Gln25, Lys54, Arg26, Arg65, Ser76. Each docking process begins with a genuine validation of the target protein ligand. This validation is performed to determine the proximity or similarity of the results between the ligand crystallography results with a ligand that has been optimized thus docking method. Coordinates a ligand interactions and protein for docking process is X = 38.8691, Y = 26.8816, Z = -12.5203. The results of this validation should be worth 0.180 with binding energy -98.696 kcal/mol. Data shows less than 2.0 indicates the method can be used. There are one hundred two compounds outcome from thirty-three substituents combination of modifications on usnic acid compounds based on the Topliss Decision Tree [16], were simulated using molecular docking on target protein of Bcl-2 breast cancer. In this study served with the five ligands smallest based on binding energy. The results are displayed in Table 2. TABLE II. THE SUBSTITUENTS OF USNIC ACID Compounds R R1 R2 Binding Energy Kcal/Mol AU3 Cyclo-C5H9 - - -115.152 AU57 C5H9 C5H9 - -106.454 AU53 C6H5 C6H5 - -106.050 AU25 - (CH2)2-C6H5 - -105.185 AU19 C5H9 - - -104.964 DRO -98,696 As shown in Table 2, compared to DRO (1-(2-((3S)-3-(aminomethyl)-3,4-dihydroisoquinolin-2(1H)-yl)carbonyl} phenyl)-4-chloro-5-methyl-N,N-diphenyl-1H-pyrazole-3 carboxamide) that act as selective inhibitors Bcl-2 protein, exhibited higher binding energy, affinity, and hydrogen bond, steric interaction on Bcl-2 breast cancer cells, indicating that five compounds has a stronger inhibitory activity against Bcl-2 protein targets. The docking of compounds outcome produced the five best ranked compounds, namely, compounds AU3, AU57, AU53, AU25, and AU19, which showed lower moldock score as binding energy value and a higher number of hydrogen bonding interaction than the others compounds. The binding energy values of compounds AU3, AU57, AU53, AU25, and AU19 are -115.152, -106.454, -106.050, -105.185, and -104.964 kcal/mol, respectively, which are better than DRO with binding energy value of -98,696 kcal/mol. These results showed that, compared to DRO as selective Bcl-2 protein, those five top-ranked compounds will form more stable complex and selective with Bcl-2, as well as, be better able to inhibit and reduce the activity of Bcl-2. The highest binding energy has shown AU3 compounds. The interaction between the best five compounds and amino acid of the Bcl-2 breast cancer cells are Lys22, Ala59, Ser64, Arg66, Tyr67, Arg68, and Tyr161. If a compound interacts with the amino acid 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 and steric interactions. The quantities of hydrogen bond interactions of the compound with the amino acid of the target protein indicate Its ability to inhibit the protein target. Figure 3 displays the ligand complex interaction of the best five compounds AU3, AU57, AU53, AU25, and AU19 and DRO with the receptor target Bcl-2 consisting of hydrogen bond and steric interaction. As shown, all the best five compounds could change the conformation of the receptor target cavity, and were able to enter the binding site of the Bcl-2 protein target. In addition, compared to DRO, those best five compounds showed more hydrogen binding interaction within Bcl-2. Hydrogen bond interactions between amino acid residues of Bcl-2 breast cancer with AU3, AU57, AU53, AU25, and AU19 and DRO are summarized in Table 3. As shown in Table 3, the best compounds AU3, AU57, AU53, AU25 have a higher number of hydrogen bonds and steric interactions to the protein target Bcl-2 than that DRO. TABLE III. THE HIDROGEN BOND AND STERIC INTERACTION OF COMPOUNDS WITH BCL-2 PROTEIN Compou nds Hidrogen Bond Steric Interaction AU3 Lys22, Ala59, Ser64, Arg66, Tyr67, Arg68 Lys 22, Ala59, Tyr67, Tyr161 AU57 Ser64, Arg68, Tyr67, Arg66, Gln25 Arg66 AU53 Arg66, Ser75, Phe71, Arg65 Ser64, Arg68 AU25 Asp62, Tyr161, Pro63, Gly162,

Ala59, Glu58, Leu160 AU19 Ser64, Arg66, Arg68, Arg65, Arg68, Tyr67 DRO Glu199 - The red color represents the amino acid of Bcl-2 In this study also has successfully qualified Lipinski's Rules, CMC like rule, MDDR like rule and WDI like rule but we only show 5 best compounds were have highest activity based on docking result in Table 4. Ligands outcome tested in this study were predicted to have good oral bioavailability in Table 5. The best five of the compounds have shown excellent permeability, while the DRO have poor permeability indicated by violated on Rule of Five and failed on CMC like rule or WDI like rule (Table 4). The physical properties like ionization potential, electronic energy and dipole plays an important role inactivity of compounds. Molecular descriptor properties The selected compounds used in this study were evaluated as selective inhibitor Bcl-2 protein target by comparis. The oral bioavailability of the compounds projected as potential drugs were evaluated by determining the molecular weight, number of rotatable bonds , number of hydrogen bonds (nON and nOHNH), and drug's polar surface (TPSA). Since the individual molecular weights of all the compounds were less than 500, the number of the rotatable bond were <10, the number of hydrogen bond donors and acceptors were < 12, and TPSA values being <140, they qualified to be an ideal oral drug. Ligands tested in this study were also predicted to have good oral bioavailability. ADME prediction In the sophisticated drug designing process, computational draw on like preADMET prediction; MDCK and Caco-2 cell permeability, etc. serve as computational screening model for the prediction of intestinal drug absorption. All the compounds under study have qualified HIA%, in vitro plasma% (>90% in all the cases) and Caco-2 cell permeability (>25 nm/Sec) to be a commonsense drug candidate. Some of the compounds have shown excellent permeability, while others have relatively less or poor permeability in relation to qualify as CNS drug and MDCK permeability as shown in Table 4. Less permeability is predicted because of the lesser solubility; and solubility, to a certain extent, depends on the arrangement of molecules in the crystal. It is to be noted that the topological aspects cannot be predicted via atom types or substructure fragments. TABLE IV. THE DATA REPRESENTING THE QUALIFICATION OF THE SUBSTITUENTS FOR DRUG LIKELINESS USING CMC LIKE RULE, MDDR LIKE RULE AND WDI LIKE RULE ALONG WITH RULE OF FIVE AS PREDICTED USING PREADMET SERVER

Compounds	CMC like rule	MDDR like rule	Rule of five	WDI like rule
AU3	Qualified	Mid-structure	Suitable 90%	AU57
AU57	Qualified	Mid-structure	Suitable 90%	AU53
AU53	Qualified	Mid-structure	Suitable 90%	AU25
AU25	Qualified	Mid-structure	Suitable 90%	AU19
AU19	Qualified	Mid-structure	Suitable 90%	DRO
DRO	Failed	Mid-structure	Violated	Failed

TABLE V. PREADME PREDICTION OF LIGANDS (a) In Comp vitro Caco-2 MDCK In vitro ounds blood nm/sec HIA% nm/se plasma barrier % AU3 0.2070 19.5653 94.6202 0.0480 94.5751 AU57 0.1193 19.3162 93.5592 0.1536 95.9790 AU53 0.0121 18.1399 85.1093 4.7135 72.2935 AU25 0.0121 18.1399 85.1093 4.7135 72.2935 AU19 0.0997 18.8938 89.8190 19.4418 87.3994 (b) DRO 0.1514 34.1907 95.9112 0.0464 100.0000

TABLE VI. TOXICITY PREDICTION AS OUTPUT OF PREADMET

Compounds	Ames_test	Carcino_Rat	in vitro hERG
AU3	non-mutagen	negative	low_risk
AU57	non-mutagen	negative	low_risk
AU53	non-mutagen	negative	low_risk
AU25	non-mutagen	negative	low_risk
AU19	non-mutagen	negative	ambiguous
DRO	non-mutagen	negative	high_risk

All the five parameters of Lipinski's rule of five are qualified by This pharmacophore and thus could be considered as a lead molecule to generate conformations or virtual screening library along with more modifications which could enhance its therapeutic index by upgrading the kind of interactions it could possibly make with the target protein. Docking process that has been carried out, generating the value energy ligand binding to plasma proteins and shows the interaction between the ligand and the target protein. These interactions involve various amino acids

and kind of bond, bonding both hydrophobic and hydrogen bonding. The number of amino acids that interact with ligand, will affect the value of the binding energy of protein ligands. The fate of a promising drug depends on its toxicity. The therapeutic index of a drug would be higher when it shows low toxicity or adverse effects. Based on this we have performed toxicity predication using pre ADMET calculator. Results amest test is the best five compounds (AU3, AU57, AU53, AU25, and AU19) revealed that the compounds are negative, it is indicated no metabolic activation and clear evidence of carcinogenic activity. Moreover, hERG inhibition in vitro shows low risk. Meanwhile, The DRO ligand on hERG inhibition of in vitro shown high risk as represent evidence to cardio toxic (d) (e) (f) Figure 3. Binding Interctions mode of (a)AU3, (b)AU57, (c)AU53, (d)AU25, (e)AU19, (f)DRO compounds with Bcl-2, blue dots (-----) indicates H bonding and red dots (.....) indicates steric interactions.

IV. Conclusion In conclusion, we have simulated one hundred and two designed compounds from thirty three substituents by molecular docking and ADME-Toxicity approach. Among them, there are the best five compunds derivatives such as AU3, AU57, AU53, AU25, and AU19, in particular, to show stronger inhibitory activity and greater interaction with amino acid residues in the binding site of Bcl-2 breast cancer compared to the original DRO as selective inhibit Bcl-2 protein breast cancer. Interestingly, there are have good oral bioavailability better than DRO was have bad and failed cathegory by ADMET approach. Moreover, there compounds have low toxicity value. The compounds were predicted to be safe (nonmutagenic as well as non-carcinogenic). Acknowledgment Authors are thankful to Prof. Dr. Siswandono, Apt, MS who has given a license Molegro Virtual Docker 5.0 Program, and the Laboratory of Phytochemistry and Pharmacognosy-Chemistry, Faculty of Pharmacy, and was funded and provided by Muslim University of Indonesia. [1] [2] [3] [4] [5] [6] [7] [8] [9] [13] Vidal, D., Garcia-Serna, R. and Mestres, J. (2011) Chemoinformatics and Computational Chemical Biology. Chemo-informatics and Computational Chemical Biology, 672, 489- 502. [14] Wang, Y., Xiao, J., Suzek, T.O., Zhang, J. and Wang, J., et al. (2009) A Public Information System for Analyzing Bio-activities of Small Molecules. Nucleic Acids Research, 37, 1-11. [15] Gehlhaar, D. K.; Verkhivker, G.; Rejto, P. A.; Fogel, D. B.; Fogel, L. J.; Freer, S. T. (1995) Docking Conformationally Flexible Small Molecules Into a Protein Binding Site Through Evolutionary Programming. Proceedings of the Fourth International Conference on Evolutionary Programming, No 123-124. [16] Porter J, Payne A, de Candole B, Ford D, Hutchinson B, Trevitt G et al. Tetrahydroisoquinoline amide substituted phenyl pyrazoles as selective Bcl-2 inhibitors. Bioorg Med Chem Lett 2009;19:230-233

About Author (s): [never give up if once surrendered then it will be a habit in your life] References Data Ministry of Health Republic of Indonesia, 2015 Wolff, M. E., 1996, Burgers Medicinal Chemistry and Drug Discovery, 5th Edition Volume 1, New York, Wiley-Interscience. Guitierrez-de-Teran H, and Aqvist J, 2012, Linear Interaction Energy: Method and Applications in Drug Design, 2012, in Baron R (Ed), Computational Drug Discovery and Design, Methods in Molecular Biology, Springer: Springer Science Business Media, 819:305-307.. Clerk Maxwell, A Treatise on Electricity and Magnetism, 3rd ed., vol. 2. Oxford: Clarendon, 1892, pp.68-73. Mooney, L.M., Al-Sakkaf, K.A, Brown, B.L., & Dobson, P.R.M., 2002, Apoptotic Mechanisms in T47D and MCF-7 Human Breast Cancer Cells, British Journal of Cancer, 87, 909 - 917.

J.B.Stark,E.D.Walter,andH.S.Owens,"Methodof isolation of usnic acid from Ramalina reticulata," Journal of the American Chemical Society, vol. 72, no. 4, pp. 1819-1820, 1950. M. Cardarelli, G. Serino, L. Campanella et al., "Antimitotic effects of usnic acid on different biological systems," Cellular and Molecular Life Sciences, vol. 53, no. 8, pp. 667-672, 1997. M. Takai, Y. Uehara, and J. A. Beisler, "Usnic acid derivatives as potential antineoplastic

agents," *Journal of Medicinal Chemistry*, vol. 22, no. 11, pp. 1380–1384, 1979. N. P. da Silva Santos, S. C. Nascimento, M. S. O. Wanderley et al., "Nanoencapsulation of usnic acid: an attempt to improve antitumour activity and reduce hepatotoxicity," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 64, no. 2, pp. 154–160, 2006. K. Ingólfssdóttir, G. A. C. Chung, V. G. Skulason, S. R. Gissurarson, and M. Vilhelmsdóttir, "Antimycobacterial activity of lichen metabolites in vitro," *European Journal of Pharmaceutical Sciences*, vol. 6, no. 2, pp. 141–144, 1998. [10] Y. Yamamoto, Y. Miura, Y. Kinoshita et al., "Screening of tissue cultures and thalli of lichens and some of their active constituents for inhibition of tumor promoter-induced Epstein-Barr virus activation," *Chemical and Pharmaceutical Bulletin*, vol. 43, no. 8, pp. 1388–1390, 1995 [11] M. C. B. Lira, M. S. Ferraz, D. G. V. C. da Silva et al., "Inclusion complex of usnic acid with β -cyclodextrin: characterization and nanoencapsulation into liposomes," *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, vol. 64, no. 3–4, pp. 215–224, 2009. [12] C. M. Batista, C. M. B. De Carvalho, and N. S. S. Magalhães, "Lipossomas e suas aplicações terapêuticas: Estado da arte," *Revista Brasileira de Ciências Farmacêuticas*, vol. 43, no. 2, pp. 167–179, 2007

View publication stats

Proc. of the Fourth Intl. Conf. [Advances in Bio-Informatics, Bio-Technology and Environmental Engineering- ABBE 2016](#) Copyright © Institute of Research Engineers and Doctors, USA .All rights reserved. ISBN: 978-1-63248-091-0 doi: 10.15224/ 978-1-63248-091-0-64 Proc. of the Fourth Intl. Conf. [Advances in Bio-Informatics, Bio-Technology and Environmental Engineering- ABBE 2016](#) Copyright © Institute of Research Engineers and Doctors, USA .All rights reserved. ISBN: 978-1-63248-091-0 doi: 10.15224/ 978-1-63248-091-0-64 Proc. of the Fourth Intl. Conf. [Advances in Bio-Informatics, Bio-Technology and Environmental Engineering- ABBE 2016](#) Copyright © Institute of Research Engineers and Doctors, USA .All rights reserved. ISBN: 978-1-63248-091-0 doi: 10.15224/ 978-1-63248-091-0-64 Proc. of the Fourth Intl. Conf. [Advances in Bio-Informatics, Bio-Technology and Environmental Engineering- ABBE 2016](#) Copyright © Institute of Research Engineers and Doctors, USA .All rights reserved. ISBN: 978-1-63248-091-0 doi: 10.15224/ 978-1-63248-091-0-64 Proc. of the Fourth Intl. Conf. [Advances in Bio-Informatics, Bio-Technology and Environmental Engineering- ABBE 2016](#) Copyright © Institute of Research Engineers and Doctors, USA .All rights reserved. ISBN: 978-1-63248-091-0 doi: 10.15224/ 978-1-63248-091-0-64 68 69 70 71 72