JTL 10 (2) December 2021
Page: 93-100

Contents list available at Jurnal Teknologi Laboratorium

JURNAL TEKNOLOGI LABORATORIUM

Journal Homepage: www.teknolabjournal.com
ISSN 2580-0191(Online) I ISSN 2338 – 5634(Print)

Original Research

OPEN ACCESS

Check for updates

In silico study in toxicity parameters of Pigment Derivated Compounds of Monascus sp. mold as a cervical anti-cancer drugs candidate

Lina Rahmawati Rizkuloh [1], Anindita Tri Kusuma Pratita [2], Mediana [2], Anna Yuliana [2*]

[1] Department of Pharmacy, Perjuangan University, Tasikmalaya, West Java, Indonesia.
[2] Department of Pharmacy, STIKes Bakti Tunas Husada, Tasikmalaya, West Java, Indonesia.

Abstract: Toxicity prediction is very important for the development and design of new drugs because computational toxicity estimates are not only faster than the determination of toxic doses in animals, but can also help reduce the number of animal trials. The test uses pkCSM, Protox II, Toxtree, preADMET and T.E.S.T. from the results of research that has been carried out on 57 pigment derivated compounds of Monascus sp. mold, the results of the pkCSM application are 39 test compounds for the Protox-II application there is 1 compound, the Toxtree application produces 1 compound, for the PreADMET application 4 safe compounds are produced, and for the T.E.S.T application produces 1 compound because it fulfills one of the aspects of ICH (International Conference on Harmonization) S9: non-clinical evaluation for anticancer pharmaceutical 2010 and has the potential as a candidate for anticancer drugs.

Keywords: Monascus sp., Toxicity, In silico.

INTRODUCTION

With the existence of modeling predictions by QSAR, it is possible to predict drug toxicity easier and perhaps even before drug synthesis. Quantitative Structure–Activity Relationships (QSAR) has been widely used in toxicology to predict the liability of novel compounds using structural features of known toxicants. During the last 40 years, many QSAR models have been published predicting carcinogenicity[1] and mutagenicity[2]. Toxic effects are generally categorized according to the site of the toxic effect. In some cases, the eff[3]-activity relationship has been widely used in the world to predict toxicity using computers[4,5].

One of the new drug candidate alternatives is the pigment produced by Monascus sp. Monascus sp. is one type of mold used for fermentation of Angkak. One of the metabolites produced by Monascus sp. is a pigment. Monascus sp. pigment research has progressed very rapidly, there are 3 main pigments from Monascus sp. namely yellow, orange and red. From these main pigments, several pigment-derived compounds are found which are widely used as natural coloring agents in the textile, food, pharmaceutical and cosmetic industries[6,7,8,9]. According to research by Singgih[10], 19 pigment derivates from Monascus sp. have been tested to have smaller bond energy value than Genistein as natural

Corresponding author.
E-mail address: lina@unper.ac.id (Lina Rahmawati R)
DOI: 10.29238/teknolabjournal.v10i2.238
Received 29 September 2021; Received in revised form 21 November 2021; Accepted 21 November 2021

ligand. Genistein is an anti-cancer drug that has strong potential in the treatment and prevention of breast cancer because it has anti-proliferative properties. Genistein has an antiesterogenic effect, namely the ability to stop the estrogen hormone binding to cells in order to prevent cancer from growing and dividing. Through binding to ERβ through inhibition of the activity of enzymes involved in estrogen metabolism. Monankarin C with the smallest bond-free energy value is -11.08 Kcal / mol, which means the most stable complex conformation, genetic compounds form hydrogen bonds with Arg346, Glu305, and His475. Monankarin C also has the same interaction so that it is expected to have a better affinity than natural ligands.

The compounds with the next lowest free energy are PP-V Monaskorubin and Monaskin, the best compounds as a candidate for breast cancer drugs, namely Monaskin pigments because they have smaller bond energies than natural ligands. However in PP-V, Monascorubrin, Monankarin C, although they have a small bond energy, this compound is seen from the toxicity test of Monarkarin C which is mutagenic, carcinogenic and toxic to reproduction, whereas in PP-V and Monascorubrin it is toxic to reproduction. Many have been shown to have anticancer activity, but their use is limited due to side effects and high toxic effects[11]. Nevertheless, toxicity can be assessed using computational resources (computational algorithms, software, and data) to organize, analyze, model, simulate, visualize, or predict chemical toxicity[12,13,14,15].

MATERIAL AND METHOD

2.1 Materials and Research Tools

The tools used are the HP brand laptop (3D248N5S laptop), Windows 10 operating system, 64 bit operating system x64- bassed processor, AMD A9-9420 RADEON 5R processor, 4.00 GB RAM, 5 COMPUTER CORES 2C + 3G 3.00 GHz. The programs used are Chem Bio Draw Ultra Version 12 (CambridgeSoft), Chem Bio 3D Ultra Version 12 (CambridgeSoft), and T.E.S.T.

Online Tools for Toxicity Test used are PreADMET (https://preadmet.bmdrc.kr), pkCSM online tool (http://biosig.unimelb.edu.au/pkcsm/prediction), ToxTree (http://toxtree.sourceforge.net) and Protox-II (http://tox.charite.de/tox/). The materials used were 57 derivated compounds of Monascus sp.6

2.2 Materials Preparation

Prior to the in silico toxicity test, 57 pigment derivated compounds of Monascus sp., their molecular structures were drawn using Chem Draw Ultra Version 12, stored in *.sdf or *.pd files and then made in *SMILES* form using the Chem Draw application.

2.3 Prediction of Toxicity

Prediction of the toxicity of the pigment derivated compounds of *Monascus* sp. using online tools [16,17,18]Prior to the *in silico* toxicity test, 57 pigment derivated compounds of *Monascus* sp. depiction of 2-D molecular structures with Chem Bio Draw Ultra Version 12, then in the Chem Bio 3D Ultra Version 12 program to create 3-D structures, then saved in the form of * .sdf or * .pd files. Next, the 57 pigment derivated compounds of *Monascus* sp., The structure is converted into the *SMILES* format with the Chem Draw application. pkCSM online tool (http://biosig.unimelb.edu.au/ pkcsm / prediction). To predict the toxicity (LD_{50}) orally on rodent and the classification of toxicity of compounds based on the Globally Harmonized System (GHS), the Protox online tool (http://tox.charite.de/tox/) was used[19]. The toxicity of the test compounds includes prediction of mutagenesis, carcinogenicity and acute toxicity (LD50). Mutagenesis prediction uses ADMET predictor, T.E.S.T, Protox-II (http://tox.charite.de/protox_II/), Toxtree (http://toxtree.sourceforge.net/),and pkCSM (http://biosig.unimelb.edu.au/ pkcsm/).

RESULTS AND DISCUSSION

Based The toxicity of drug impurities is also closely related to their structure. Structure-activity relationships have been widely used to predict toxicity using[4,5]. The determination of toxicity testing criteria is based on a protocol issued by the ICH (International Conference on Harmonization) S9: non-clinical evaluation for pharmaceutical anticancer, 2010, which includes: General toxicity includes MTD (Maximum Tolerated Dose), and LOAEL (Low observed adverse effect level), toxicity to reproduction or fetal development (DTP = Developmental toxicity potency), mutagenicity and carcinogenicity.

There are many tools for predicting the toxicity of a molecule, some of which are commercial in nature, some of which are online web servers and some of which can be downloaded freely. In the prediction of toxicity[20] in silico, the type of toxicity assessment uses computational resources (algorithms, software, and data) to organize, analyze, model, simulate, visualize, or predict chemical toxicity20. In predicting the toxicity of in silico, pigment derivated compounds of Monascus sp. mold was performed using pkCSM, Protox II, Toxtree, preADMET and T.E.S.T. The data generated from this in silico test can be used to design further testers.

The performance of the pkCSM software in the external validation dataset showed 83.8% accuracy in the mutagenicity test. There are several pkCSM end points, LD50, AMES test, maximum daily dose, and hepatotoxicity[17]. Based on our research, the toxicity data obtained from the pkCSM application for liver toxicity (hepatotoxic) contained 15 positive compounds that could cause damage to the liver, for mutagenesis data on the pkCSM application used the AMES method namely mutagenesis validation test method. There are 5 compounds that are mutagen positive.

The prediction of ProTox-II hepatotoxicity has a balanced accuracy of 82.00% on cross validation and 86.00% on external validation. In our research for hepatotoxicity, it is not stated that there are compounds that cause damage to the liver. Drug-induced hepatotoxicity is a significant cause of acute liver failure and one of the main reasons for drug withdrawal from the market.

Drug-induced liver injury (DILI) is a chronic process or rare event. The conceptual mechanism of DILI is direct cell stress, direct mitochondrial damage and specific immune reactions for the carcinogenicity test, 19 compounds were identified that could affect genes and damage normal cells so that they could become cancer cells. For mutagenesis data carried out using this application, it was found that there were no compounds that could cause mutations. For LD50, there are 2 compounds belonging to class II, 24 compounds belonging to class III, 28 compounds belonging to class IV and there are 3 compounds belonging to class VI based on the Globally Harmonized System[21,22.]

Toxtree's performance in the external validation dataset shows 70% accuracy and 78.3% sensitivity in the carcinogenicity test and 78% accuracy for the mutagenicity test. Toxtree represents the end point of various toxicities, namely the Cramer Rules to see from its functional groups, Kroes TTC to estimate the exposure threshold for drug compounds in humans, Benignin Bosa rules for carcinogenicity (genotoxic and non-genotoxic) and mutagenicity in vitro (AMES test)[23,24.]

In the Toxtree test according to Cramer's rules, there is 1 compound belonging to class I, which is the lowest level of toxicity, while 56 other compounds are included in class III, which means that the highest level of toxicity contains a functional group, the substituent ring which is a marker for the compound is toxic and even possible to have toxicity which is significant and it is estimated that this compound is not guaranteed its safety because it is a substance with a chemical structure that does not allow initial assumptions of safety or may even suggest significant toxicity or has reactive functional groups[25]. For the Benignin / Bossa rules parameter, which is to determine which

compounds can cause carcinogenicity and mutagenicity, 57 compounds tested positive for mutagens were obtained. This is intended as an initial stage of mutagenic in vivo screening, molecular functional groups that are known to be related are aromatic polycyclic hydrocarbons and heterocyclic molecules that can form non-covalent interactions with proteins or DNA bonds that have the potential to be genotoxic[26]. PreADMET provides 62.5% accuracy and 52.2% sensitivity for carcinogenicity tests[27]. PreADMET predicts toxicity based on AMES mutagenicity parameters, the actual predictive value is "positive" or "negative"[21].

Mutagenicity testing used the PreADMET application using the AMES method and the results obtained were 21 compounds that were positive for mutagens, and for carcinogenicity (Carcino Rat) there were 9 compounds that were negative for carcinogens. The compounds tested using the T.E.S.T application contained 9 positive mutagens and 7 N/A compounds (Not Applicated) and 41 other compounds were negative mutagenic, in this application there were N/A results because using the FDA method: Predictions for each chemical test were made using a new model corresponding to the chemical most similar to the test compound. Each model is generated at runtime which is the reason these 7 compounds cannot be translated into this application.

The toxicity test carried out with 5 different applications for toxicity parameters, it can be concluded that there are 4 compounds: N-glutaryl Monascurobamine, Monankarin F, Monaphilones A and Monaphilones B, that meet the criteria issued by ICH (International Conference on Harmonization) S9: non-clinical evaluation for pharmaceutical anticancer (2010) because they have a low level of toxicity from the 57 compounds tested. Whereas for testing each application, the best compound results for anticancer drug candidates according to ICH is as follows: for the application of pkCSM, the best compounds that have the potential as anticancer drug candidates are 39 test compounds, for the Protox-II application there is 1 safe compound; Xantomiscin B because it is included in class VI for LD50 and negative for carcinogens and mutagens, the Toxtree application produces 1 safe compound; N-glucosyl rubropuctamine because according to Creamer rules it is categorized as class 1 which means has a low level of toxicity, for the application of PreADMET 4 safe compounds are produced, namely *Monaspyridine* A, *Monaspyridine* B, *Monaspyridine* C, and *Monaspyridine* D, while for the T.E.S.T application produced 1 safe compound, namely Monaphilol A with negative mutagen and LD50 toxicity category including in class V.

CONCLUSION

Research on 57 pigment derivated compounds of *Monascus* sp. mold, the results were obtained for the application of pkCSM, the best compound that has the potential as an anticancer drug candidate are 39 test compounds, for the Protox-II application there is 1 safe compound, namely *Xantomiscin* B, the Toxtree application produces 1 safe compound, namely *N-glucosyl rubropuctamine*, for PreADMET applications 4 Safe compounds which are *Monaspyridine* A, *Monaspyridine* B, *Monaspyridine* C, and *Monaspyridine* D are produced, while for the T.E.S.T application, 1 safe compound is produced, namely *Monaphilol* A because it fulfills one of the aspects of ICH (International Conference on Harmonization) S9: non-clinical evaluation for anticancer pharmaceutical 2010 and has potential as an anticancer drug candidate.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to this work.

ACKNOWLEDGEMENT

The authors would like to thank the Human Genetic Perjuangan University Tasikmalaya, STIKes Bakti Tunas Husada Tasikmalaya and to all of my supervisor.

FOUNDING INFORMATION

Self funding

DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

REFERENCE

1. Mombelli E. DJ. Evaluation of the OECD (Q)SAR Application Toolbox and Toxtree for predicting and profiling the carcinogenic potential of chemicals. *SAR QSAR Env Res*. 2010;21:731–752.
2. Devillers J. ME. Evaluation of the OECD QSAR Application Toolbox and Toxtree for estimating the mutagenicity of chemicals. Part 1. *Aromat Amin SAR QSAR Env Res*. 2010;21:753–769.
3. RI B. *Peraturan Kepala Badan Pengawas Obat Dan Makanan Republik Indonesia Nomor 7 Tahun 2014 Tentang Pedoman Uji Toksisitas Nonklinik Secara In Vivo*. BPOM; 2014.
4. Greene, N., Dobo, K. L., Kenyon, M. O., Cheung, J., Munzner, J., Sobol, Z., & Wichard J. A practical application of two in silico systems for identification of potentially mutagenic impurities. *Regul Toxicol Pharmacol*. 2015;72(2):335-349.
5. Gautam, P. K., Gautam, R. K., Banerjee, S., Chattopadhyaya, M. C., & Pandey JD. Heavy metals in the environment: fate, transport, toxicity and remediation technologies. *Nov Sci Publ*. 60:101-130.
6. Yuliana, A., Singgih, M., Julianti, E., & Blanc PJ. Derivates of azaphilone Monascus pigments. *Biocatal Agric Biotechnol*. 2017;9:183-194.
7. Dikshit, R., & Tallapragada P. Monascus purpureus: A potential source for natural pigment production. *J Microbiol Biotechnol Res*. 2011;1(4):164-174.
8. Mostafa, M.E., Abbady M. Secondary metabolites and Bioactivity of the Monascus pigments review article. *Glob Biochem J Biotechnol*. Published online 2014.
9. Singgih, W. JE. Food colorant from microorganisms. M.-T. Liong (ed.) Beneficial Microorganism in Food and Nutraceuticals. *Microbiol Monogr 27*. Published online 2015.
10. Singgih M., Benny, P., Selvira, A., Yuliana A. Study In Silico Metabolit Sekunder Kapang Monascus sp. Sebagai Kandidat Obat Antikolesterol dan Antikanker. *ALCHEMY J Penelit Kim*. 2019;(15(1)):104-123.
11. Malchers, F., Ercanoglu, M., Schütte, D., Castiglione, R., Tischler, V., Michels, S.,& George J. Mechanisms of primary drug resistance in FGFR1-amplified lung cancer. *Clin Cancer Res*. 2017;23(18):5527-5536.
12. Narko, T., Benny, P., Riska, P., Dang, S., & Faridhatul K. Molecular Docking Study of Bulb of Bawang Dayak (Eleutherine Palmifolia (L) Merr) Compound as Anti Servical Cancer. *J Ilm Farm Bahari*. 2017;8(2):1-14.
13. VB. RA dan B. In silico toxicology: computational methods for the

prediction of chemical toxicity. *WIREs Comput Mol Sci*. Published online 2016.
14. Yuliana, A., Zannah, S. U., Rahmiyani, I., & Amin S. Fermentation and Determination of Anti-Cholesterol Monakolin K from Different Monascus purpureus Isolates. In: In 2nd Bak. Atlantis Press; 2020:225-227.
15. Yuliana, A., Rahmiyani, I., Amin, S., & Fathurohman M. *Isolation and Determination Antibacterial Citrinin From Various Fungal Monascus Purpureus Using Rice as a Fermentation Substrate*. Vol 1179(1). In Journal. IOP Publishing; 2019.
16. Yuliana, A., Wibowo, M. S., & Julianti E. Solubility and toxicity level of Monascus pigments. Trends Tech Sci Res. 2018;2(4):1-3.
17. Hassan SF., Rashid U., Ansari FL. U-HZ. Bioisosteric Approach in Designing New Monastrol Derivatives: An Investigation on Their ADMET Prediction Using In Silico Derived Parameters. *J Mol Graph Mode*. Published online 2017.
18. Pires DEV, Blundell TL AD. pkCSM Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *J Med Chem*. Published online 2015.
19. Saeed A, Rehman S, Channar PA, Larik FA, Abbas Q, Hassan M, Raza H, Flörke, U SS. Long chain 1-acyl-3-arylthioureas as jack bean urease inhibitors, synthesis, kinetic mechanism and molecular docking studies. *J Taiwan Inst Chem Eng*. Published online 2017.
20. Ruswanto, Siswandono, Richa, M., Tita, N., Tresna L. Molecular Docking of 1Benzoyl-3-methylthiourea as Anti Cancer Candidate and Its Absorption, Distribution, and Toxicity Prediction. *J Pharm Sci Res*. Published online 201AD.
21. Banerjee P., Andreas O., Eckert., Anna K. S dan RP. ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic Acids Research. *2018*.
22. Nation. U. A Guide to The Globally Harmonized System of Classification and Labeling of Chemicals (GHS). *United Nation*. Published online 2019.
23. El-Din, H. M. A., Loutfy, S. A., Fathy, N., Elberry, M.H, Mayla, A.M., Kassem, S., Naqvi A. Molecular docking based screening of compounds against VP40 from Ebola virus. *Bioinformation*. Published online 2016.
24. Yeni, Yeni & Supandi, Supandi & Merdekawati F. In silico toxicity prediction of 1-phenyl-1-(quinazolin-4-yl) ethanol compounds by using Toxtree, pkCSM and preADMET. *Pharmaciana*. Published online 2018. doi:8. 216. 10.12928/pharmaciana.v8i2.9508
25. Lestari T. Studi Interaksi Senyawa Turunan 1, 3-Dibenzoiltiourea sebagai Ribonukleotida Reduktase Inhibitor. *J Farm Indones*. 2015;7(3).
26. Benigni, R., and Bossa C. Mechanisms of Chemical Carcinogenicity and Mutagenicity: A Review with Implications for Predictive Toxicology,Chemical Reviews. Published online 2011.
27. Zhang, L., Ai, H., Chen, W., Yin, Z., Hu, H., Zhu, J., ... & Liu H. CarcinoPred-EL: novel models for predicting the carcinogenicity of chemicals using molecular fingerprints and ensemble learning methods. *Sci Rep*. 7(1):1-14.

Table 1. Toxicity Prediction Results of 57 Derivated Compounds of Monascus sp. Using pkCSM, Protox II, Toxtree PreADMET, and T.E.S.T

No.	Compounds	pkCSM		Protox-II		ToxTree	PreADMET	T.E.S.T
		Mutagenicity	Hepatotoxicity	Mutagenicity	Hepatotoxicity	Mutagenicity	Mutagenicity	Mutagenicity
1	*N-glucosyl rubropunctamine*	+	+	-	-	+	-	-
2	*N-glucosyl monascurobamine*	-	+	-	-	+	-	-
3	*N-glutaryl Monascurobamine*	-	-	-	-	+	-	-
4	*N-glutaryl Rubropuctamine*	-	-	-	-	+	-	-
5	Red Derivat 1	-	+	-	-	+	-	-
6	Red Derivat 2	-	+	-	-	+	-	+
7	Red Derivat 3	-	-	-	-	+	-	-
8	Red Derivat 4	-	-	-	-	+	-	-
9	Red Derivat 5	-	+	-	-	+	-	-
10	Red Derivat 6	-	+	-	-	+	-	-
11	Red Derivat 7	-	+	-	-	+	+	N/A
12	Red Derivat 8	-	+	-	-	+	+	N/A
13	Isolat MPs 4	-	-	-	-	+	+	N/A
14	Isolat MPs 3	-	-	-	-	+	-	N/A
15	Isolat MPs 2	-	+	-	-	+	+	N/A
16	Isolat MPs 1	-	-	-	-	+	+	N/A
17	New Red Pigment	+	-	-	-	+	-	+
18	Compound 3	-	-	-	-	+	-	+
19	*Monaspyridine A*	-	-	-	-	+	-	-
20	*Monaspyridine B*	-	-	-	-	+	-	-
21	*Monaspyridine C*	-	-	-	-	+	-	-
22	*Monaspyridine D*	-	-	-	-	+	-	-
23	Red Shandong 1	-	+	-	-	+	+	-
24	Red Shandong 2	-	+	-	-	+	-	-
25	PP-V	-	-	-	-	+	+	N/A
26	*Glycyl-rubropuntatin*	-	+	-	-	+	-	-
27	Un Named	-	-	-	-	+	-	+
28	*Xantomanascin A*	-	-	-	-	+	+	-
29	*Xantomanascin B*	-	-	-	-	+	+	-
30	Yellow II	-	-	-	-	+	+	-
31	*Monankarin A-B*	-	+	-	-	+	-	+
32	*Monankarin C-D*	-	+	-	-	+	-	-
33	*Monankarin E*	+	+	-	-	+	-	-
34	*Monankarin F*	-	-	-	-	+	-	-
35	*Monapurones A*	-	-	-	-	+	-	-
36	*Monapurones B*	-	-	-	-	+	+	-
37	*Monapurones C*	-	-	-	-	+	+	-
38	*Monaphilones A*	-	-	-	-	+	-	-
39	*Monaphilones B*	-	-	-	-	+	-	-
40	*Monaphilones C*	-	-	-	-	+	-	-
41	*Monashexonone*	-	-	-	-	+	-	-
42	*Rubropuctin*	-	-	-	-	+	-	-
43	*Monarubrin (Y,BF)*	-	-	-	-	+	-	-
44	*Purpureus One*	-	-	-	-	+	-	-
45	*Monascuspiloin*	-	-	-	-	+	-	-
46	*Monascusone A*	-	-	-	-	+	-	-
47	*Monascusone B*	+	-	-	-	+	+	+
48	FK-17-P2B2	+	-	-	-	+	+	-
49	Y3	-	-	-	-	+	+	-
50	*Monaphilol A*	-	-	-	-	+	+	-
51	*Monaphilol B*	-	-	-	-	+	+	+
52	*Monaphilol C*	-	-	-	-	+	-	-
53	*Monaphilol D*	-	-	-	-	+	-	+
54	*Monasfluor A*	-	-	-	-	+	+	-
55	*Monasfluor B*	-	-	-	-	+	+	-
56	*Monascuskaodione A*	-	-	-	-	+	+	+
57	*Monascuskaodione B*	-	-	-	-	+	+	-

(+) : Positive
(-) : Negative
N/A : Not Applicable