ALCHEMY Jurnal Penelitian Kimia

Official Website: https://jurnal.uns.ac.id/alchemy

ISSN 1412-4092
EISSN 2443-4183

Synthesis and Molecular Docking Study of 4-(3-(2-Chlorophenyl)-5-(2-Methoxyphenyl)-4,5-Dihydro-1*H*-Pyrazol-1-yl) Benzenesulfonamide as Antibreast Cancer Agent

Eka Marisa Putri[a], Noval Herfindo[b], Guntur Guntur[a], Neni Frimayanti[b], Adel Zamri[a*]

[a]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Riau*
Jalan H.R. Subrantas Km. 12.5, Pekanbaru, 28293, Indonesia
[b]*Department of Pharmacy, Sekolah Tinggi Ilmu Farmasi Riau*
Jalan Kamboaja, Pekanbaru, 28289, Indonesia

[*]*Corresponding author: adel.zamri@lecturer.unri.ac.id*

DOI: 10.20961/alchemy.18.1.48298.30-36

Received 04 February 2021, Accepted 03 August 2021, Published 01 March 2022

Keywords:
anticancer;
molecular docking;
pyrazoline;
sulfonamide;
tyrosine kinase.

ABSTRACT. Breast cancer is a disease in which cells in the breast tissue change and divide in an uncontrolled way. Pyrazoline is a promising agent reported against cancer. In this work, we have synthesized pyrazoline 4-(3-(2-chlorophenyl)-5-(2-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) benzenesulfonamide (**EMP-1**). The reaction was successfully carried out in one-pot three components from 2-chloroacetophenone, 2-methoxybenzaldehyde, and 4-hydrazinylbenzenesulfonamide as starting materials. The reaction was conducted by assisting the irradiation of Monowave 50 (Anton-Paar) with a high yield of 91%. Its potential anti-breast cancer was investigated by molecular docking and dynamic studies. The molecular docking study showed that **EMP-1** had binding energy of -7.17 kcal/mol. The spatial arrangement of **EMP-1** was similar to the positive control of doxorubicin. These results indicate that **EMP-1** compound potentially developed as anti-breast cancer.

INTRODUCTION

Breast cancer is a disease in which cells in the breast tissue divide in an uncontrolled way and change. Based on the annual report by WHO, in 2018, an estimated 18.1 million people worldwide had cancer, and 9.6 million died because of it, of which 11.6% cases were caused by breast cancer (WHO, 2018). Then, in 2020, breast cancer is the most common cancer in American women, with an estimated 276,480 cases and 42,170 of them were died because of this cancer (ACS, 2020). Human epidermal growth factor receptor HER-2 is one of tyrosine kinase. Activation of HER-2 causes conversion in gene expressions, including transcription, protein stability, and translation conversions. These can affect cell growth and proliferation (Meric-Bernstam *and* Hung, 2006). In breast cancer cases, HER-2 was over-expressed at 15% – 25% (Piccart-Gebhart *et al.*, 2005). Tyrosine Kinase Inhibitor (TKI) can be a treatment for this over-expression through HER-2 pathway. Lapatinib is used as TKI inhibitor in the treatment of breast cancer. However, like other commercial drugs, it has side effects such as diarrhea and vomiting (Higa *et al.*, 2007). Therefore, the study to find new or alternative anticancer agents via HER-2 pathway as a TKI inhibitor is necessary to develop.

Pyrazolines become privileged scaffolds with many biological activities (Varghese *et al.*, 2017). Their biological activities have been investigated, such as antidiabetic (Jasril *et al.*, 2019), antiviral (Havrylyuk *et al.*, 2013), anticancer (Adamus-Grabicka *et al.*, 2020), antimicrobial (Cuartas *et al.* 2020), antioxidant (Teruna *et al.*, 2019). These indicate that pyrazolines have promising agents in the medicinal chemistry field. Moreover, some patented pyrazoline was reported against colorectal cancer (Altisen *et al.*, 2007). The most simple pathway to synthesis pyrazoline is from α,β-unsaturated ketone with hydrazine derivates under reflux. Some literature reported the modification method using ultrasound irradiation (Ji-Tai *et al.*, 2007) and microwave irradiation (Jasril *et al.*, 2016).

In previous work, we have synthesized 1,3,5-triaryl pyrazole with anti-proliferative activity against MCF-7 cell line (Herfindo *et al.*, 2020). However, that work showed that the compounds are not active as anti-proliferative.

Cite this as: Putri, E., Herfindo, N., Guntur, G., Frimayanti, N., & Zamri, A., 2021. Synthesis and Molecular Docking Study of 4-(3-(2-Chlorophenyl)-5-(2-Methoxyphenyl)-4,5-Dihydro-1H-Pyrazol-1-yl) Benzenesulfonamide as Antibreast Cancer Agent. *ALCHEMY Jurnal Penelitian Kimia, 18*(1), 30-36. http://dx.doi.org/10.20961/alchemy.18.1.48298.30-36.

Thus, based on the literature above, we are interested in synthesizing pyrazoline as an anticancer agent. In this work, we used sulfonamide and chlorine substitutions to increase its anticancer activity. This modification was based on the pharmacophore of sulfonamide and chlorine substituents on indisulam. Ozawa *et al.* reported indisulam (E7070) bearing sulfonamide and chlorine moiety showed potential as anticancer drug candidate (Ozawa *et al.*, 2012). Based on indisulam, the sulfonamide group possibly creates the hydrogen bond, which is important for interaction among receptor and ligand. In addition, electron-withdrawing groups such as chlorine can make ligand-binding pockets around protein. The modification is illustrated in Figure 1.

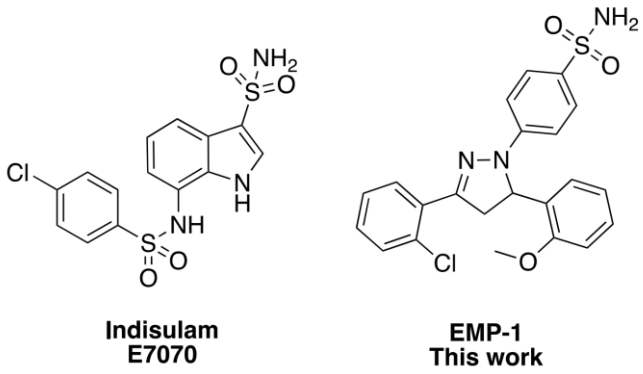

Figure 1. Design of targeted pyrazoline compounds.

Therefore, this research aims to synthesize pyrazoline (**EMP-1**) from 3-chloroacetophenone, 2-methoxybenzaldehyde, and 4-hydrazinylbenzenesulfonamide in one-pot three component reaction with a high yield. The structure of the compound synthesized is determined by IR, MS, ^{1}H, and ^{13}C NMR spectrum analysis. Further study, its anti-breast cancer activity is evaluated through molecular docking and dynamic molecular studies to receptor PDB ID: 1TP6.

EXPERIMENTAL

Materials and Instrumentation

All reagents used in this research were purchased from Merck and Sigma Aldrich (for synthesis grade) and were used without further purification. The synthesis reaction was carried out using a sealed-vessel reactor Monowave 50 (Anton-Paar, Graz, Austria). The melting point was determined on a Fisher-Johns apparatus (Fisher Scientific, Waltham, MA, USA) (uncorr). Thin Layer Chromatography (TLC) analysis was carried out using GF254 (Merck Millipore, Darmstadt, Germany) under an UV lamp 254/365 nm (Cole-Elmer®, Vernon Hills, IL, USA). The UV spectrum was recorded on a Genesys™ 10S UV–visible spectrophotometer (Thermo Scientific™, Waltham, MA, USA). The FTIR spectrum was recorded in KBr powder on a Shimadzu® FTIR Prestige-21 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The Mass spectrum was measured using Water Xevo QTOFMS instrument (Waters, Milford, MA, USA). The ^{1}H and ^{13}C NMR spectra were recorded on an Agilent® (Agilent Technologies, Santa Clara, CA, USA) at 500 MHz and 125 MHz, respectively.

Procedure

Synthesis of 4-hydrazinylbenzenesulfonamide

Synthesis of 4-hydrazinylbenzenesulfonamide was conducted according to the previously reported method (Soliman, 1979). The sulfanilamide (20 mmol), crushed ice (20 mL), and concentrated hydrochloric acid (10 mL) were stirred and added by dropwise sodium nitrite (20 mmol) for 30 minutes. After the diazonium salt was formed, cold stannous chloride (10 g) in hydrochloric acid (10 mL) was added with vigorously stirring. The mixture reaction was left in an ice bath overnight. The precipitated 4-hydrazinylbenzenesulfonamide was filtered in vacuum and dried at room temperature.

Synthesis of 4-(3-(2-chlorophenyl)-5-(2-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl) benzenesulfonamide

A mixture of 2-chloroacetophenone (1 mmol), 2-methoxybenzaldehyde (1 mmol) and 4-hydrazinylbenzene sulfonamide (1.2 mmol) was dissolved in 5 mL of ethanol in a closed pressurized tube equipped with a stirbar. Then 1 mL of natrium hydroxide 1N was added to the mixture. The mixture was reacted using sealed-vessel reactor Monowave 50 at 80 °C for 3 hours. After the completion of the reaction, the mixture into beaker glass containing

crushed ice 20 g. The precipitate formed was filtered and washed with water followed by *n*-hexane. The solid products are recrystallized in methanol to obtain compound **EMP-1** as a yellow crystal (0.401 g, 91% yield). The characterization data of compound **EMP-1** are as follows: m.p. 212–214 °C; ^{1}H NMR (500 MHz, DMSO) δ 7.78 (dd, *J* =6.0, 3.5 Hz, 1H), 7.60 (d, *J* =8.7 Hz, 2H), 7.54 (dd, *J* =5.8, 3.5 Hz, 1H), 7.41 (dd, *J* =6.0, 3.5 Hz, 2H), 7.26 (t, *J* =7.7 Hz, 1H), 7.09 (d, *J* =8.2 Hz, 1H), 7.00 (d, *J* =8.6 Hz, 2H), 6.91 (d, *J* =7.2 Hz, 1H), 6.85 (t, *J* =7.4 Hz, 1H), 5.75 (dd, *J* =12.2, 5.0 Hz, 1H), 4.09 (dd, *J* =17.6, 12.2 Hz, 1H), 3.89 (s, 3H), 3.18 (dd, *J* =17.5, 5.0 Hz, 1H); ^{13C} NMR (126 MHz, DMSO) δ 156.6, 148.9, 146.1, 133.8, 131.6, 131.4, 131.1, 131.0, 130.9, 130.8, 129.5, 128.3, 127.8, 127.7, 126.9, 126.5, 123.6, 121.1, 119.1, 112.4, 112.1, 110.6, 57.9, 56.2, 55.3, 44.6. HRMS (ESI): m/z 442.0991, $[M + H]^+$ (calcd for $C_{22}H_{21}N_3O_3SCl$: 442.0992). FTIR (KBr) ῡ (cm−1): 3100 (m), 3066 (m), 3011 (s), 2837 (s), 1596 (s), 1507 (s), 1490 (s), 1332 (s), 1245 (s), 1157 (s). UV–vis (EtOH) λmax: 353 nm.

Molecular Docking

The protein structure was downloaded from the protein databank (www.pdb.org with PDB ID: 1T46), then the water molecules and initial ligands in the protein were removed. Further preparation was carried out by adding hydrogen atoms, polar only, and merged non-polar. A grid box of the protein structure was then achieved with a grid spacing of 1 Å, dimensions of 31 x 25 x 35 points along the x, y, and z-axes, and centered on the protein active site. Docking was performed using Autodock vina software packages (Frimayanti *et al.*, 2021).

Molecular Dynamic

The molecular dynamic was executed using NAMD (Nanoscale Molecular Dynamics program v 2.9), with CHARMM27 (Chemistry at Harvard Macromolecular Mechanics) was selected as the best force field. Achieved modeled protein was determined using TIP3P water box with 2.5 Å water layer for each direction of the coordinated structure.

NVT ensemble from 0 to 300 K over 100 ps was applied to heat the system gradually. MD simulations were conducted on 50 ns time scale for each system in an isothermal isobaric ensemble (NPT) with periodic boundary conditions. The coupling of temperature and pressure parameters were set at 1.0 ps. The coordinates were saved at every 0.1 ps during the sampling process. The simulations have performed some conformations for then they were used for further binding free energy calculations and decomposition process.

RESULTS AND DISCUSSIONS

Synthesis

The compound **EMP-1** has been successfully synthesized as Figure 1. The 4-hydrazinylbenzenesulfonamide **3** was prepared by diazonium reaction of sulfanilamide **1** using sodium nitrite in HCl to form diazonium salt intermediate **2**, followed by its reduction using stannous chloride to yield hydrazine **3** (Soliman, 1979). The targeted compound was obtained through a one-pot reaction of hydrazine **3**, 4-methoxybenzaldehyde **4**, and 2-chloroacetophenone **5** in a sealed-vessel reactor with 91% yield (Zamri *et al.*, 2021). The reaction mechanism of one-pot pyrazoline synthesis is initiated by the formation of chalcone compound as intermediate via Claisen-Schmidt reaction. Subsequently, the cyclization reaction occurs between chalcone with hydrazine to form pyrazoline compounds (Herfindo *et al.*, 2020).

The chemical structure of the title compound was confirmed by IR, NMR, and mass spectroscopy. IR spectrum showed no vibration at around 1650 cm^{-1} because of the absence of the C=O bond, which showed the α,β-unsaturated ketone of intermediate chalcone compound had been reacted into pyrazoline core. The presence of the sulfanilamide group was indicated by absorption at 3100 cm^{-1} of N–H vibration and 1157 cm^{-1} of S=O vibration. Additionally, the vibration of the methoxy group was observed at 1245 cm^{-1}.

Confirmation of the pyrazoline ring can be seen by the appearance of the ABX proton pattern in the upfield area. In this case, the protons H_A and H_B are protons that are in the geminal position of the pyrazoline ring methylene carbon, whereas H_X is the proton of the pyrazoline ring methine carbon. The peak of protons ABX was observed as doublet of doublets at δ_H 3.18 (dd, *J* =12.2, 5.0 Hz), δ_H 4.09 (dd, *J* =17.6, 12.2 Hz) and δ_H 5.75 (dd, *J* =17.5, 5.0 Hz), respectively. The difference in chemical shifts of AB protons is due to the anisotropic effect of the atoms attached to the chiral carbon atom. Moreover, a singlet signal at δ_H 7.00 showed the protons of the sulfanilamide group.

The existence of the pyrazoline ring was also confirmed by the ^{13}C NMR spectrum, where the spectrum of the compound 1,3,5-triaryl pyrazoline showed 25 signals in total. Specifically, the signal for the bonding of carbon atoms C=N was observed at δ_C 156.6 ppm. Furthermore, the signal at δ_C 65.17 ppm indicated methine carbon, and the signal at δ_C 44.65 ppm indicated methylene carbon of pyrazoline ring. The HRMS spectrum of **EMP-1** showed molecular ion peak as $[M+H]^+$ at m/z = 442.0991 with abundance 100%. This molecular weight corresponds to the calculated mass of $C_{22}H_{21}N_3O_3SCl$ with m/z = 442.0992. Based on this data, it was sure that the target compound was synthesized in accordance with the structure shown in the Scheme of Figure 2.

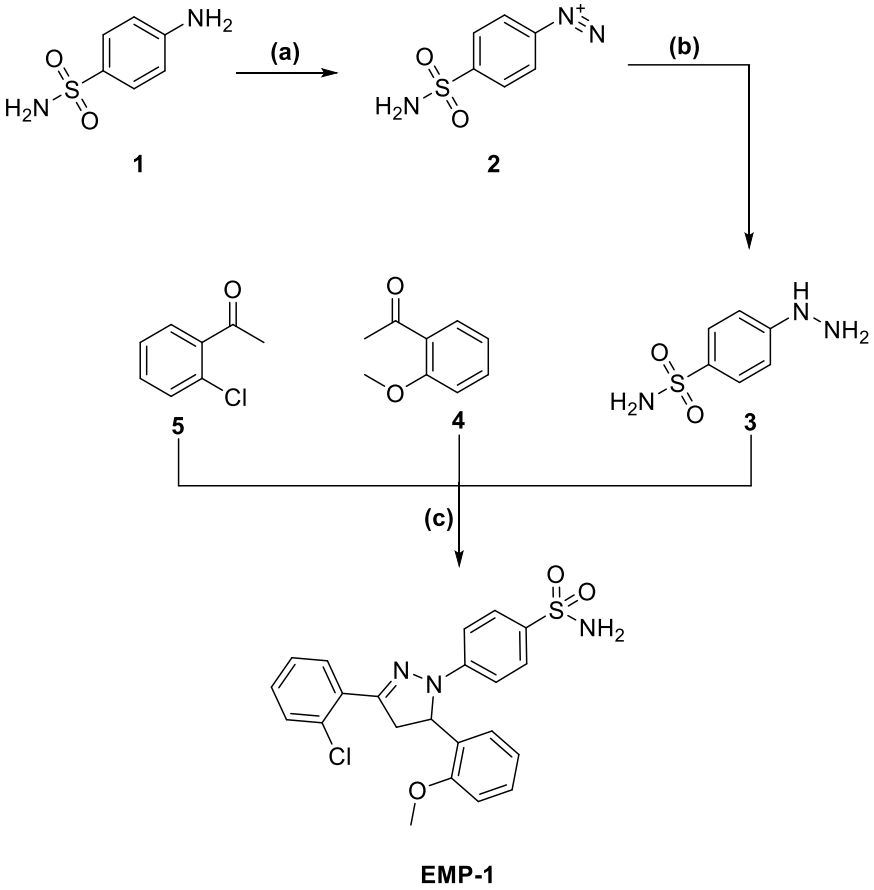

Figure 2. Synthesis pathway of **EMP-1** compound. Reactions conditions: (a) $NaNO_2$, HCl, stirred at 0 °C 30 minutes; (b) $SnCl_2$, HCl, stirred at room temperature and (c) reacted in ethanol at 80 °C using sealed-vessel reactor.

Molecular Docking

Based on the docking results (see Table 1), it was found that doxorubicin has a binding free energy value of -8.14 kcal/mol and an RMSD value of 0.000. Docking visualization results showed that doxorubicin (i.e., positive control) could bind to 8 amino acid residues on the receptor's active site, namely Ala636, Glu640, Val643, Leu644, Ile789, His790, Arg791, and Asp810. It interacts with receptors through hydrogen bonds with Ala636, Ile789, and Arg791 residues, van der Waals interaction with Glu640 and His790 residues, pi-alkyl bonds with Leu644 residue, pi-sigma bonds with Val643 residue, and pi-pi T-shaped with His790 residue. Figure 3 is depicted the spatial arrangement for doxorubicin.

Table 1. Docking results of synthesized compound.

Compound	Parameters			
	Binding Free Energy (kcal/mol)	**RMSD Value**	**Hydrogen Bond**	**Factor of Binding**
EMP-1	-7.17	0.000	Glu640, Leu813	-
Doxorubicin	-8.14	0.000	Ala636, Ile789, Arg791	

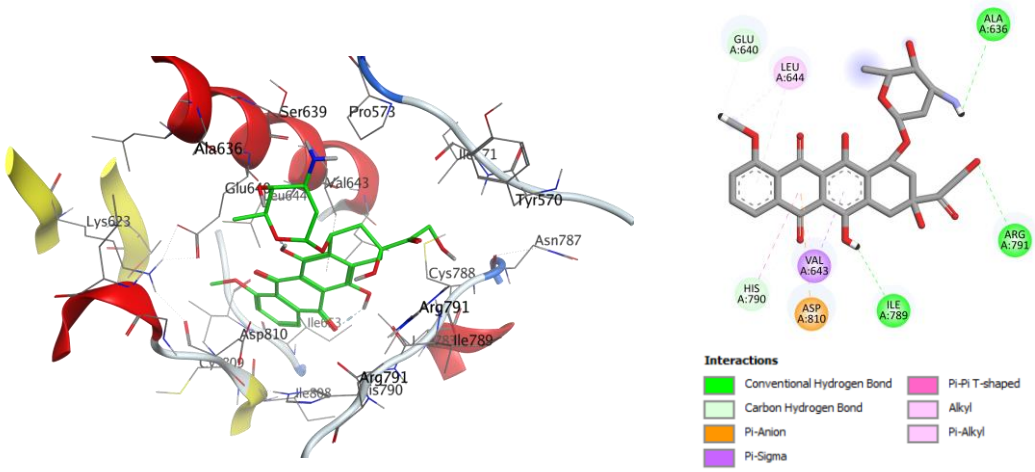

Figure 3. The spatial arrangement of doxorubicin.

Compound **EMP-1** has a binding free energy of -7.17 kcal/mol. It has some interactions with amino acids, such as having hydrogen bond with Glu640 and Leu813 residues, van der Waals interaction with Tyr570 and Ile808 residues, pi-alkyl interaction with Ala643 and Leu644 residues, and pi-pi T-shaped interaction with His790 residue. Furthermore, **EMP-1** compound also had hydrophilic interaction through Pi-anion interaction with Asp810 residue. The synthesized compound shows quite a similar binding mode compared to doxorubicin. Although there are few interaction differences, mainly hydrogen-bonded residues, these compounds seem to have the potency to become inhibitors for breast cancer MCF7. The spatial arrangement of compound 1 (i.e., protein's sequence) appears close to the spatial arrangement of positive control (i.e., protein's sequence) (Frimayanti *et al.*, 2020). In addition, the binding free energy of this compound is also close with the positive control. The spatial arrangement of compound **EMP-1** is presented in Figure 4.

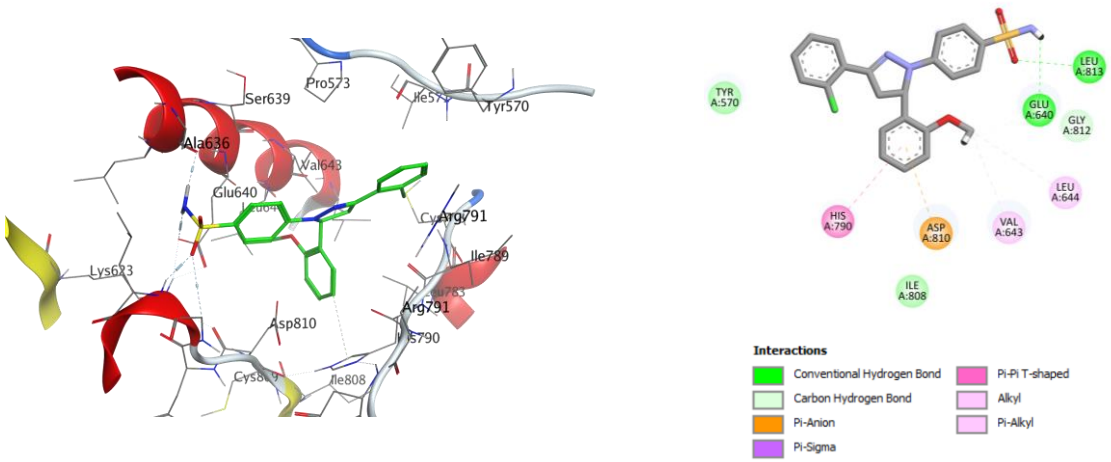

Figure 4. The spatial arrangement of **EMP-1** compound.

MD Simulation

The efficiency of hydrogen binding was used to examine the stability of docking results on the condition (NPT) before and after simulation at 50 ns and 300 K. For compound **EMP-1**, the conformation was maintained to binding well with the same residues before and after simulation, and the hydrogen bond was observed with the distance of 2.9 Å (Duan *et al.*, 2019). The interactions with amino acids before and after MD simulation are presented in Table 2. Based on MD simulation, compound **EMP-1** seemed to be stable and maintain the interaction with the receptor. Thus, it can be used as a potential inhibitor, and an in vitro assay is needed to confirm. Visualization of MD simulation is depicted in Figure 5.

Table 2. Ligand interaction with amino acid residues.

After Docking	MD Simulation	Distance of Hbond
Glu633, Glu640, His790 Asp810	**Asp810**	2.9 Å

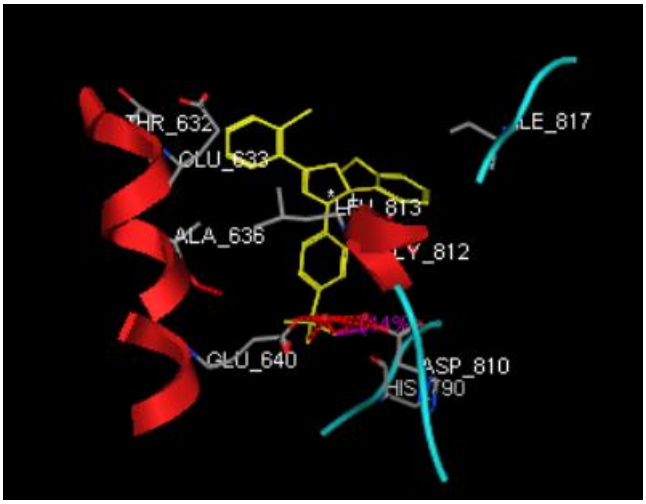

Figure 5. Visualization of MD simulation for compound **EMP-1**.

CONCLUSIONS

The pyrazoline compound, 4-(3-(2-chlorophenyl)-5-(2-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) benzenesulfonamide **(EMP-1)** was successfully synthesized through a one-pot reaction with 91% yield. Furthermore, a molecular docking study showed that the binding energy of **EMP-1** was -7.17 kcal/mol, which was higher than the positive control. Therefore, the spatial arrangement of **EMP-1** was similar to the positive control, so this compound potentially developed to become an anti-breast cancer agent.

ACKNOWLEDGMENTS

This work was funded by Direktorat Riset dan Pengabdian Masyarakat (DPRM) KEMENRISTEK DIKTI through Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) grant with contract number 407/UN.19.5.1.3/PT.01.03/2020.

REFERENCES

Adamus-Grabicka, A.A., Markowicz-Piasecka, M., Cieślak, M., Królewska-Golińska, K., Hikisz, P., Kusz, J., Małecka, M., and Budzisz, E., 2020. Biological Evaluation of 3-benzylidenechromanones and Their Spiropyrazolines-based Analogues. *Molecules*, *25*(7), 1613. doi: 10.3390/molecules25071613.

Altisen, R. C., Constansa, J. F. C., Bafalluy, R. M., and Rigal, I. C., 2007. Pyrazoline Derivates Useful for the Treatment of Cancer, US 2007/00, 1–12.

American Cancer Society, "Cancer Facts & Figures 2020," *American Cancer Society*. 2020, [Online]. Available: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2020/cancer-facts-and-figures-2020.pdf.

Cuartas, V., Robledo, S.M., Vélez, I.D., Crespo, M.D.P., Sortino, M., Zacchino, S., Nogueras, M., Cobo, J., Upegui, Y., Pineda, T. and Yepes, L., 2020. New Thiazolyl-Pyrazoline Derivatives Bearing Nitrogen Mustard as Potential Antimicrobial and Antiprotozoal Agents. *Archiv der Pharmazie*, *353*(5), e1900351. doi: 10.1002/ardp.201900351.

Duan, L., Guo, X., Cong, Y., Feng, G., Li, Y., and Zhang, J.Z., 2019. Accelerated Molecular Dynamics Simulation for Helical Proteins Folding in Explicit Water. *Frontiers in Chemistry*, *7*(540), 1–18. doi: 10.3389/fchem.2019.00540.

Frimayanti, N., Yaeghoobi, M., Ikhtiarudin, I., Rizki, D., and Putri, W. 2021. Insight on the In Silico Study and Biological Activity Assay Molecular docking. 20(1), 1–11. doi: 10.12982/CMUJNS.2021.019.

Frimayanti, N., Ikhtiarudin, I., Rahma, D.O.N.A., Agustini, T.T., Murdiya, F., and Zamri, A., 2020. A Computational Approach to Drug Discovery: Search for Chalcone Analogues as the Potential Candidates for Anti-Colorectal Cancer (HT29). *Walailak Journal of Science and Technology (WJST)*, *17*(2), 64–74.

Havrylyuk, D., Zimenkovsky, B., Vasylenko, O., and Lesyk, R., 2013. Synthesis and Anticancer and Antiviral Activities of New 2-Pyrazoline-Substituted 4-Thiazolidinones. *Journal of Heterocyclic Chemistry*, 50(S1), E56–E62. doi: 10.1002/jhet.5570100628.

Herfindo, N., Prasetiawati, R., Sialagan, D., Frimayanti, N., and Zamri, A., 2020. Synthesis, Anti-proliferative Activity and Molecular Docking Studies of 1, 3, 5-triaryl Pyrazole Compound as Estrogen a Receptor Inhibitor Targeting MCF-7 Cells Line. *Molekul*, *15*(1), 18–25. doi: 10.20884/1.jm.2020.15.1.585.

Higa, G.M. and Abraham, J., 2007. Lapatinib in the Treatment of Breast cancer. *Expert Review of Anticancer Therapy*, *7*(9), 1183–1192. doi: 10.1586/14737140.7.9.1183.

Jasril, J., Ikhtiarudin, I., Hasti, S., Indah, A.I., and Frimayanti, N., 2019. Microwave-Assisted Synthesis, in Silico Studies and in vivo Evaluation for the Antidiabetic Activity of New Brominated Pyrazoline Analogs. *Thai Journal of Pharmaceutical Sciences*, *43*(2), 83–89.

Jasril, J., Zamri, A., Ikhtiarudin, I. and Teruna, H.Y., 2016. 5-(4-Fluorophenyl)-3-(naphthalen-1-yl)-1-Phenyl-4, 5-dihydro-1H-pyrazole. *Molbank*, *2016*(2), 1-5. doi: 10.3390/M891.

Ji-Tai, L., Xiao-Hui, Z., and Zhi-Ping, L., 2007. An Improved Synthesis of 1, 3, 5-Triaryl-2-Pyrazolines in Acetic Acid Aqueous Solution. *Beilstein J ournal Organic Chemistry*, *3*, 1–4. doi: 10.1186/1860-5397-3-13.

Meric-Bernstam, F. and Hung, M.C., 2006. Advances in Targeting Human Epidermal Growth Factor Receptor-2 Signaling for Cancer Therapy. *Clinical Cancer Research*, *12*(21), 6326–6330. doi: 10.1158/1078-0432.CCR-06-1732.

Ozawa, Y., Kusano, K., Owa, T., Yokoi, A., Asada, M., and Yoshimatsu, K., 2012. Therapeutic Potential and Molecular Mechanism of a Novel Sulfonamide Anticancer Drug, Indisulam (E7070) in Combination with CPT-11 for Cancer Treatment. *Cancer Chemotherapy and Pharmacology*, *69*(5), 1353–1362. doi: 10.1007/s00280-012-1844-8.

Piccart-Gebhart, M.J., Procter, M., Leyland-Jones, B., Goldhirsch, A., Untch, M., Smith, I., Gianni, L., Baselga, J., Bell, R., Jackisch, C., and Cameron, D., 2005. Trastuzumab After Adjuvant Chemotherapy in HER2-Positive Breast Cancer. *New England Journal of Medicine*, *353*(16), 1659–1672. doi: 10.1056/NEJMoa052306.

Soliman, R., 1979. Preparation and Antidiabetic Activity of Some Sulfonylurea Derivatives Of 3, 5-Disubstituted Pyrazoles. *Journal of Medicinal Chemistry*, *22*(3), 321–325. doi: 10.1021/jm00189a022.

Teruna, H.Y., Aisyah, A., Nurlaili, N., and Hendra, R., 2019. Microwave Assisted Synthesis and Evaluation of Toxicity and Antioxidant Activity of Pyrazoline Derivatives. *Indonesian Journal of Chemistry*, *19*, 583–91. doi: 10.22146/ijc.34285.

Varghese, B., Al-Busafi, S.N., Suliman, F.O., and Al-Kindy, S.M., 2017. Unveiling A Versatile Heterocycle: Pyrazoline–A Review. *RSC Advances*, *7*(74), 46999−47016. doi: 10.1039/C7RA08939B.

World Health Organization, "Latest Global Cancer Data: Cancer Burden Rises to 18.1 Million New Cases and 9.6 Million Cancer Deaths in 2018," *International Agency for Research on Cancer*, pp. 1–3, 2018, [Online]. Available: https://www.who.int/cancer/PRGlobocanFinal.pdf.

Zamri, A., Teruna, H.Y., Wulansari, S., Herfindo, N., Frimayanti, N. and Ikhtiarudin, I., 2019. 3-(3, 4-Dimethoxyphenyl)-5-(2-fluorophenyl)-1-phenyl-4, 5-dihydro-1*H*-pyrazole. *Molbank*, *2019*(4),1-8. doi: 10.3390/M1088.