European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 10, 2020

Binding Affinity Of Omega 3 Fatty Acid As An Agonist PPAR-γ And GPR120 Receptor For Obesity Using Molecular Docking And ADME Prediction

Ginna Megawati[1], Dewi M.D Herawati[1], Ida Musfiroh[2*]

[1]*Division of Medical Nutrition, Department of Public Health, Faculty of Medicine Universitas Padjadjaran, Sumedang, Indonesia.*
[2]*Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia.*

E-mail: ida.musfiroh@unpad.ac.id

Abstract: Obesity is a complex disease involving excessive amounts of body fat due to problems with lipid metabolism and catalysis by PPARγ and GPR120. It is currently known that full-agonist drugs PPRγ have cardiovascular side effects and are closely related to lipid metabolism by GPR120. The selected of omega-3 derivative compounds is based on an important role in morphological, biochemical, molecular brain development and has been shown to control body weight by reducing body fat accumulation. The aim of this study was to obtain information regarding the binding affinity of 9 selected compounds from omega-3 to PPARγ and GPR120 either as full agonist or partial agonist by showing that these compounds using absorption and distribution prediction (ADME) sufficiently reasonable. Docking analysis was performed using Auto Dock 4.2, and ADME prediction using PreADMET software. The results showed that DPA and DHA have most higher binding affinity of molecular docking at the active site of the partial agonis and full agonist for PPARγ with free energy -9.26 kcal/mol and -8.92, respectively. DPA showed capabilities as partial agonist is characterized by a hydrogen bond in the form of Ser342 such as telmisartan, while DHA has a similar hydrogen bond in the form of Ile281 such as rosiglitazone. Whereas the results for GPR120 showed that DHA, EPA, and ETA compounds had good potential activity as agonists by binding to the same amino acid residues Arg327 and Tyr146, and the compounds have the lowest bond energies were -9.4, -8.72 and -8.15 kcal/ mol, respectively compared to the neurotensin ligand is -6.31 kcal/mol. All compounds meet absorption and distribution parameters, so that the selected compounds have the potential to prevent obesity through PPARγ and GPR120.
Keywords : Omega-3, PPAR-γ, GPR120, Obesity, ADME

1. INTRODUCTION

Obesity is one of the complex diseases characterised by an expansion of the mass of adipose tissue and dramatic changes in its distribution in the body[1]. The search for both safe and efficacious pharmacological therapies that support weight loss and prevent chronic metabolic conditions related to obesity has proven difficult[2]. Omega-3 is an essential

nutrition that has been proved can control body weight by reducing the body fat accumulation. Omega-3 fatty acid has a significant role as anti-inflammatory sensors and control lipid metabolism. The mechanism to prevent obesity comorbidity remains unclear but it's have been reported to improve insulin resistance known as the cause of obesity-associated metabolic disorders by binds to protein peroxisome proliferator-activated receptor PPARγ and GPR120[3][4].

The core receptors of the Peroxisome Proliferator-Activated Receptors (PPARs) group are the ones that play a role in regulating adipocyte differentiation, lipids and glucose homeostasis[5]. PPARγ which actively acts on adipose tissue and macrophages triggers the differentiation of fat cells and regulates fatty acid storage and glucose metabolism by influencing related genes[6]. While, GPR120 is a receptor for long-chain fatty acids and is expressed in small intestinal endocrine cells, L cells and adipose tissue. Activation of GPR120 promotes the secretion of incretin GLP-1, which is known to have an effect on anti-metabolic syndrome[7][8]. In other words, PPAR-γ plays a role in insulin sensitivity while the GPR120 receptor plays a role in lipid metabolism.

Free fatty acids as endogenous ligands play a role in modulating the expression of genes and proteins that regulate various ranges of proteins that function physiologically and pathophysiologically, including those associated with energy homeostasis[9]. It is currently known that full-agonist drugs PPRγ have cardiovascular side effects and are closely related to lipid metabolism by GPR120. In a Goyal et al. administration of full partial agonist drugs exerts cardioprotective effects not only through its ARB class but also on myocardial treatment ischaemia/reperfusion (I/R) injury in diabetic rats[10]. Thiazoledinediones (TZDs) are fully agonists used in the treatment of type 2 diabetes (T2D) since 1997. It has the ability to reduce insulin resistance. Treatment with TZD takes a long time to lower plasma glucose levels and administration of TZD can have an effect in the form of increasing body fat resulting in weight gain[11]. Based on this, the two drugs have their respective disadvantages, but for partial agonists it is better because it can treat hypertensive patients who also suffer from type II diabetes mellitus. So that this study was carried out to study the interaction of omega-3 compounds with PPAR-γ and GPR 120 protein receptors, compared to the current type-2 DM drugs which are known to have side effects.

Until now, the mechanism of the role of omega-3, especially in lipid and glucose metabolism in obesity cases, still opens up opportunities for further exploration. The various published systematic reviews have not been able to provide consistent results regarding the utilization of omega-3 in obesity cases. The results of studies in experimental animals and humans have not been concluded consistently, the exact mechanism of action of how omega-3 in obesity cases is still unclear, but the provision of omega-3 supplementation shows the potential to prevent the occurrence of comorbids in obesity. Low grade inflammation has been identified as a key factor in the development of metabolic syndrome in obese patients. EPA and DHA, which are important components of omega-3, are able to modulate fat cell insulin sensitivity and glucose utilization[12]. This is related to its ability to stimulate PPAR-γ and GPR 120 activity. So that omega-3 can be an alternative supplementation option that is safe and effective in preventing obesity.

2. MATERIALS AND METHODS

A. Selection of Compounds

Nine chemical compounds from omega-3 fatty acids were selected including hexadecatrienoic acid (HTA), alpha-linolenic acid (ALA), stearidonic acid (SDA),

eicosatrienoic acid (ETE), eicosatetraenoic acid (ETA), eicosapentaenoic acid (EPA), heneicosapentaenoic acid (HPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA)[13]. In ligand preparation, the structure is designed using the ChemDraw Ultra 12.0 program. Then the compound structure is saved with the format (.pdb). The Nine Chemical Compounds from omega-3 fatty acids can be seen in Figure 1.

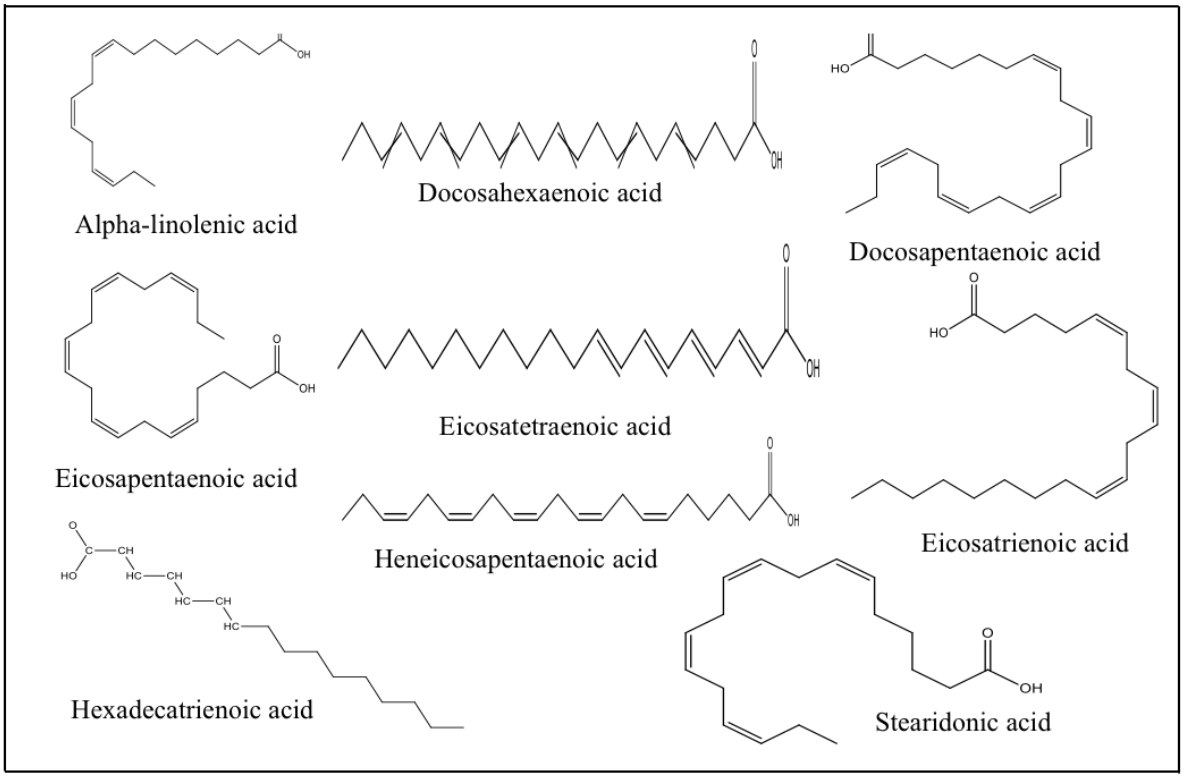
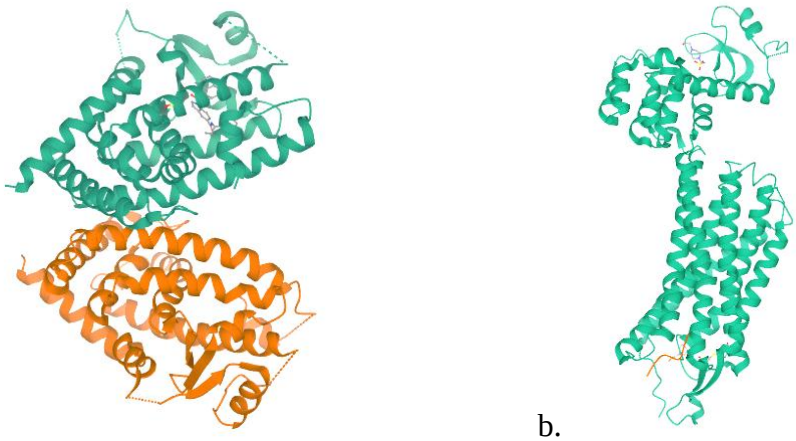


Fig. 1: Two-dimensional chemical structures of selected compounds from omega-3 fatty acids

B. Protein Model

PPARγ protein structures (PDB ID: 4R06) and GPR120 (PDB ID: 4GRV) were retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank. All data files are saved in .pdb file format. Water molecules and ligands from proteins were removed using the Discovery Studio Visualizer 2016 Client® program. The result will be a pure receptor which is then stored in the Protein Data Bank (.pdb) format. The crystal structure of 4R06 was first determined by Marrewijk et al (2016)[14]. The crystal structure consists of two chains, namely chain A and B with a chain length of 273 amino acids for each chain. The crystal structure of 4R06 also contains a small compound in the form of a sulfate ion. For the 4GRV Crystal Structure was first determined by Noinaj et al (2012)[15]. The crystal structure consists only of chain A with a chain length of 510 amino acids for the chain. In the 4R06 crystal structure there are no other small compounds that are on the crystal side. The protein can be seen in Figure 2.



a. b.

Fig. 2: (a). Receptor PPARγ (4R06), and (b). Receptor GPR120 (4GRV)

C. Molecular Docking

Molecular analysis of docking was performed using Auto Dock 4.222 auto docking tool. The polar hydrogen atom and Gasteiger partial charge are added to the 3D protein structure. The protein structure is written in.pdbqt file format for further analysis. The grid box was set to 40 × 40 × 40, coordinates of 18.225; 30.291; 15.008 (as x, y, and z center of mass, respectively), and spacing 0.375 Å for PPARγ. Then on the active side of GPR120 coordinates (x, y, z) 90.024; -11.196; 68.538 with a distance of 0.375 Å. Genetics algorithm was set to run 100 times while remaining parameters were kept as default. AutoDock 4.2 was used to simulate the docking process. The binding affinities of the compounds were studied using Discovery Studio Visualizer 2016 Client® program.

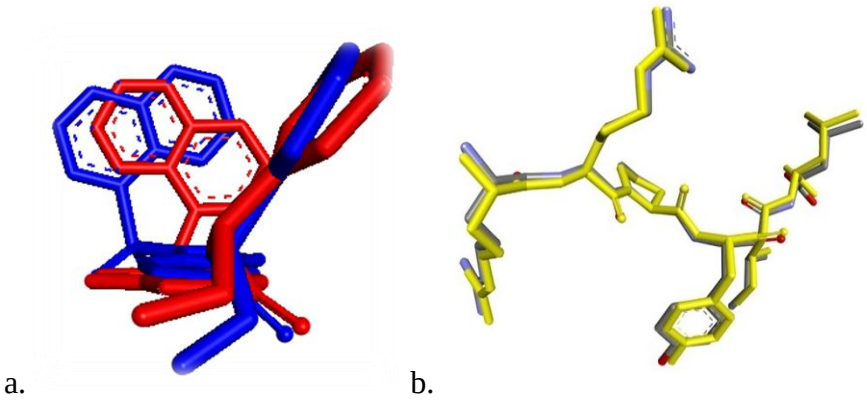
D. ADME Prediction

ADME parameters are predicted using the preADMET® program which is accessed via the website (https://preadmet.bmdrc.kr/adme/). The chemical structure of the compounds is drawn or uploaded in Mol file (.mol) format. The program automatically calculates the predicted value of the selected parameters, namely: Human colon adenocarcinoma (Caco-2) cell permeability, Human Intestinal Absorption (HIA), Plasma Protein Binding, and Blood Brain Barrier.

3. RESULTS

A. Molecular Docking

Molecular docking is a computational method that aims to mimic the interaction of a ligand molecule with the protein that is its target in an in-vitro test[16]. Validation of the docking process is done by redocking using Autodock 4.2. Validation was carried out on the active side of Novel SR2067 co-crystal ligands and neurotensin on crystallographic results of PPARγ and GPR120, respectively. The redocking results show RMSD values of 1.743 Å A and 0.20 Å, which indicate that the positions of the atoms in the ligands from the redocking results are not too different from the positions of the crystallographic ligands[17]. These results indicate that the PPARγ and GPR120 receptors can be used for molecular docking processes. The overlap between the initial pose and the redocking pose is shown in Figure 3. The docking parameters are accepted when the RMSD value is not greater than 2.0 Å[18].



a. b.

Fig. 3: (a). Novel SR2067 with receptor PPARγ (Red = crystallographic results and blue = redocking pose), (b). Neurotensin with GPR120 (gray = crystallography results and yellow = redocking pose).

Analysis of molecular docking results was assessed on the value of free energy and the similarity of interactions between amino acids and the target receptor protein. The analysis results based on the best energy value docking on PPARγ showed that docosapentaenoic acid (DPA) had free energy -9.26 kcal/mol, telmisartan -13.34 kcal/mol, docosahexaenoic acid (DHA) -8.92 kcal/mol, and rosiglitazone -9.04 kcal/mol. While the docking at the GPR120 receptor showed docosahexaenoic (DHA) -9.4 kcal/mol, eicosapentaenoic (EPA) -8.72 kcal/mol, and eicosatrienoic (ETE) -8.15 kcal/mol. The more negative value for free energy indicates a strong bond with the receptor. Based on research by Ganou et al, 2018 the value of free energy is less than -5.5 kcal/mol has the potential to do activity with target receptors. The value of free energy is the ability of the ligands to form interactions with macromolecules, so that the more negative the value is, the process will occur spontaneously, as a result, the easier it will be for the ligands to interact on the active side of the macromolecule. The simulation results of the docking of compound molecules to the PPARγ and GPR120 receptors can be seen in Table 1 and 2.

Table 1. The Result of Molecular Docking with The 4R06 Receptor

No	**Compounds**	**Ki (μM)**	**ΔG° (kcal/mol)**	**Pharmacophores**	**Amino Acid Residue**
1.	Rosiglitazone (full agonists)	0.2359	-9.04	Carboxylate Group	ILE281, ARG288
2.	Telmisartan (Partial agonist)	0.00016	-13.34	Carboxylate Group	GLY284, SER342
3	ALA	4.02	-7.36	Carboxylate Group	LEU340
4.	DHA	0.2897	-8.92	Carboxylate Group	ILE281
5.	DPA	0.1625	-9.26	Carboxylate Group	SER342
6.	EPA	0.5177	-8.58	Carboxylate Group	HIS449, TYR573
7.	ETA	0.8169	-8.31	Carboxylate Group	HIS449
8.	ETE	1.98	-7.78	Carboxylate	ARG288

				Group	
9.	HPA	1.14	-8.11	Not Bonding	Not Bonding
10.	HTA	3.99	-7.37	Carboxylate Group	ARG288
11.	SDA	2.21	-7.72	Carboxylate Group	PHE282

Table 2. The Result of Molecular Docking with The 4GRV Receptor

No	**Compounds**	**Ki (µM)**	**ΔG° (kcal/mol)**	**Pharmacophores**	**Amino Acid Residue**
1	Native ligand (Neurotensin)	23.57	-6.31	Carboxylate Group	ARG327, TYR146
2	ALA	1.17	-8.09	Carboxylate Group	ARG328
3	DHA	0.27	-8.94	Carboxylate Group	ARG328, ARG327
4	DPA	0.30	-8.89	Carboxylate Group	ARG213
5	EPA	0.40	-8.72	Carboxylate Group	ARG327, TYR146
6	ETA	0.61	-8.87	Carboxylate Group	ARG327, ARG328
7	ETE	1.06	-8.15	Carboxylate Group	ARG327, ARG149, TYR351, TYR146
8	HPA	0.38	-8.75	Not Bonding	ARG327, ARG328
9	HTA	0.73	-8.37	Carboxylate Group	ARG327, ARG149, TYR351
10	SDA	0.62	-8.46	Carboxylate Group	ARG327

B. ADME Prediction

ADME properties of new drug candidate compounds must have a good value that meets the preADMET rules[19] against the body. The predicted pharmacokinetic parameters included absorption (Human Intestinal Absorption (HIA) and Caco-2) and distribution (Plasma Protein Binding). In terms of toxicity, the mutagenic and carcinogenic properties were seen. Human Intestinal Absorption (HIA) has a percentage of intestinal absorption in humans, namely if it is more than 80%, the absorption is better and if it is less than 30%, the absorption is poor. Based on the prediction results, all compounds have a Human Intestinal Absorption (HIA) value above 80% which indicates that all these compounds can be absorbed through the intestine and have a good absorption rate in the intestine (well absorption). Meanwhile, all compounds showed moderate permeability in Caco-2 cells, namely 4 to 70. Caco-2 cells are derivatives of human adenocarcinoma colon and have various drug transport routes through the intestinal epithelium. Plasma Protein Binding, more than 90% indicated that strong drugs were bound to plasma protein, less than 90% of weak drugs were bound to plasma proteins[20][21].

4. DISCUSSION

Based on Ritia Rahmawati's research in 2016, amino acids Gly284, Cys285, Arg288, Glu291, Ala292, Ile326, Met329, Leu330, Leu333, Ile341, Ser342 are active site amino acids that interact in the PPARγ region[22][11]. In addition, in terms of its bonds with amino acids on the active side of PPARγ, DPA compounds have hydrogen bonds in the form of Ser342

such as telmisartan, while DHA also has hydrogen bonds in the form of Ile281 such as rosiglitazone. In this study DPA had the best binding affinity for the active site of PPARγ. On the active side of GPR120, it was found that the hydrogen bond between the ligand carboxylic acid residue and the guanidine group of the Arginine (R99) residue in GPR120 has an important role for ligand activity[23]. Three omega-3 derivative compounds, namely docosahexaenoic (DHA) eicosapentaenoic (EPA) and eicosatrienoic (ETE) compounds have amino acids similar to neurotensin ligands, namely Arg327 and Tyr146. Where in the interaction the amino acids that are formed are in the carboxylic group so that this is what is responsible for biological activity as an inhibitor of obesity. Amino acid interactions can be seen in Figure 4.

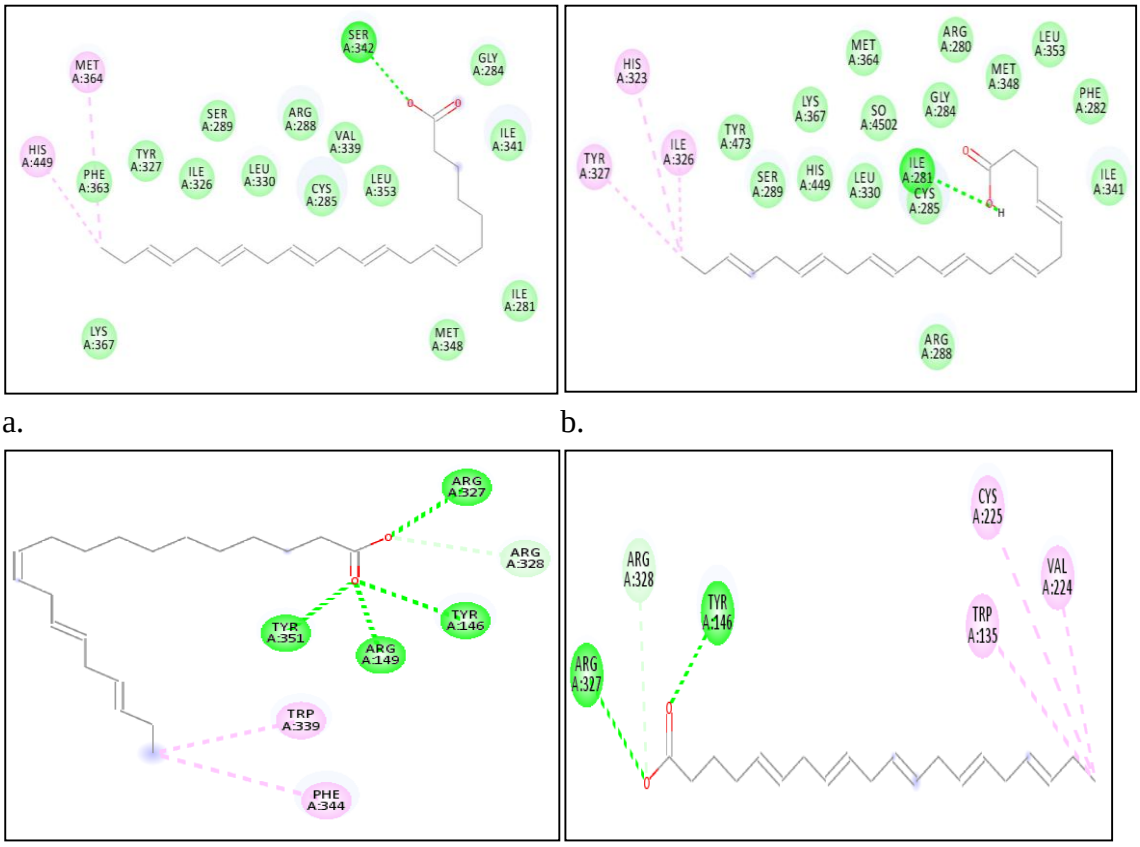


Fig. 4: Ligand interaction of DPA (a) and DHA (b) with the PPARγ, (c) ETE and (d) EPA with the GPR120

The results of visualization comparisons showed the similarity of the bound amino acids between DPA and telmisartan and DHA and rosiglitazone. Telmisartan, an AT1R antagonist, has been reported as a partial agonist of peroxisome proliferator-activated receptor-γ (PPARγ)[24]. It has the unique ability to bind and activate PPARγ and has a beneficial effect on insulin sensitivity in humans[25]. DPA compounds are able to provide activity such as telmisartan as a partial agonist although it is not too large based on the energy affinity produced. Likewise, DHA has the ability as a full agonist against PPARγ such as rosiglitazone by binding to similar amino acids but slightly higher energy affinity. Rosiglitazone is an antidiabetic drug that is classified as a full agonist which is included in the thiazolidinedione class[26]. It works as an insulin sensitizer, by binding to PPARs in fat

cells and making cells more responsive to insulin[27]. Meanwhile, DHA, EPA and ETE have better abilities than neurotensin ligands in providing full agonist activity against GPR120.

Table 3. Results Of Pharmacokinetic Predictions

Compound	**Absorption**		**Distribution**	
	%HIA	**Caco2**	**PPB**	**BBB**
ALA	98.27	27.97	100	6.69
DHA	97.87	32.32	98.82	7.435
DPA	97.87	32.43	100	9.206
EPA	97.94	30.08	100	6.599
ETA	98.01	30.18	100	11.177
ETE	98.10	30.30	100	10.393
HPA	97.90	31.26	73.28	7.577
HTA	95.14	38.41	100	8.859
SDA	98.16	27.86	100	4.355

Based on the ADME predictions in Table 3, the compound hits the virtual screening results with the best interactions, namely DPA, DHA, EPA and ETE. The prediction of absorption in HIA (human Intestinal Absorption) DPA, DHA, EPA and ETE had a percentage of 97.87%, 97.87%, 97.94% and 98.10% which indicated that these compounds were absorbed very well. Caco-2 cells are derived from human adenocarcinoma colon and have various drug transport routes through the intestinal epithelium. All compounds showed moderate permeability in Caco-2 cells. Plasma Protein Binding (PPB) shows that the compound is 100% DPA, 98.82% DHA, 100% EPA and 100% ETE, thus indicating that the compound is strongly bound to plasma proteins. The ability of drugs to cross the Blood Brain Barrier (BBB) is an important parameter to consider to help reduce side effects and toxicity or to increase the efficacy of drugs whose pharmacological activity is present in the brain. According to Pires et al. (2015) stated that a compound is said to be able to penetrate the blood brain barrier well if it has a Log BB value> 0.3, and cannot be properly distributed if log BB <-1[28]. Based on the prediction results that all of these compound derivatives are able to penetrate the blood brain barrier moderately.

ACKNOWLEDGMENT

This research was supported by Universitas Padjadjaran Research Grant trough the RDPD scheme, 2020.

5. REFERENCES

[1] Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, et al. *Dietary Factors and Low-Grade Inflammation in Relation to Overweight and Obesity*. Br J Nutr;106(SUPPL.3) : 1-80, 2011.

[2] Schroeder LE, Leinninger GM. *Role of Central Neurotensin in Regulating Feeding: Implications for The Development and Treatment of Body Weight Disorders.* Biochim Biophys Acta;1864(3):900–16, 2018.

[3] Calder PC. V*ery Long Chain Omega-3 (N-3) Fatty Acids and Human Health*. Eur J Lipid Sci Technol;116(10):1280–300, 2014.

[4] Song T, Yang Y, Zhou Y, Wei H, Peng J. *GPR120: A Critical Role in Adipogenesis, Inflammation, and Energy Metabolism in Adipose Tissue.* Cell Mol Life

Sci;74(15):2723–33, 2017.

[5] Senthil R, Sakthivel M, Usha S. *Structure-Based Drug Designo of Peroxisome Proliferator-Activated Receptor Gamma Inhibitors: Ferulic Acid and Derivatives*. J Biomol Struct Dyn: 1-17, 2020.

[6] Cui J, Lee DY. *Synthesis and Biological Activity of Piperine Derivatives as Potential PPAR γ* Agonists; 14 : 2069-2078, 2020.

[7] Halder S, Kumar S, Sharma R. *The Therapeutic Potentialo of GPR120: A Patent Review*. Expert Opin Ther Pat;23(12):1581–90, 2013.

[8] Hara T, Hirasawa A, Ichimura A, Kimura I, Tsujimoto G. *Free Fatty Acid Receptors FFAR1 and GPR120 as Novel*;100(9):3594–601. 2011.

[9] Ichimura A, Hirasawa A, Hara T, Tsujimoto G. *Free Fatty Acid Receptors Act as Nutrient Sensors to Regulate Energy Homeostasis Prostaglandins and Other Lipid Mediators Free Fatty Acid Receptors Act as Nutrient Sensors to Regulate Energy Homeostasis*. J. Prostaglandins; 89 (3-4): 82-8, 2009.

[10] Goyal SN, Bharti S, Bhatia J, Nag TC, Ray R, Arya DS. *Telmisartan, A Dual ARB/Partial PPAR-Γ Agonist, Protects Myocardium from Ischaemic Reperfusion Injury in Experimental Diabetes.* Diabetes, Obes Metab;13(6):533–41, 2011.

[11] Larsen TM, Toubro S, Astrup A. *PPARγ Agonists in The Treatment Of Type II Diabetes: is Increased Fatness Commensurate with Long-Term Efficacy*. Int J Obes;27(2):147–61, 2003.

[12] Huang F, del-Río-Navarro BE, Leija-Martinez J, Torres-Alcantara S, Ruiz-Bedolla E, Hernández-Cadena L, et al. *Effect of Omega-3 Fatty Acids Supplementation Combined with Lifestyle Intervention on Adipokines and Biomarkers of Endothelial Dysfunction in Obese Adolescents with Hypertriglyceridemia.* J Nutr Biochem;64:162–9, 2019.

[13] Idrus S. *Dengue Omega 3 Fatty Acid and 6 As Potential Inhibitors Of Dengue Virus Fusion*. Majalah BIAM;11(2):49–56, 2015.

[14] Van Marrewijk LM, Polyak SW, Hijnen M, Kuruvilla D, Chang MR, Shin Y, et al. *SR2067 Reveals A Unique Kinetic And Structural Signature For PPARγ Partial Agonism.* ACS Chem Biol;11(1):273–83, 2016.

[15] White JF, Noinaj N, Shibata Y, Love J, Kloss B, Xu F, et al. *Structure of The Agonist-Bound Neurotensin Receptor.* Nature;490(7421):508–13, 2012.

[16] Motiejunas D, Wade R. *Structural, Energetic, and Dynamic Aspects of Ligand-Receptor Interactions.* Comprehensive Medicinal Chemistry II: 193-213, 2007.

[17] Bissantz C, Kuhn B, Stahl M. *A Medicinal Chemist's Guide to Molecular Interactions.* J Med Chem;53(14):5061–84, 2010.

[18] Musfiroh I, Nursamsiar N, Tinggi S, Farmasi I, Muhtadi A, et al. *In Silico Study of Asiatic Acid Interaction with Inducible Nitric Oxide Synthase (Inos) and Cyclooxygenase-2 (COX-2).* IJPPS; 5(1): 204-207, 2013.

[19] Muchtaridi M, Dermawan D, Yusuf M. *Prediction of Alpha Mangostin and Its Derivatives against Estrogen Receptor Molecular Docking, 3D Structure-Based Pharmacophore Modeling, and ADME Prediction of Alpha Mangostin and its Derivatives against Estrogen Receptor Alpha.* Journal Young Pharmacist; 10(3): 252-259, 2018.

[20] Jasril, J. Frimayanti, N. Ikhtiarudin, I. In I. *In Silico Studies Of Fluorinated Chalcone and Pyrazoline Analogues as Inhibitors for Cervical Cancer.* Proceeding of ISCPMS:040008-8, 2019.

[21] Kartasasmita RE, Musfiroh I, Muhtadi A, Ibrahim S. *Binding Affinity of Asiatic Acid Derivatives Design Against Inducible Nitric Oxide Synthase and ADMET Prediction.* JAPS;4(02):75–80, 2014.

[22] Rahmawati R, Amin M, Rahmawati R, Amin M, Lestari U, Universitas P, et al. *Virtual Screening Analysis of Natural Compounds of PPAR Agonist Candidates*. In: National Seminar on Education and Science; 498–503, 2016.

[23] Shimpukade B, Hudson BD, Hovgaard CK, Milligan G, Ulven T. *Discovery of a Potent and Selective GPR120 Agonist.* J Med Chem; 55(99): 4511-5, 2012.

[24] Wang Z-F, Li J, Ma C, Huang C, Li Z-Q. *Telmisartan Ameliorates Aβ Oligomer-Induced Inflammation Via Pparγ/PTEN Pathway In BV2 Microglial Cells*. Biochem Pharmacol;171:113674, 2020.

[25] Kolli V, Stechschulte LA, Dowling AR, Rahman S, Czernik PJ, Lecka-Czernik B. *Partial Agonist, Telmisartan, Maintains PPARΓ Serine 112 Phosphorylation, And Does Not Affect Osteoblast Differentiation And Bone Mass.* Journal Plos One;9(5):e96323, 2014.

[26] Sikora-Wiorkowska A, Smolen A, Czechowska G, Wiorkowski K, Korolczuk A. *The Role of PPAR Gamma Agonists-Rosiglitazone and 15-Deoxy-Delta12, 14-Prostaglandin J2 in Experimental Cyclosporine A Hepatotoxicity*. J Physiol Pharmacol;70(6):885–93, 2019.

[27] Malinowski, M.J. *Rosiglitazone in The Treatment of Type 2 Diabetes Mellitus : A Critical Review.* Clinical Therapeutics; 22(10): 1151-1168, 2000.

[28] Pires DE V, Blundell TL, Ascher DB. *Pkcsm: Predicting Small-Molecule Pharmacokinetica and Toxicity Properties Using Graph-Based Signatures.* J Med Chem;58(9):4066–72, 2015.