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QSAR OF ACYL ALIZARIN RED BIOCOMPOUND DERIVATIVES OF *RUBIA TINCTORUM* ROOTS AND ITS ADMET PROPERTIES AS ANTI-BREAST CANCER CANDIDATES AGAINST MMP-9 PROTEIN RECEPTOR: *IN SILICO* STUDY

Mochamad R. T. Alifiansyah[1], Mochammad A. Herdiansyah[2], Rahma C. Pratiwi[1], Ragil P. Pramesti[1], Nisriena W. Hafsyah[1], Athaya. P. Rania[1], Justitia E. R. P. Putra[2], Putri A. Cahyono[2], Litazkiyyah[2], Sirojuddin K. Muhammad[3], Ahmad A. A. Murtadlo[2,4], Viol D. Kharisma[2,4], Arif N. M. Ansori[4,5,6]*, Vikash Jakhmola[6], Praveen K. Ashok[7], Jyoti M. Kalra[8], Hery Purnobasuki[2]*, Intan Ayu Pratiwi[2]

[1] Department of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2] Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surbaya, Indonesia
[3] Department of Physics, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia
[4] Division of Research and Development, Jalan Tengah, Surabaya, Indonesia
[5] Postgraduate School, Universitas Airlangga, Surabaya, Indonesia
[6] Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University, Dehradun, India
[7] Gyani Inder Singh Institute of Professional Studies, Dehradun, India
[8] School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Dehradun, India

KEY WORDS:
ADMET prediction, alizarin derivatives, breast cancer, medicine, QSAR

ABSTRACT

Alizarin is a polycyclic compound isolated from roots of *Rubia tinctorum* that has potential as a breast anticancer candidate. Increasing anticancer activity can be done through structural modification to produce derivatives in the form of group substitution in the meta position using acyl. The purpose of this work is to forecast the anticancer activity of alizarin and its derivatives on the MMP-9 receptor using. Important biological activity factors will be identified by Quantitative Structure Activity molecular docking Relationship (QSAR) and projected absorption, distribution, metabolism, elimination, and toxicity (ADMET). Using Molegro Virtual Docker (MVD), molecular docking was carried out on the MMP 9 receptor (4WZV.pdb). LogP, Etot, and MR are the physicochemical parameters that are examined in order to produce QSAR. Statistical Package for the Social Science (SPSS) was used for the QSAR analysis. The pkCSM was utilized to determine ADMET prediction. The acyl alizarin derivatives have a lower rerank score than alizarin, according to the docking results so that they are predicted to have more potent anticancer activity. The QSAR analysis's findings indicated that logP and Etot had the greatest effects on the alizarin compound's and its derivatives' activity. The results of the ADMET prediction indicate that acyl alizarin is less harmful and superior to alizarin. Research findings show that it is possible to synthesize acyl alizarin derivatives, especially alizarin octanoate, which will then be tested in vitro or in vivo to determine its anti-breast cancer activity and toxicity.

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КОЛИЧЕСТВЕННОЕ СООТНОШЕНИЕ «СТРУКТУРА-АКТИВНОСТЬ» (QSAR) БИОСОЕДИНЕНИЯ АЦИЛАЛИЗАРИНА КРАСНОГО, ПРОИЗВОДНОГО КОРНЕЙ МАРЕНЫ КРАСИЛЬНОЙ, И ЕГО СВОЙСТВ В АСПЕКТАХ ADMET В КАЧЕСТВЕ ВЕЩЕСТВА-КАНДИДАТА, ПРЕПЯТСТВУЮЩЕГО РАЗВИТИЮ РАКА МОЛОЧНОЙ ЖЕЛЕЗЫ, В СРАВНЕНИИ С БЕЛКОВЫМ РЕЦЕПТОРОМ ММП-9: ИССЛЕДОВАНИЕ *IN SILICO*

Мохамад Р. Т. Алифансья[1], Мохаммад А. Хердиансья[2], Рахма К. Пративи[1], Рагил П. Прамести[1], Нисриена В. Хафсия[1], Атайя. П. Рания[1], Джустиция Э. Р.П. Путра[2], Путри А. Кахионо[2], Литазкийя[2], Сироджуддин К. Мухаммад[3], Ахмад А. А. Муртадло[2,4], Виол Д. Харисма[2,4], Ариф Н. М. Ансори[4,5,6],* Викаш Джахмола6, Правин К. Ашок[7], Джьоти М. Калра[8], Хери Пурнобасуки[2],* Интан Аю Пративи[2]

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[1]Кафедра фармацевтических наук, фармацевтический факультет, Университет Эйрлангга, Сурабая, Индонезия
[2]Кафедра биологии, факультет науки и технологий, Universitas Airlangga Университет Эйрлангга,, Сурбая, Индонезия
[3]Кафедра физики, факультет науки и технологий, Университет Эйрлангга,, Сурабая, Индонезия
[4]Отдел исследований и разработок, Джалан Тенга, Сурабая, Индонезия
[5]Аспирантура, Университет Эйрлангга,, Сурабая, Индонезия
[6]Институт фармацевтических наук Уттаранчала, Университет Уттаранчала, Дехрадун, Индия
[7]Институт профессиональных исследований Гьяни Индера Сингха, Дехрадун, Индия
[8]Школа фармацевтических наук, Гари Гурурам Рам Раи Университет, Дехрадун, Индия

КЛЮЧЕВЫЕ СЛОВА:
прогноз параметров ADMET, производные ализарина, рак молочной железы, медицина, количественное соотношение «структура-активность» (QSAR)

АННОТАЦИЯ

Ализарин представляет собой полициклическое соединение, выделенное из корней растения марена красильная (Rubia tinctorum), которое потенциально может представлять интерес как средство в лечении рака молочной железы. Повысить противораковую активность можно путем модификации структуры вещества с получением производных в виде замещения группы в метаположении с применением ацила. Цель данной работы — прогнозирование противоракового действия ализарина и его производных на рецепторе ММП-9 при помощи молекулярного докинга. Важные факторы биологической активности будут определены с помощью количественного соотношения структура-активность (QSAR) и прогнозируемых параметров всасывания, распределения, метаболизма, выведения и токсичности (ADMET). С помощью инструмента MVD был осуществлен молекулярный докинг рецептора MMP 9 (4WZV.pdb). LogP, Etot и MR — это физико-химические параметры, которые исследуются для получения QSAR. Для анализа QSAR использовался пакет программ обработки статистических данных (SPSS). Программа pkCSM использовалась для определения прогнозируемых параметров ADMET. Согласно результатам докинга, ацильные производные ализарина имеют более низкую степень переранжирования, нежели ализарин. Результаты анализа параметров QSAR показали, что logP и Etot оказали наибольшее влияние на активность соединения ализарина и его производных. Результаты прогноза параметров ADMET показывают, что ацилализарин менее вреден, и превосходит ализарин по своим полезным свойствам. Показано, что можно синтезировать производные ацилализарина, в частности октаноат ализарина, для их использования в качестве средства лечения рака молочной железы.

БЛАГОДАРНОСТИ: Автор выражает благодарность профессору, доктору наук Сисвандоно, магистру наук, который предоставил лицензию на использование инструмента MVD. Мы также благодарим Джалана Тенгаха, Индонезия (jalantenah.site) за редактирование рукописи.

1. Introduction

Breast cancer is an abnormal condition of the body that allows cells in the breast to grow uninterruptedly and thus trigger the formation of cancer cells. The main mechanisms of cancer progression include inhibition of apoptosis, involving the unlimited capacity for cells to divide, increased angiogenesis, resistance to growth-suppressing signals and excessive induction of cells to continue their growing [1]. With estimated 2.3 million of new cases worldwide, breast cancer is the fifth most common malignancy to cause mortality [2]. According to data obtained from the International Agency for Research on Cancer (IARC), Global Burden of Cancer, the global burden of cancer accounted for 18.1 million new cases of cancer worldwide in 2018, and 9.6 million of cancer-caused deaths. It is predicted that the number of cancer cases will rise from 18.1 million to 22 million over the next 20 years. According to WHO forecasts, 26 million of individuals will be diagnosed with cancer by 2030, and 17 million of those cases would result in cancer-caused deaths.

Matrix metalloproteinases, also known as MMPs, are zinc-dependent endopeptidases that are a part of the metzincin superfamily, which has been linked to a number of clinical conditions, including cancer. The largest member of the MMP superfamily linked to onset of the medical condition and metastasis in breast cancer is MMP-9. MMP-9 can activate various proteins involved in inflammatory pathways and can act as a pro-inflammatory factor [3,4]. This activation causes chronic inflammation which can promote tumorigenesis [5]. Apart from that, MMP is also known for its ability to activate tumor growth factors and trigger the mechanisms that can inhibit cell apoptosis and trigger increased cell proliferation [6]. MMP-9 is known for its ability to activate vascular endothelial growth factor (VEGF) so that it can cause angiogenesis in tumor cells [7]. Numerous substances found in the natural goods have shown their possession of anticancer properties. It is well known that *Rubia tinctorum* root features antibacterial, anti-inflammatory, and antioxidant properties. One of the primary components of the *Rubia tinctorum* plant is alizarin, which has proven to be useful in the therapy of bone cancer as an osteotropic medication [8,9,10]. Based on previous research, it was shown that alizarin is able to inhibit the growth of pancreatic cancer cells and cause apoptosis by inhibiting nuclear translocation of NF-kB, whereas activation of NF-kB will trigger gemcitabine resistance in the therapy of pancreatic cancer [11,12]. Based on an *in vitro* study, the IC50 value of alizarin for type 4T1 breast cancer cells are equal to 495 μM and these results indicate that alizarin is cytotoxic to type 4T1 breast cancer cells. In addition, the same study also showed that alizarin was able to reduce MMP-9 expression compared to the control group [13].

The modification of the organic structure of alizarin by molecular docking is one of the efforts that can be made to increase the anticancer activity of alizarin compounds in a body. Molecular docking simulation, else known as *in silico,* is carried out before the compound synthesis process. The advantages of this *in silico* study are reducing costs, research time, errors, using the experimental animals, and preventing contamination by chemicals [14,15]. Molecular docking is the tethering of a ligand or test compound in a certain conformation to a receptor target [16]. The result of molecular docking is a reranking of score (RS) parameters, while these parameters describe the binding energy needed by the ligand to bind to the receptor so as to produce biological activity. The smaller the RS value, the stronger the bond formed between the ligand and the receptor, so that the biological activity also increases [17,18,19].

In this study, dry lab tests were carried out to predict the anticancer activity of alizarin compounds and their derivatives against breast cancer receptor MMP-9 using molecular docking simulations. The structure was modified in the form of a derivatization process targeted to produce compound derivatives by substituting the meta position of the structure with the help of an acyl group. The addition of acyl groups to alizarin is suggested to increase biological activity in the form of anticancer potency because acyl groups can increase lipophilicity along with increasing the number of C atoms [20]. The existence of structural modifications in the form of adding acyl groups to alizarin will cause differences in physicochemical properties so that the resulting biological activity will also be different [21]. Molecular docking simulation was performed using MVD on MMP-9 receptor (4WZV.pdb). From the docking results, the rerank score parameters were obtained to proceed to the QSAR study. QSAR studies have the aim of designing and identifying the new drug compounds that are likely to possess preset biological activity or to improve the pharmacokinetic and toxicity characteristics of the new drug compounds [22]. The physicochemical parameters used in the QSAR study are logP, Etot, and MW. The QSAR study aims to obtain a mathematical equation that correlates the physicochemical characteristics of the drug compounds with their biological activity [23,24].

Furthermore, an assessment of the toxicity and pharmacokinetic parameters (absorption, distribution, metabolism, elimination and toxicity, ADMET) of acyl alizarin derivatives was conducted. This evaluation was implemented because most of the new drug candidates were predicted to have good activity but failed in the preclinical and clinical trial phases due to poor ADMET parameters [25]. The results of the implemented dry lab tests were aimed to predict the anticancer activity of alizarin and its derivatives against MMP-9 breast cancer cells as well as its being a source of reference data in the future for the other pharmaceutical research.

2. Objects and methods

2.1. Materials

The type of the hardware and software used is a Lenovo Laptop, Windows 11 operating system, 64-bit, Intel Core i3–1115G4, CPU @ 3 GHz 3 Ghz, 8.00 GB RAM. The application used is ChemDraw ver.19, Chem3D ver.19, Molegro Virtual Docker (MVD) ver. 6.0, SPSS ver. 25, SMILES Translator, and pkCSM online tool.

2.2. Protein preparation

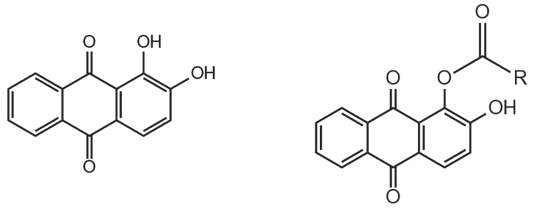
The protein in the form of the MMP-9 receptor (Figure 1) was downloaded from the Protein Data Bank (https://www.rcsb.org/). From among the several existing receptors, the MMP-9 receptor with PDB code 4WZV was selected. The MMP-9 receptor with PDB code 4WZV was chosen because the native ligand has a carbonyl group (C=O) as a pharmacophore group. The alizarin compound also has carbonyl group.

2.3. Ligand preparation

The 2D structures of the test compounds, in particular alizarin and acyl alizarin derivatives (A and AA1–9) (Table 1) were drawn using the ChemDraw Ver. 19 software. Structures from ChemDraw were copied to Chem 3D Ver. 19 to view the 3D structure and measure the minimum energy with MMFF94. Furthermore, these structures are stored in the format *mol2* {SYBYL2(*.Mol2)}.

Table 1. **The chemical structure of alizarin and acyl alizarin derivatives**

Таблица 1. **Химическое строение производных ализарина и ацилализарина**



	Alizarin	Acyl alizarin derivatives
Code	**Compounds name**	**Functional group**
A	Alizarin	—
AA1	Alizarin acetate	CH3 (methyl)
AA2	Alizarin propionate	C2H5 (ethyl)
AA3	Alizarin butyrate	C3H7 (propyl)
AA4	Alizarin pentanoate	C4H9 (butyl)
AA5	Alizarin hexanoate	C5H11 (pentyl)
AA6	Alizarin heptanoate	C6H13 (hexyl)
AA7	Alizarin octanoate	C7H15 (heptyl)
AA8	Alizarin nonanoate	C8H17 (octyl)
AA9	Alizarin decanoate	C9H19 (nonyl)

2.4. Docking method validation

Validation was carried out by docking the native ligand on MMP-9 receptor (4WZV) based to the cavity. Docking method is valid if RMSD score is less than 2 A [14,26]. RMSD is a parameter that takes into account the distance deviation that occurs between the orientation of the native ligand before its re-docking and after re-docking [27].

2.5. Docking method

The molecular docking compound test was carried out on the same cavity of protein as the native ligand in the MMP-9 receptor based on the results of method validation using MVD. The results obtained from the molecular docking are expressed in the form of a rerank score (RS) which parameter describes the energy required to form a compound bond with the receptor. The smaller the RS value produced, the more stable the bond formed between the compound and the receptor so that the biological activity also increases. In addition, the interaction between the test compound and the amino acid residues of the MMP-9 receptor was observed using the Discovery Studio 2021 Client visualization software.

2.6. QSAR study

The QSAR study uses physicochemical parameters obtained from ChemDraw Ver.18 — these are logP as a lipophilic parameter, and molecular weight as a steric parameter. The logP and MW values are obtained by selecting the menu function "view", then selecting "show chemical properties window". Meanwhile, the total energy value (Etot) as an electronic parameter was obtained from the software Chem 3D Ver.18 by copying the 2D structure from ChemDraw then pasting it into Chem 3D. Next, the function "calculation" was selected, then "MMFF94", and "perform MMFF94 minimization" as last. The Etot value can be seen at the bottom of the output window. QSAR research uses the SPSS software by entering these three parameters as the independent variables and the RS value as the dependent variable. From these variables, linear regression and nonlinear regression results were obtained.

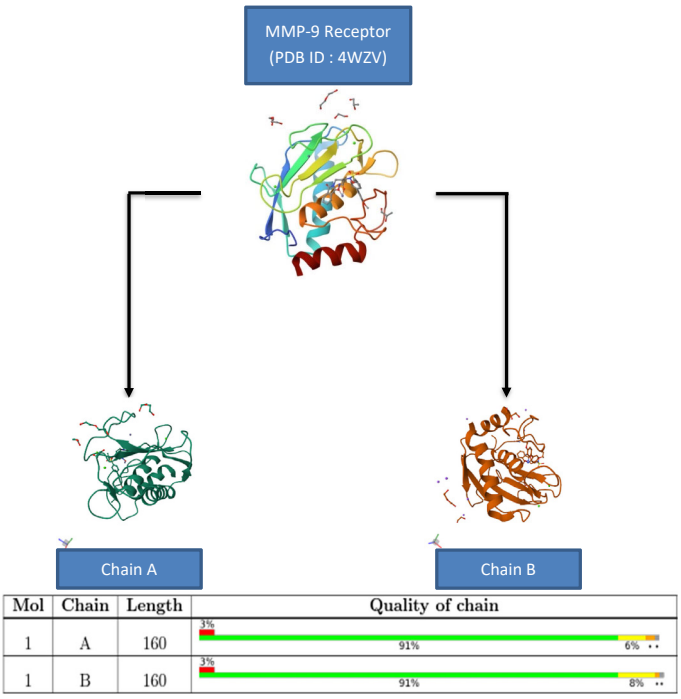


Figure 1. **Detail of MMP-9 Receptor (PDB ID: 4WZV)**

Рисунок 1. **Состав рецептора ММП-9 (идентификатор согласно системе учета банка белковых структур: 4WZV0)**

2.7. ADMET prediction

ADMET prognostication of alizarin compounds and acyl alizarin derivatives properties can be done with the pkCSM online tool. Software pkCSM is known to have quite high accuracy, for example the AMES toxicity predictor in pkCSM has an accuracy rate of 83.8%, while in ToxFree it has an accuracy rate of 75.8% [28]. The first step taken was to draw the 2D structure of the compound to be analyzed using the ChemDraw Ver. 19.1, then the 2D structure was copied to the Chem3D Ver. 19.1 to obtain the 3D structure and saved in *.sdf file format. Furthermore, the compound file was translated using SMILES translator (https://cactus.nci.nih.gov/translate/). Then, the translated compound can be prognosticated for its pharmacokinetic and toxicity properties using the pkCSM online tool (https://biosig.lab.uq.edu.au/pkcsm/). The results obtained from these prognostications can be interpreted through pkCSM theory.

2.8. Statistical analysis

Statistical analysis was carried out for QSAR study with the help of SPSS software. QSAR study use regression analysis to look for correlations between physicochemical property parameters that represent the structure of chemical compounds and the resulting biological activity. From the regression analysis, the HKSA equation and several statistical criteria are produced. The best HKSA equation is selected based on statistical criteria. The statistical criteria used in QSAR research are significance, r, F, and Standard Error (SE). The significance value of less than 0.05 (<0.05) indicates the presence of significant relation between the physicochemical property parameters and the resulting biological activity. The r value ranges between 0 and 1, where the higher the r value, the stronger the relationship between physicochemical property parameters and biological activity. The F value shows the significance of the relation, where the higher the F value, the higher the degree of significance. The SE value shows variations or errors in the study.

3. Results and discussion

Molecular docking of acyl alizarin derivative compounds was carried out using the receptor MMP-9 (PDB code: 4WZV). Before carrying out the molecular docking trials on the acyl alizarin derivative test compound, method validation process is required to be run on the selected receptor. Due to the validation process of the docking method, an RMSD value will be obtained which analyzes changes in the interaction between the ligand and the protein or the receptor structure before and after docking. The docking method is considered to be valid if the resulting RMSD value is less than 2 Å [14,26]. Validation of the docking method was carried out in 2 replications and the RMSD values obtained for each replication were equal to 0.7773 Å and 0.6239 Å, respectively. From these results it was concluded that the docking method and MMP-9 receptor, designated with PDB code: 4WZV, can be used for molecular docking of the acyl alizarin derivative test compound.

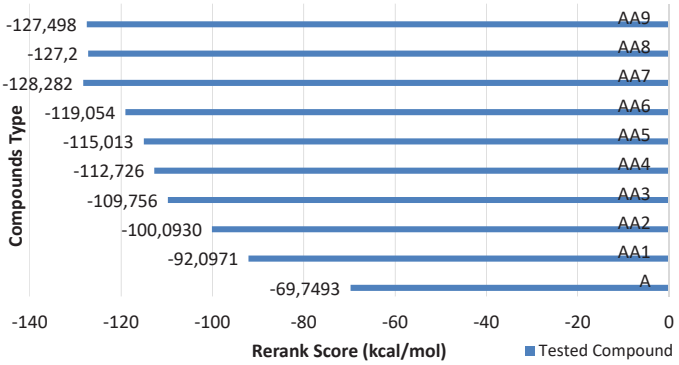


Figure 2. **Prognosticated results of biological activity of alizarin derivative compounds via using MVD**

Рисунок 2. **Прогностические результаты биологической активности производных соединений ализарина, проанализированных с помощью MVD**

From Figure 2, the results show that the alizarin derivative compound feature a lower RS value than its parent compound, namely alizarin. Alizarin derivative compounds are considered to have a higher cytotoxic effect on breast cancer cells than alizarin. The compound alizarin octanoate (AA7) is known to have the lowest RS value compared to other alizarin derivative compounds.

Table 2. **Results of interaction of alizarin compounds and their derivatives with amino acid residues of the MMP-9 receptor**

Таблица 2. **Результаты взаимодействия соединений ализарина и их производных с аминокислотными остатками рецептора ММП-9**

Code	Hydrogen Bond	Steric Bond	Amount of Hydrogen Bond	Amount of Steric Bound
A	Ala 242; Ala 242; Met 247	Leu 188; His 226; His 226; Tyr 245; Leu 243; Val 223; Val 223	3	7
AA1	Met 247	His 226; His 226; Val 223; Val 223; Tyr 248; Leu 222; Leu 243	1	7
AA2	Met 247	His 226; His 226; Val 223; Val 223; Tyr 248; Leu 222; Leu 243; Tyr 245; Arg 249; Met 247	1	10
AA3	—	His 226; His 226; Val 223; Tyr 248; Leu 222; Leu 243; Met 247; Tyr 245; Tyr 245	0	9
AA4	—	His 226; His 226; Tyr 245; Tyr 245; Met 247; Glu 241; Leu 222; Leu 243; Tyr 248; Val 223; Val 223	0	11
AA5	Tyr 245	Met 247; His 226; Val 223; Leu 188; Glu 241	1	5
AA6	Arg 249	Pro 240; Arg 249; Arg 249; Met 247; Met 247; Leu 188; Val 223; Val 223; His 226; Tyr 248; Pro 255	1	11
AA7	Tyr 245	Met 247; Tyr 248; Ala 189; Leu 188; Val 223; His 226; Glu 241	1	7
AA8	Tyr 245; Arg 249	Leu 188; Val 223; His 226; Tyr 248; Met 247; Glu 241	2	6
AA9	Arg 249; Arg 249	Val 223; Ala 189; Leu 188; Leu 188; Arg 249; Arg 249; Met 247; His 226; His 226	1	9

The alizarin compound and acyl alizarin derivatives were also observed for their interactions with the MMP-9 receptor, coded under PDB4WZV, through visualization software *Discovery Studio 2021 Client*. The interactions between the compounds and receptors that could be seen include hydrogen bonds and steric bonds. Hydrogen bonds are intermolecular bonds between hydrogen atoms bonded to highly electronegative atoms (F, O, and N) and highly electronegative atoms from the other molecules. Hydrogen bonds are very strong bonds with bond energies reaching 40 kJ/mol. In Table 2, the alizarin compound has a greater number of hydrogen bonds than all acyl alizarin derivative compounds. Steric bonds include Van der Walls bonds and hydrophobic bonds. Van der Walls bonds consist of several types, namely dipole-dipole bonds, London bonds, and ion-dipole bonds [29]. Hydrophobic bonds are the bonds that occur between amino acids from non-polar protein side chains and lipophilic groups from the ligands [30]. Based on the Table 2 it can be seen that alizarin pentanoate (AA4) and alizarin heptanoate (AA6) have the highest number of steric bonds compared to alizarin and other acyl alizarin derivatives. Figure 3 shows a visualization of the interactions that occur in the alizarin derivative that has the lowest RS, namely alizarin octanoate (AA7) with the MMP-9 receptor amino acid residue (PDB: 4WZV).

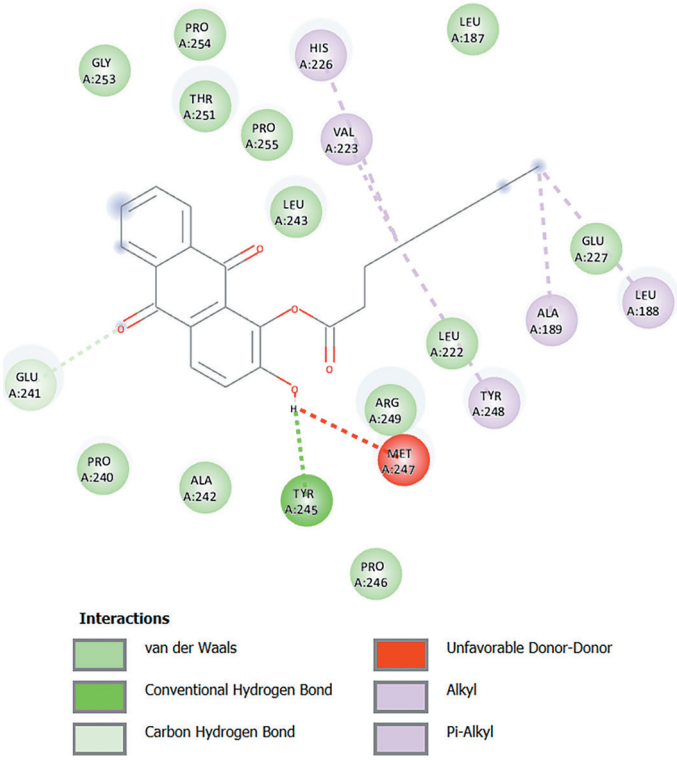


Figure 3. **Visualization of the Interaction of Alizarin Octanoate with MMP-9 Receptor Amino Acid Residues (PDB: 4WZV) (Discovery Studio 2021 Client)**

Рисунок 3. **Визуализация взаимодействия октаноата ализарина с аминокислотными остатками рецептора ММП-9 (идентификатор согласно системе учета банка белковых структур: 4WZV) (программа Discovery Studio 2021 Client)**

The physicochemical parameters (descriptors) of alizarin derivatives and acyl alizarin were determined using Chem Draw Ver. 18 and Chem 3D Ver. 18 which can be seen in the Table 3. The prognosticated descriptor values and anticancer activity of the molecular docking later were analyzed using linear and non-linear regression by SPSS. The equation is presented in the Table 4.

Table 3. **Physicochemical parameters (descriptors) of alizarin and acyl alizarin derivatives**

Таблица 3. **Физико-химические параметры (дескрипторы) ализарина и производных ацилализарина**

Code	LogP	E_{tot} (kkal/mol)	MW (g/mol)
A	1.64	48.6696	66.38
AA1	1.62	61.5911	75.77
AA2	2.27	61.313	80.51
AA3	2.69	60.9239	85.11
AA4	3.10	60.8187	89.71
AA5	3.52	60.8902	94.31
AA6	3.94	60.5841	98.91
AA7	4.36	60.8082	103.51
AA8	4.77	60.7794	108.10
AA9	5.19	60.8725	112.7

Table 4. **Linear and non-linear regression equation of alizarin and acyl alizarin derivatives**

Таблица 4. **Линейное и нелинейное уравнение регрессии ализарина и производных ацилализарина**

No.	Regression equation	n	r	s	F	Sig
	One parameter					
1.	RS = –13.319 logP – 66.062	10	0.908	8.22	37.658	0.000
2.	RS = –1.192 MR – 1.035	10	0.954	5.92	80.088	0.000
3.	RS = –3.443 E_{tot} + 95.489	10	0.724	13.56	8.806	0.018
4.	RS = 3.856 $logP^2$–38.928 logP – 29.076	10	0.947	6.74	30.508	0.000
	Two parameters					
5.	RS = –2.771 MR + 18.756 logP + 81.335	10	0.975	4.634	68.430	0.000
6.	RS = –1.999 E_{tot} – 10.771 logP + 44.912	10	0.986	3.56	118.395	0.000
7.	RS = –1.359 E_{tot} — 0.996 MR + 62.146	10	0.983	3.87	99.774	0.000
8.	RS = 2.817 log^2P — 4.094 logP — 2.413 MR + 89.317	10	0.994	2.50	162.062	0.000
9.	RS = 2.056 log^2P — 24.790 logP — 1.713 E_{tot} + 48.719	10	0.994	2.40	176.982	0.000

The QSAR study demonstrated that the best equation with one parameter is equation No.2 and the best equation with two parameters is equation No. 9 which is presented in the Table 4. From these equations, the conclusion can be drawn that there is a significant parabolic relation between logP and Etot with the prognostication of breast anticancer activity so that lipophilic and electronic parameters are the most influential parameters on the prognostication of breast anticancer activity.

Table 5 showed the ADMET properties of the alizarin and its derivatives. ADMET properties divided into five group parameters that are absorption, distribution, metabolism, and toxicity. The process of drug absorption has significant importance within the realm of pharmacokinetics, a scientific discipline focused on comprehending the body's interactions with the administered substances throughout their entire presence within the body system [31]. Drug absorption refers to the mechanism through which a drug transitions from its point of its administering into the body to its circulation throughout the system. Multiple mechanisms contribute to the effective assimilation of drugs, encompassing passive diffusion, facilitated diffusion, active transporting, and endocytosis. The quantification of drug absorption is expressed as bioavailability, which denotes the degree of successful absorption. In simpler terms, bioavailability is referred to the portion of the administered drug that remains unchanged and reaches the circulation system [32].

Water solubility, permeability through Caco-2 cell layers, and intestinal absorption in humans were presented among the reported absorption parameters. These properties are all crucial to the medication absorption process. Another crucial aspect of oral administering of the medications is their degree of solubility in the body, particularly in the intestinal fluid, since insufficient solubility might impede or prevent intestinal absorption of the drug via the portal vein system [33]. Based on the results presented in the Table, acyl alizarin derivative compounds have lower water solubility values than alizarin compounds. It is explained by the longer carbon chain that decreases the solubility of a compound in water because of its non-polar properties. Furthermore, the $CaCO_2$ permeability value possessed by these compounds feature shows a high value (>0.90 logPapp). When administering medications orally, the Caco-2 cell monolayer is frequently used as an *in vitro* model of the human intestinal mucosa to prognosticate the rate of absorption. The high result suggests that the $CaCO_2$ membrane is highly permeable for alizarin chemicals and acyl alizarin derivatives. The intestinal absorption parameter estimates the proportion of a medicine that is absorbed through the human intestine, which is often the major route of absorption for drugs taken orally. The investigated substances have an intestinal absorption value of >30%, thus indicating that the gut can effectively absorb them, according to the data obtained. When it comes to oral medications, they have to be capable to pass through the biological membranes in the gastrointestinal tract in order to enter the bloodstream. Transporter-mediated pathways, paracellular diffusion, and transcellular diffusion are some of the ways that penetration can occur through [34].

The drug distribution process involves the movement of the drug after absorption into various bodily compartments, including the spaces such as the interstitial and intracellular areas. This distribution process is vital, as it leads to the target organ exposure to the administered drug, thus ensuring its impact [34]. Distribution involves the transfer of the drug from the general bloodstream to bodily tissues. It's necessary for the drug to be spread over the target location where it exerts its intended effect in a potent concentration to bring about therapeutic benefits. The distribution process primarily revolves around the blood circulatory system, with minor lymphatic glands engagement, distributing drugs throughout tissues, except for the brain and testes due to their membrane barriers. Consequently, the dosage needed can be influenced by the varying blood flow rates to different tissues [35].

The distribution-related characteristics, that were observed, included the blood-brain barrier's permeability and distribution volume, both of which play significant roles in the overall mechanism of drug's distribution. Volume known as VDss, which is uniformly present in blood plasma, is necessary for processing of the entire dosage of a medicine. In cases of the larger VDss, the drug will be more widely distributed in the tissue than in the plasma. If a compound's VDss value is more than 0.45, it has a good distribution. Meanwhile the log VDss<–0.15 and log VDss>–0.45, VDss is evaluated as low and high, respectively. The more the medicine is dispersed in tissue as opposed to plasma, the higher the volume of distribution value. Alizarin compounds and acyl alizarin derivatives have low VDss values (logVDss < –0.15), according to the data collected. This suggests that it is challenging for these substances to disperse throughout

Table 5. ADMET properties prediction of alizarin and acyl alizarin derivatives predicted using the pkCSM online tool

Таблица 5. **Прогнозирование ADMET свойств производных ализарина и ацилализарина, спрогнозированных с помощью онлайн-инструмента pkCSM**

Code	Absorption			Distribution		Metabolism		Excretion		Toxicity		
	Water solubility (logS)	$CaCO_2$ permeability (logPapp)	HIA (%)	VDss (Human) (log L/kg)	BBB Permeability (logBB)	CYP2D6 substrate	CYP3A4 substrate	Renal OCT Substrate	Total Clearance (log ml/min/kg)	Rat LD_{50} (mol/kg)	AMES Toxicity	Hepatotoxicity
A	–2.705	1.027	94.268	–0.146	–0.081	No	No	No	0.095	2.238	Yes	No
AA1	–3.793	1.102	98.656	–0.297	–0.142	No	No	No	0.274	2.71	Yes	Yes
AA2	–4.326	1.062	98.365	–0.182	–0.153	No	No	No	0.314	2.746	Yes	No
AA3	–5.634	0.95	95.542	–0.045	–0.177	No	Yes	No	1.189	2.723	No	No
AA4	–5.114	1.015	96.579	–0.076	–0.161	No	Yes	No	0.368	2.754	Yes	No
AA5	–4.867	1.031	96.936	–0.107	–0.158	No	Yes	No	0.349	2.754	Yes	No
AA6	–5.504	0.972	95.886	–0.045	–0.172	No	Yes	No	1.165	2.741	No	No
AA7	–5.329	0.994	96.229	–0.055	–0.167	No	Yes	No	1.142	2.751	No	No
AA8	–4.601	1.042	97.531	–0.143	–0.158	No	No	No	0.331	2.752	Yes	No
AA9	–4.054	1.091	98.746	–0.237	–0.145	No	No	No	0.298	2.731	Yes	Yes

the tissues. Compounds that have a low volume of distribution cause the compound to be more likely accumulate in the intravascular compartment so that the dose required is not high to achieve the target therapeutic concentration [36]. The degree at which a medication binds blood proteins can affect the degree of its efficiency; the more bound the medication is, the less effectively it can diffuse or cross cellular membranes.

Furthermore, blood-brain barrier (BBB) permeability is an important parameter to consider as it is used to help reduce the side effects and toxicity as well as increase the therapeutic effect of the drug in the brain. Molecule must cross the BBB by transcellular passive diffusion and/or due to active transport mechanisms [37]. The logarithmic ratio of brain to plasma drug concentrations is used to calculate the rate of permeability. The value obtained in this parameter indicates that the drug easily passes through the blood brain barrier if logBB > 0.3 is obtained, while logBB value < −1 indicates that the drug is not well supplied to the brain. Based on the values obtained it is shown that alizarin compounds and acyl alizarin derivatives have a log BB value < −1 which indicates these compounds cannot be properly distributed to the brain. This means that these compounds do not affect the brain so they are relatively safer to administer because the concentration of compounds distributed to the brain is estimated to be little [25].

Drug metabolism is a chemical modification of drugs into their metabolites, which can be divided into active metabolites, inactive metabolites, and toxic metabolites [38,39]. Active metabolites are biochemically active compounds that have therapeutic effects. Furthermore, inactive metabolites are biochemically inactive compounds that do not have therapeutic effects or are toxic. Meanwhile, toxic metabolites are biochemically active compounds that are similar to active metabolites, but feature distinctive harmful effects [40]. Drug metabolism is very important for the pharmacokinetic process of drugs because drugs are chemical substances that are not produced by the body (xenobiotics) so they must be metabolized to reduce their toxicity and easily excreted [38,39]. The metabolic parameters analyzed were CYP2D6 substrate and CYP3A4 substrate, which have important roles in the process of drug metabolism. Based on the data obtained, it is known that there are no acyl alizarin derivatives compounds that can be metabolized by CYP2D6 substrates. However, compounds from AA3 to AA7 can be metabolized by CYP3A4 substrates.

Drug excretion is the process of removing the drugs from the body, both those that are not fully metabolized, as well as the results of metabolic biotransformation [40,41]. This excretion process mainly occurs via the kidneys, but other organ systems are also involved [42,43]. The excretion parameters observed were renal OCT2 substrate which holds an important role in the drug excretion process in the kidneys, and total clearance which is a combination of hepatic clearance and renal clearance. Renal OCT2 substrate also has the potential to provide adverse interactions with OCT2 inhibitors when administered at the same time, such as cimetidine, trimethoprim, ranitidine, levofloxacin, and propranolol. Meanwhile, total clearance has an important role in determining the dosing rate in order to obtain steady status levels [30].

Based on the data obtained it was found that all tested compounds are not the substrates of OCT2 so it can be predicted that there is no interaction with compounds that act as OCT2 inhibitors. Meanwhile, the total clearance value of acyl derivatives of alizarin is greater than the total clearance value of alizarin, so it can be prognosticated that all of these compounds are more easily excreted than alizarin compounds.

Hepatotoxicity, AMES toxicity, and rat LD_{50} can all be used to quantify toxicity parameters. The common way to estimate acute toxicity and compare the relative toxicity of several compounds is to use the lethal dosage values, or LD_{50}. The amount of chemical administered all at once that results in 50% of the test group animal death is known as the LD_{50}. The model estimates the LD_{50} in mol/kg and was developed using more than 1000 chemicals that were evaluated in rats. Rats were expected to have an alizarin LD_{50} of 2.238 mol/kg, whereas the generated compounds had a range of 2.71 to 2.731 mol/kg. This proves that the alizarin derivative compound is not more hazardous than the alizarin itself. The AMES test is a commonly used technique to evaluate a compound's potential for mutagenicity tested on the array of bacteria. A positive test results in the substances being mutagenic mean they could cause cancer. The table shows that the compounds A, AA1, AA2, AA4, AA5, AA8, and AA9 are the carcinogenic compounds, while the compounds AA3, AA6, and AA7 are not carcinogenic. Drug-induced hepatotoxicity is a significant safety issue in the drug research and a leading reason for the rejection of potential drugs. This model was composed on the base of an analysis of 531 drugs' liver-related side effects seen in human studies. If a substance induced at least one liver-related event that significantly interfered with the liver's normal functioning, it was classified as hepatotoxic. From the table it can be seen that AA1 and AA9 are hepatotoxic, while A, AA2, AA3, AA4, AA5, AA6, AA7, and AA8 are not hepatotoxic. From the toxicity parameter data obtained, it can be concluded that the acyl alizarin derivatives have high toxicity, but there are several derivatives that are not highly toxic, including AA3, AA6, and AA7.

The limitation of this research is that it is still prognostic the tests are still being run *in silico*. There is hope that the results of this research can serve as supporting data in determining which alizarin derivative compounds are suitable for their synthesis and *in vitro* preclinical testings to determine their cytotoxic activity.

4. Conclusion

Acyl alizarin derivatives have more potent anti-breast cancer activity than alizarin compounds. Apart from that, acyl alizarin derivative compounds also have better pharmacokinetic aspects, and some acyl alizarin derivatives are also less toxic than alizarin. From the results of this study, the best QSAR equation was also obtained: RS = 2.056log2P — 24.790logP — 1.713Etot + 48.719 which concluded that lipophilic and electronic characteristics are involved into the anti-breast cancer effect generated by alizarin derivatives. Additionally, acyl alizarin derivatives, especially alizarin octanoate, are recommended for their synthesis. Later the drug candidate should be tested *in vitro* or *in vivo* to determine its activity and toxicity for the living creatures.

REFERENCES

1. Hanahan, D., Weinberg, R.A. (2000). The Hallmarks of Cancer. *Cell*, 100(1), 57–70. https://doi.org/10.1016/S0092-8674(00)81683-9
2. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A. et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 71(3), 209–249. https://doi.org/10.3322/caac.21660
3. Yabluchanskiy, A, Ma, Y, Iyer, R.P., Hall, M.E., Lindsey, M.L. (2013). Matrix metalloproteinase-9: Many shades of function in cardiovascular disease. *Physiology,* 28(6), 391–403. https://doi.org/10.1152/physiol.00029.2013
4. Greenlee, K.J., Corry, D.B., Engler, D.A., Matsunami, R.K., Tessier, P., Cooc, R.G. et al. (2006). Proteomic identification of in vivo substrates for matrix metalloproteinases 2 and 9 reveals a mechanism for resolution of inflammation. *The Journal of Immunoljgy*, 177(10), 7312–7321. https://doi.org/10.4049/jimmunol.177.10.7312
5. Khandia, R, Munjal, A. (2020). Interplay between inflammation and cancer. Chapter in a book: Advances in Protein Chemistry and Structural Biology, 119, 199–245. https://doi.org/10.1016/bs.apcsb.2019.09.004
6. Kessenbrock, K., Plaks, V., Werb, Z. (2010). Matrix metalloproteinases: Regulators of the tumor microenvironment. *Cell*, 141(1), 52–67. https://doi.org/10.1016/j.cell.2010.03.015
7. Quintero-Fabián, S., Arreola, R., Becerril-Villanueva, E., Torres-Romero, J.C., Arana-Argáe, V., Lara-Riegos, J. et al. (2019). Role of matrix metalloproteinases in angiogenesis and cancer. *Frontiers in Oncology,* 9, Article 1370. https://doi.org10.3389/fonc.2019.01370
8. Nelson, A. R., Fingleton, B., Rothenberg, M. L., Matrisian, L. M. (2000). Matrix metalloproteinases: Biologic activity and clinical implications. *Journal of Clinical Oncology*, 18(5), 1135–1149. https://doi.org/10.1200/jco.2000.18.5.1135
9. Merdad, A., Karim, S., Schulten, H. J., Dallol, A., Buhmeida, A., Al-Thubaity, F. et al. (2014). Expression of matrix metalloproteinases (MMPs) in primary human breast cancer: MMP-9 as a potential biomarker for cancer invasion and metastasis. *Anticancer Research*, 34(3), 1355–1366.
10. Meng, Q., Liang, C., Hua, J., Zhang, B., Liu, J., Zhang, Y. et al. (2020). A miR-146a-5p/TRAF6/NF-kB p65 axis regulates pancreatic cancer chemoresistance: functional validation and clinical significance. *Theranostics*, 10(9), 3967–3979. https://doi.org/10.7150/thno.40566
11. Li, K., Zhang, Z., Mei, Y., Yang, Q., Qiao, S., Ni, C. et al. (2021). Metallothionein-1G suppresses pancreatic cancer cell stemness by limiting activin A secretion NF-κB inhibition. *Theranostics*, 11(7), 3196–2212. https://doi.org/10.7150/thno.51976
12. Lindenmeyer, F., Legrand, Y., Menashi, S. (1997). Upregulation of MMP-9 expression in MDA-MB231 tumor cells by platelet granular membrane. *FEBS Letters*, 418(1–2), 19–22. https://doi.org/10.1016/s0014-5793(97)01336-7
13. Mishra, S. R., Nandhakumar, P., Yadav, K. P., Barik, S., Kumar, A., Saini, M. et al. (2017). In vitro analysis of alizarin as novel therapeutic agent for murine breast cancer. *The Pharma Innovation Journal*, 6(10), 345–350.
14. Ekowati, J., Diyah, N. W., Nofianti, K. A., Hamid, I. S., Siswandono (2018). Molecular Docking of Ferulic Acid Derivatives on P2Y12 Receptor and their ADMET Prediction. *Journal of Mathematical and Fundamental Sciences*, 50(2), 203–219. https://doi.org/10.5614/j.math.fund.sci.2018.50.2.8
15. Kamath, V., Pai, A. (2017). Application of molecular descriptors in modern computational drug design-an overview. *Research Journal of Pharmacy and Technology*, 10(9), 3237–3241. http://doi.org/10.5958/0974-360X.2017.00574.1
16. Habeela, J.N., Maruga, R.M.K.M. (2018). In silico molecular docking studies on the chemical constituents of clerodendrum phlomidis for its cytotoxic potential against breast cancer markers. *Research Journal of Pharmacy and Technology*, 11(4), Article 1612–1618. http://doi.org/10.5958/0974-360X.2018.00300.1

17. Hardjono, S. (2012). Modification of the structure of 1 — (benzoyloxy) ureaand quantitative relationship of its structure-cytotoxic activity. Author's abstract of the thesis. Universitas Airlangga, Indonesia, 2012. (In Indonesian)
18. Hardjono, S., Siswodihardjo, S., Pramono, P., Darmanto, W. (2016). Quantitative structure-cytotoxic activity relationship 1-(benzoyloxy) urea and its derivative. *Current Drug Discovery Technologies*, 13(2), 101–108. https://doi.org/10.2174%2F1570163813666160525112327
19. Pinzi, L., Rastelli, G. (2019). Molecular docking: Shifting paradigms in drug discovery. *International Journal of Molecular Sciences*, 20(18), Article 4331. https://doi.org/10.3390/ijms2018433
20. Park, K. D., Lee, S. G., Kim, S. U., Kim, S. H., Sun, W. S., Cho, S. J. et al. (2004). Anticancer activity of 3-O-acyl and alkyl-(−)-epicatechin derivatives. *Bioorganic and Medicinal Chemistry Letters*, 14(20), 5189–5192. https://doi.org/10.1016/j.bmcl.2004.07.063
21. Hoque, I., Chatterjee, A., Bhattacharya, S., Biswas, R. (2017). An approach of computer-aided drug design (CADD) tools for *in silico* pharmaceutical drug design and development. *International Journal of Advanced Research in Biological Sciences*, 4(2), 60–71. http://doi.org/10.22192/ijarbs.2017.04.02.009
22. Abdel-Ilah, L., Veljović, E., Gurbeta, L., Badnjević, A. (2017). Applications of QSAR study in drug design. *International Journal of Engineering Research and Technology (IJERT)*, Vol. 6(6), 582–587.
23. Verma, J., Khedkar, V. M., Coutinho, E. C. (2010). 3D-QSAR in drug design-a review. *Current Topics in Medicinal Chemistry*, 10(1), 95–115. https://doi.org/10.2174/156802610790232260
24. Pathan, S., Ali, S. M., Shrivastava, M. (2016). Quantitative structure activity relationship and drug design: A review. *International Journal of Research in Biosciences*, 5(4), 1–5.
25. Pires, D. E. V., Blundell, T. L., Ascher, D. B. (2015). pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *Journal of Medicinal Chemistry*, 58(9), 4066–4072. https://doi.org/10.1021/acs.jmedchem.5b00104
26. Pagadala, N. S., Syed, K., Tuszynski, J. (2017). Software for molecular docking: A review. *Biophysical Reviews*, 9(2), 91–102. https://doi.org/10.1007/s12551-016-0247-1
27. Ramírez, D., Caballero, J. (2018). Is it reliable to take the molecular docking top scoring position as the best solution without considering available structural data? *Molecules*, 23(5), Article 1038. https://doi.org/10.3390/molecules23051038
28. Patlewicz, G., Jeliazkova, N., Safford, R. J., Worth, A. P., Aleksiev, B. (2008). An evaluation of the implementation of the Cramer classification scheme in the Toxtree software. *SAR and QSAR in Environmental Researc,* 19(5–6), 495–524. https://doi.org/10.1080/10629360802083871
29. McMurry, J., E., Fay, R., C. (2012). Chemistry. Boston: Prentice Hall, 2012.
30. Klebe, G. (2013). Protein–Ligand Interactions as the Basis for Drug Action. Chapter in a book: Drug Design. Springer, Berlin, Heidelberg, 2013. https://doi.org/10.1007/978-3-642-17907-5_4
31. Grogan, S, Preuss, C.V. (2023). Pharmacokinetics. Chapter in a book: StatPearls [Internet]. StatPearls Publishing LLC., 2023.
32. Paul, A. (2019). Drug Absorption and Bioavailability. Chapter in a book: Introduction to Basics of Pharmacology and Toxicology. Springer, Singapore, 2019. https://doi.org/10.1007/978-981-32-9779-1
33. Chevillard, F., Lagorce, D., Reynès, C., Villoutreix, B. O., Vayer, P., Miteva, M. A. (2012). *In silico* prediction of aqueous solubility: A multimodel protocol based on chemical similarity. *Molecular Pharmaceutics*, 9(11), 3127–3135. https://doi.org/10.1021/mp300234q
34. Gleeson, M. P. (2008). Generation of a set of simple, interpretable ADMET rules of thumb. *Journal of Medicinal Chemistry*, 51(4), 817–834. https://doi.org/10.1021/jm701122q
35. Currie, G. M. (2018). Pharmacology, part 2: Introduction to pharmacokinetics. *Journal of Nuclear Medicine Technology*, 46(3), 221–230. https://doi.org/10.2967/jnmt.117.199638
36. Smith, D. A., Beaumont, K., Maurer, T. S., Di, L. (2015). Volume of distribution in drug design. Miniperspective. *Journal of Medicinal Chemistry*, 58(15), 5691–5698. https://doi.org/10.1021/acs.jmedchem.5b00201
37. Jeffrey, P., Summerfield, S. (2010). Assessment of the blood–brain barrier in CNS drug discovery. *Neurobiology of Disease*, 37(1), 33–37. https://doi.org/10.1016/j.nbd.2009.07.033
38. Wilde, M., Pichini, S., Pacifici, R., Tagliabracci, A., Busardò, F. P., Auwärter, V. et al. (2019). Metabolic Pathways and Potencies of New Fentanyl Analogs. *Frontiers in pharmacology*, 10, Article 238. https://doi.org/10.3389%2Ffphar.2019.00238
39. Rizzieri, D., Paul, B., Kang, Y. (2019). Metabolic alterations and the potential for targeting metabolic pathways in the treatment of multiple myeloma. *Journal of Cancer Metastasis and Treatment*, 5, 26. https://doi.org/10.20517/2394-4722.2019.05
40. Garza, A. Z., Park, S. B., Kocz, R. (2023). Drug Elimination. Chapter in a book: StatPearls [Internet]. StatPearls Publishing LLC., 2023.
41. Herdiansyah, M. A., Ansori, A. N. M., Kharisma V. D., Alifiansyah, M. R. T., Anggraini, D., Priyono, Q. A. P., Yusniasari, P. A., Fetty, A. J. T., Zainul, R., Rebezov, M., Kolesnik, E., Maksimiuk, N. (2024). *In silico* study of cladosporol and its acyl derivatives as anti-breast cancer against alpha-estrogen receptor. *Biosaintifika*, 15(1), 1–13.
42. Zainul, R., Kharisma, V. D., Ciuputri, P., Ansori, A. N. M., Herdiansyah, M. A., Sahadewa, S., Durry, F. D. (2024). Antiretroviral activity from elderberry (*Sambucus nigra* L.) flowers against HIV-2 infection via reverse transcriptase inhibition: A viroinformatics study. *Healthcare in Low-resource Settings*, 1(2024), 1–12. https://doi.org/10.4081/hls.2024.12047
43. Krihariyani, D., Haryanto, E., Sasongkowati, R. (2021). *in silico* analysis of antiviral activity and pharmacokinetic prediction of brazilein sappan wood (*Caesalpinia sappan* L.) against SARS-CoV-2 spike glycoproteins. *Indonesian Journal of Medical Laboratory Science and Technology*, 3(1), 26–37. https://doi.org/10.33086/ijmlst.v3i1.1854

AUTHOR INFORMATION	СВЕДЕНИЯ ОБ АВТОРАХ
Affiliation	**Принадлежность к организации**
Mochamad R. T. Alifiansyah, Bachelor of Pharmacy, Department of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia Tel.: +628–573–042–07–56 E-mail: mochamad.radika.tory-2020@ff.unair.ac.id ORCID: http://orcid.org/0009-0009-3873-3756	**Алифиансия Мохамад Радика Тори** — бакалавр фармакологии, кафедра фармацевтических наук, фармацевтический факультет, Университет Айрлангга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, ул. Мулиорехо Тел.: +628–573–042–07–56 E-mail: mochamad.radika.tory-2020@ff.unair.ac.id ORCID: http://orcid.org/0009-0009-3873-3756
Mochammad A. Herdiansyah, Bachelor of Biology, Department of Biology, Faculty of Science and Technology, Universitas Airlangga Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia Tel.: +628–880–700–02–22 E-mail: aqilahherdiansyah123@gmail.com ORCID: http://orcid.org/0009-0006-9383-6203	**Хердиансиах Мохаммад Акилах** — бакалавр биологии, отделение биологии, факультет науки и технологии, Университет Айрлангга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, ул. Мулиорехо E-mail: aqilahherdiansyah123@gmail.com ORCID: http://orcid.org/0009-0006-9383-6203
Rahma C. Pratiwi, Bachelor of Pharmacy, Department of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia Tel.: +628–121–743–58–26 E-mail: rahma.cintya.pratiwi-2020@ff.unair.ac.id ORCID: http://orcid.org/0009-0001-1673-8646	**Пративи Рахма Синтия** — бакалавр фармакологии, кафедра фармацевтических наук, фармацевтический факультет, Университет Айрлангга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, ул. Мулиорехо Тел.: +918–126–00–96–20 E-mail: rahma.cintya.pratiwi-2020@ff.unair.ac.id ORCID: http://orcid.org/0009-0001-1673-8646
Ragil P. Pramesti, Bachelor of Pharmacy, Department of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia Tel.: +628–533–419–33–18 E-mail: ragil.putri.pramesti-2020@ff.unair.ac.id ORCID: http://orcid.org/0009-0002-0018-6565	**Прамести Рагил Путри** — бакалавр фармакологии, кафедра фармацевтических наук, фармацевтический факультет, Университет Айрлангга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, ул. Мулиорехо Тел.: +628–533–419–33–18 E-mail: ragil.putri.pramesti-2020@ff.unair.ac.id ORCID: http://orcid.org/0009-0002-0018-6565
Nisriena W. Hafsyah, Bachelor of Pharmacy, Department of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia Tel.: +628–223–034–06–86 E-mail: nisriena.wijdan.hafsyah-2020@ff.unair.ac.id ORCID: http://orcid.org/0009-0007-3955-5336	**Хафсиах Нисриена Вийдан** — бакалавр фармакологии, кафедра фармацевтических наук, фармацевтический факультет, Университет Айрлангга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, ул. Мулиорехо Тел.: +628–223–034–06–86 E-mail: nisriena.wijdan.hafsyah-2020@ff.unair.ac.id ORCID: http://orcid.org/0009-0007-3955-5336

AUTHOR INFORMATION	СВЕДЕНИЯ ОБ АВТОРАХ
Affiliation	**Принадлежность к организации**
Athaya P. Rania, Bachelor of Pharmacy, Department of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia Tel.: +628–223–329–02–94 E-mail: athaya.putri.rania-2020@ff.unair.ac.id ORCID: http://orcid.org/0009-0005-7772-9769	**Рания Атайя Путри** — бакалавр фармакологии, кафедра фармацевтических наук, фармацевтический факультет, Университет Айрлангга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, ул. Мулиорехо Тел.: +628–223–329–02–94 E-mail: athaya.putri.rania-2020@ff.unair.ac.id ORCID: http://orcid.org/0009-0005-7772-9769
Justitia E. R. P. Putra, Bachelor of Biology, Department of Biology, Faculty of Science and Technology, Universitas Airlangga Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia Tel.: +628–122–612–38–60 E-mail: justitia.eka.rizqi-2020@fst.unair.ac.id ORCID: http://orcid.org/0009-0000-7537-4734	**Путра Джустиция Эка Ризки Пердана** — бакалавр биологии, отделение биологии, факультет науки и технологии, Университет Айрлангга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, ул. Мулиорехо Тел.: +628–122–612–38–60 E-mail: justitia.eka.rizqi-2020@fst.unair.ac.id ORCID: http://orcid.org/0009-0000-7537-4734
Putri A. Cahyono, Bachelor of Biology, Department of Biology, Faculty of Science and Technology, Universitas Airlangga Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia Tel.: +628–180–373–91–28 E-mail: putri.aminanda.cahyono-2022@fst.unair.ac.id ORCID: http://orcid.org/0009-0008-3575-1126	**Кахионо Путри Аминанда** — бакалавр биологии, отделение биологии, факультет науки и технологии, Университет Айрлангга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, ул. Мулиорехо Тел.: +628–180–373–91–28 E-mail: putri.aminanda.cahyono-2022@fst.unair.ac.id ORCID: http://orcid.org/0009-0008-3575-1126
Litazkiyyah, Bachelor of Biology, Department of Biology, Faculty of Science and Technology, Universitas Airlangga Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia Tel.: +628–579–946–30–57 E-mail: litazkiyyah-2021@fst.unair.ac.id ORCID: http://orcid.org/0009-0002-7872-1687	**Литацкия,** бакалавр биологии, отделение биологии, факультет науки и технологии, Университет Айрлангга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, ул. Мулиорехо Тел.: +628–579–946–30–57 E-mail: litazkiyyah-2021@fst.unair.ac.id ORCID: http://orcid.org/0009-0002-7872-1687
Sirojuddin K. Muhammad, Bachelor of Biomedical Engineering, Department of Physics, Faculty of Science and Technology, Universitas Airlangga Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia Tel.: +628–151–578–86–71 E-mail: sirojudin-2020@fst.unair.ac.id ORCID: http://orcid.org/0009-0005-3031-4099	**Мухаммад Сироджуддин Холил** — бакалавр биомедицинской инженерии, кафедра физики, факультет науки и технологии, Университет Айрлангга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, ул. Мулиорехо Тел.: +628–151–578–86–71 E-mail: sirojudin-2020@fst.unair.ac.id ORCID: http://orcid.org/0009-0005-3031-4099
Ahmad A. A. Murtadlo, Bachelor of Biology, Master Student, Department of Biology, Faculty of Science and Technology, Universitas Airlangga Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia Tel.: +628–581–653–43–15 E-mail: ahmad.affan.ali-2023@fst.unair.ac.id ORCID: http://orcid.org/0000-0002-7942-875X	**Ахмад Аффан Али Муртадло** — бакалавр биомедицинской инженерии, магистрант кафедры биологии факультета науки и технологий, Университет Айрлангга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, ул. Мулиорехо Тел.: +628–581–653–43–15 E-mail: ahmad.affan.ali-2023@fst.unair.ac.id ORCID: http://orcid.org/0000-0002-7942-875X
Viol D. Kharisma, Master of Science, PhD Student, Department of Biology, Faculty of Science and Technology, Universitas Airlangga Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia Tel.: +628–121–78–70–02 E-mail: viol.dhea.kharisma-2022@fst.unair.ac.id ORCID: https://orcid.org/0000-0001-9060-0429	**Харишма Виол Дхеа** — магистр наук, аспирант, кафедра биологии, факультет науки и техники, Университет Айрланга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, ул. Мулиорехо Тел.: +628–121–78–70–02 E-mail: viol.dhea.kharisma-2022@fst.unair.ac.id ORCID: https://orcid.org/0000-0001-9060-0429
Arif N. M. Ansori, Ph.D. in Veterinary Science, Researcher, Postgraduate School, Universitas Airlangga Jl. Airlangga 4–6, Surabaya, East Java, 60115, Indonesia Tel.: +628–214–464–78–32 E-mail: ansori.anm@gmail.com ORCID: https://orcid.org/0000-0002-1279-3904 * corresponding author	**Анзори Ариф Нур Мухаммад** — PhD, Доктор ветеринарных наук, научный сотрудник аспирантуры, Университет Айрлангга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, Джл. Айрлангга 4–6 Тел.: +628–214–464–78–32 E-mail: ansori.anm@gmail.com ORCID: https://orcid.org/0000-0002-1279-3904 * автор для контактов
Vikash Jakhmola, Professor, Professor, Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University Chakrata Rd, Prem Nagar, Dehradun, Uttarakhand, 248007, India Tel.: +918–126–00–96–20 E-mail: jakhmola.1979@gmail.com ORCID: https://orcid.org/0000-0002-8108-006X	**Яхмола Викаш** — профессор, Уттаранчальский институт фармацевтических наук, Уттаранчальский университет 248007, Индия, Уттаракханд, Дехрадун, ул. Чакрата Тел.: +918–126–00–96–20 E-mail: jakhmola.1979@gmail.com ORCID: https://orcid.org/0000-0002-8108-006X
Praveen K. Ashok, PhD in Pharmaceutical Sciences, Lecturer, Gyani Inder Singh Institute of Professional Studies Sinola, Dehradun, Uttarakhand, 248003, India Tel.: +91–941–169–90–52 E-mail: pkashok157@gmail.com ORCID: http://orcid.org/0000-0001-7150-3981	**Ашок Правин Кумар** — доктор фармацевтических наук, преподаватель, Институт профессиональных исследований Гьяни Индер Сингха 248003, Индия, Уттаракханд, Синола, Дехрадун, Тел.: +91–941–169–90–52 E-mail: pkashok157@gmail.com ORCID: http://orcid.org/0000-0001-7150-3981
Jyoti Maithani Kalra, PhD in Pharmaceutical Sciences, Lecturer, School of Pharmaceutical Sciences, Shri Guru Ram Rai University Dehradun, Uttarakhand, 248001, India Tel.: +91–989–752–65–68 E-mail: jyotimk123@gmail.com ORCID: http://orcid.org/0000-0002-4960-6551	**Калра Джьоти Майтани** — доктор фармацевтических наук, преподаватель Школы фармацевтических наук Университета Шри Гуру Рам Рай 248001, Индия, Дехрадун, Уттаракханд Тел.: +91–989–752–65–68 E-mail: jyotimk123@gmail.com ORCID: http://orcid.org/0000-0002-4960-6551
Hery Purnobasuki, PhD in Biology, Professor, Department of Biology, Faculty of Science and Technology, Universitas Airlangga Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia Tel.: +08–170–385–08–79 E-mail: hery-p@fst.unair.ac.id ORCID: http://orcid.org/0000-0002-0562-2058 * corresponding author	**Пурнобасуки Хери** — доктор биологических наук, профессор кафедры биологии факультета науки и технологий Университета Эйрлангга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, Джл. Айрлангга 4–6 Тел.: +08–170–385–08–79 E-mail: hery-p@fst.unair.ac.id ORCID: http://orcid.org/0000-0002-0562-2058 * автор для контактов

AUTHOR INFORMATION	СВЕДЕНИЯ ОБ АВТОРАХ
Affiliation	**Принадлежность к организации**
Intan A. Pratiwi, Master in Biology, Lecturer, Department of Biology, Faculty of Science and Technology, Universitas Airlangga Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia Tel.: +08–573–250–18–61 E-mail: intan.ayu.pratiwi@fst.unair.ac.id ORCID: http://orcid.org/0000-0003-0362-5113	**Пративи Интан Аю** — магистр биологии, преподаватель, профессор кафедры биологии факультета науки и технологий Университета Эйрлангга 60115, Индонезия, Восточная Ява, Сурабая, округ Мулиорехо, Джл. Айрлангга 4–6 Тел.: +08–57–325–018–61 E-mail: intan.ayu.pratiwi@fst.unair.ac.id ORCID: http://orcid.org/0000-0003-0362-5113
Contribution	**Критерии авторства**
Authors are equally relevant to the writing of the manuscript and equally responsible for plagiarism.	Авторы в равных долях имеют отношение к написанию рукописи и одинаково несут ответственность за плагиат
Conflict of interest	**Конфликт интересов**
The authors declare no conflict of interest.	Авторы заявляют об отсутствии конфликта интересов.