In Silico Study of Novel Ketorolac as selective Cyclooxygenase-2 (COX-2)

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ABSTRACT

Ketorolac is a non-selective non-steroidal anti-inflammatory drug (NSAID). Ketorolac works by inhibiting the enzymes Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2(COX-2). Because it also interacts with it, it gives several side effects, especially on digestion. The aim of this study was to design and obtain a novel ketorolac, a modification of the ketorolac compound that is selective and does not interact with. Modify the structure by adding a substituent at the para position in the benzene ring. The method was carried out in silico with molecular docking, and predictions were made from the physicochemical properties, ADME as a pharmacokinetic profile and its toxicity using pkCSM and Protox online. The results showed that compound J had a better affinity than ketorolac for the Cyclooxygenase-2(COX-2) receptor and had no affinity for the Cyclooxygenase-1 (COX-1) receptor because it showed a positive docking score, but on the other hand it showed different results. lack of adherence to Lipinski's 5th law as a physicochemical basis and provides predictions of hepatotoxicity

Key words: Novel ketorolac, In Silico, COX-1, COX-2

INTRODUCTION

Inflammation (inflammation) is a normal protective response to tissue injury involving various physiological processes in the body such as enzyme activation, mediator release, diapedesis or blood cell movement white through the capillaries to the area of inflammation, cell migration, damage and network repair. The mechanism of inflammation is strongly influenced by compounds and mediators produced by arachidonic acid. When the cell membrane suffers damage by a chemical, physical, or mechanical stimulus phospholipase is activated to change the phospholipids present in the cell membrane into prostaglandins and thromboxanes (Nørregaard, Kwon and Frøkiær, 2015). The cyclooxygenase (COX) enzyme involved in the reaction has 2 isoforms, namely COX-1 and COX-2. Although the two enzymes work

essentially the same way, selective inhibition can make a difference in terms of side effects. COX-1 is considered a constitutive enzyme, found in most mammalian cells. COX-2 is not detected in most normal tissues. COX-2 is usually specific for inflamed tissue (Łanocha-Arendarczyk *et al.*, 2018).

In general, the treatment used to overcome the occurrence inflammation is a modern drug from the class of Non-Steroid Anti-Inflammatories (NSAID) and steroid groups that are useful for reducing swelling and feeling pain due to inflammation. NSAID consist of a group of non-selective NSAID works by inhibiting cyclooxygenase enzymes (COX-1 and COX-2) thus reducing the production of prostaglandins, while the other NSAID groups (selective COX-2 inhibitor) works by inhibiting the COX-2 enzyme.(Desmedt *et al.*, 2018) Ketorolac is a non-selective non-steroidal anti-inflammatory drug (NSAID). Ketorolac works by inhibiting cyclo-oxygenase (COX) 1 and 2 enzymes(Andy, Santoso and Supraptomo, 2020) . However, ketorolac still has many weaknesses in terms of non-selective activity so that it can bind to 2 COX-1 and COX-2 receptor isoforms, where COX-1 is present in the stomach, which can cause digestive disorders so further research is needed to find new drug candidates that are selective only on COX-2 (Zahra and Carolia, 2017).

One that can be developed is derived from drug-like compound components from ketorolac itself. Modifications in some of these drug compound groups will help find new drug candidates that are selective for COX-2. One of the in silico methods is molecular docking. The development of active compounds from ketorolac derivatives as anti-inflammatories (COX enzyme inhibitors) can also be carried out by molecular docking.These developments include the design of compounds and interactions compounds with enzymes or receptors (Katsila *et al.*, 2016). In this case, compound compounds ketorolac derivative needs to be tested for its interaction with the COX enzyme receptor with 2 isoforms, namely COX-1 and COX-2 through in silico study, so that we can find out how ketorolac derivative compounds can bind to COX receptors and has a better affinity than ketorolac. In silico it is also possible to determine compounds according to pharmacokinetic parameters (ADME) through Lipinski's Five Law rules using the pkCSM Online Tool application and compound toxicity parameters using the Protox Online Tool application.

MATERIALS AND METHODS

Materials

The material used is the Cyclooxygenase-1 (PDB ID: 1EQG) and Cyclooxygenase-2 (PDB ID: 1PXX) as protein target which was downloaded from https:// www.rcsb.org/. The Novel ketorolac designed being structure A-J whose molecular structures were drawn using *Chem Bio Draw Ultra* version 12 (*CambridgeSoft*). The tools used include hardware in the form of a set of laptops with specifications for *Processor type Intel® CoreTM i3* and 8 GB RAM and operating system software *Windows 10 Home Single Language*, *Chem Bio Draw Ultra* version 12 (*CambridgeSoft*), *Chem Bio 3D Ultra* version 12 (*CambridgeSoft*), *Molegro Virtual Docker* version 6.0 (*Molegro ApS*), *SwissADME, pkCSM*, and *Protox Online Tool*.

Method

Validation method of docking

The validation method of *docking* is done by docking a native ligand in the cavity receptor using the application *Molegro Virtual Docker* version 6.0. The results of the receptor validation were interpreted with the value of *Root Mean Square Deviation* (RMSD). Receptors can be said to be valid if they meet the criteria for the RMSD value 2Å (angstrom).

Ligand preparation

Ten compounds from the modified ketorolac structure were drawn with ChemDraw and then converted into 3D form. Then doing energy minimalizing dengan by pressing *MMFF94 → Calculations→ Perform → MMFF94 → Minimization*. This is to determine the most stable stereochemical form of the compound. Then saved in mol2 {SYBYL2(*.mol2)} format.

In silico docking novel ketorolac

The first thing to do is import the target protein with Import molecule, this stage is done before docking using the Molegro Virtual Docker (MVD) application. In this study using Cycloxygenase-1 (COX-1;PDB ID: 1EQG) and Cyclooxygenase-2 (COX-2;PDB ID: 1PXX) as target proteins. Next is to search for the cavity (hole) contained in the receptor which will later be used as a place for interaction between the target receptor and the compound. Place the 3-Dimensional structure of the compound into *cavities* selected. The *docking* of compounds on the receptor is done automatically by *Molegro Virtual Docker.*

ADME and physicochemical properties Prediction

Prediction of ADME (Absorption, Metabolism, Distribution, Excretion) and physicochemical properties using the pkCSM tool (http://biosig.unimelb.edu.au/pkcsm/). pkCSM Tool is a new method for predicting and optimizing the pharmacokinetic properties and toxicity of small molecules. pkCSM Tool is a web service that can be used for the analysis and optimization of

the pharmacokinetic properties (Absorption, Distribution, Metabolism and Excretion) of a compounds.

Toxicity Prediction

Predicted toxicity values using Protox II (https://tox-new.charite.de/protox_II/). ProTox-II is a free web service and users can make toxicity predictions for test compounds. Prediction of compound toxicity is a classification of toxicity classes based on the Globally Harmonized System (GHS). Using the application starts with obtaining the compound SMILE code from ChembioDraw Ver.13. then the SMILE code is copied to the column listed on the web which will be processed and related data will be obtained. Then, predictions of *Ames toxicity* and *Hepatotoxicity* were carried out using the website pkCSM (http://biosig.unimelb.edu.au/pkcsm/ prediction)

RESULT AND DISCUSSION

Validation method of docking

In this study, the native ligand was first docked using Molegro Virtual Docking. The result of the native ligand docking besides the docking value is the Root Mean Square Deviation (RMSD) value. The RMSD value is used as a validation method used. RMSD is a measurement of two poses through comparison the structure by which the protein is docked with the positions of the atoms between the structures experimental. RMSD value ≤ 2Å indicates that the method used has been valid. In addition, the smaller the resulting RMSD value shows the ligand pose is getting closer to the conformation of the native ligand so that it shows the results are getting better. Based on the results of the study, the best RMSD values were obtained from the docking results of native ligands, both in cyclooxygenase-1 and Cyclooxygenase-2 target proteins that met the RMSD requirements so that the test compound could be docked, namely the modified novel ketorolac(Jain and Nicholls, 2008).

Figure.1 Native Ligand (Ibuprofen) of Cyclooxygenase-1 (COX-1;PDB ID: 1EQG)
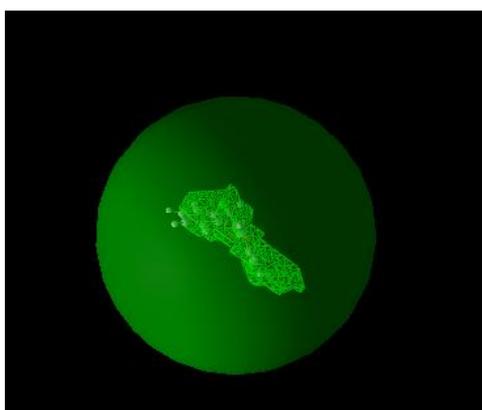
Figure.2 Native Ligand (Diclofenac) of Cyclooxygenase-2 (COX-2;PDB ID: 1PXX)
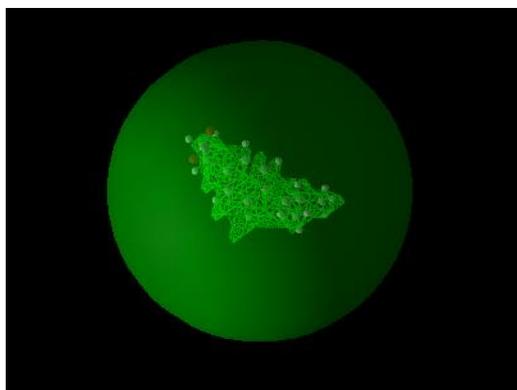
Table. 1 Result of Docking Validation

Protein Target and Native Ligand	RMSD			Average
	Replication 1	Replication 2	Replication 3	(Angstrom)
Cyclooxygenase-1 (1EQG) and Ibuprofen	0.7289	0.8169	0.8046	0.7835
Cyclooxygenase-2 (1PXX) and Diclofenac	0.8122	0.8878	1.426	1.042

Table. 2 Result Docking of Native Ligand

Protein Target and Native Ligand	Docking Score			Average (kkal/ mol)
	Replication 1	Replication 2	Replication 3	(Angstrom)
Cyclooxygenase-1 (1EQG) and	-81.5342	-80.9807	-80.9325	-81.1491

Ibuprofen			
Cyclooxygenase-2 (1PXX) and Diclofenac	-82.9688	-82.2587	-84.2138	-83.1471

Ligand preparation

The structure in 2 Dimension and 3 Dimension is a form of structure that must be made during molecular docking. after being drawn in 2 dimensions, then the structure is changed to 3 dimensions because in order to carry out an in molecular docking the structure of the compound must be in 3 dimensions. Geometry optimization was carried out to obtain the structure with the most stable conformation. Modification of the structure of the novel ketorolac by adding a substituent on the benzene ring in the para position (1,4) and ten design compounds were obtained. The 2 Dimension and 3 Dimension results can be seen in Table 3.

Table 3. Structure 2 Dimension and 3 Dimension of ten Compound Novel Ketorolac

Compound	Compound in 2D	Compound in 3D	IUPAC Name
Ketorolac			(S)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid
A			(S)-5-(4-(4,4,4-trifluorobutyl)benzo y l)-2,3-dihydro-1H- pyrrolizine-1-carboxylic acid
B			(S)-5-(4-(2-methoxyethyl)benzo y l)-2,3-dihydro-1H- pyrrolizine-1-carboxylic acid
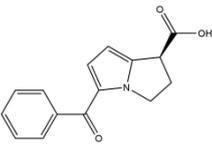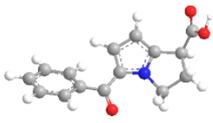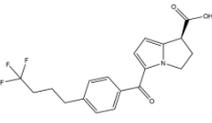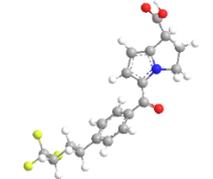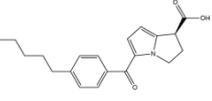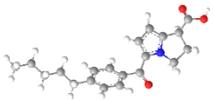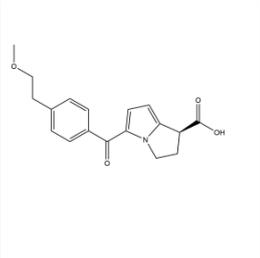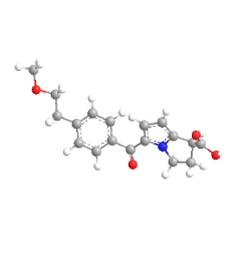
C			(S)-5-(4-pentylbenzoyl)-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid
D			(S)-5-(4-(4-aminobutyl)benzoyl)-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid
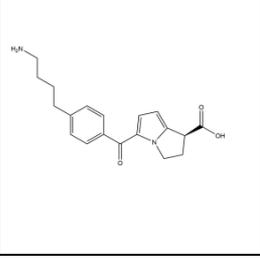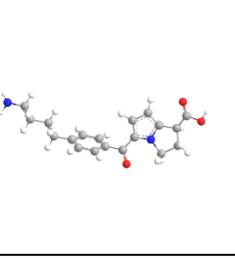
In silico docking novel ketorolac

This study aims to design and search for a novel ketorolac that works selectively on cyclooxygenase-2 (COX-2). The desired result is a compound that has good drug-receptor interaction on the cyclooxygenase-2 (COX-2) receptor and does not form a good enough interaction on the cyclooxygenase-1 (COX-1) receptor, so it is expected that it only works to inhibit cyclooxygenase-2 (COX-2). Rerank score is the value of the docking result which is the amount of Gibbs free energy, which indicates a reaction that runs spontaneously when the value is negative. The lower the rerank score indicates the most stable drug-receptor interaction and the strongest potential for binding (Kesuma *et al.*, 2018).

Table 4. Docking result Novel Ketorolac with cyclooxygenase-1 (COX-1)

Protein Target	Compound	Rerank Score			Average (kkal/mol)
		Replication 1	Replication 2	Replication 3	
Cyclooxygenase-1 (1EQG)	Ketorolac	-99.4635	-99.5073	-99.3210	-99.4306
	A	-118.3620	-118.1120	-116.9780	-117.8173
	B	-109.6870	-109.3740	-111.1570	-110.0727
	C	-114.2710	-114.2270	-94.0829	-107.5270
	D	-104.8670	-108.3370	-73.7253	-95.6431
	E	-88.7753	-102.6200	-94.2930	-95.2294
	F	-48.9542	-35.1179	-92.1185	-58.7302
	G	-94.6600	-92.7531	-81.5169	-89.6433
	H	-60.9938	-13.0961	-72.6971	-48.9290

| | I | -72.7981 | -71.3858 | -70.5416 | -71.5752 |
| | J | 3.9046 | 33.0841 | 4.9649 | 13.9845 |

Based on the results of docking (Table 4) with the cyclooxygenase-1 (COX-1) receptor, it was found that almost all compounds provide an affinity for the target protein. Ketorolac itself as a comparator compound has a rerank score of -99.4306. Some compounds that show lower rerank scores than ketorolac are compounds A, B, C . While the compounds that showed a higher rerank score than ketorolac were compounds E, F, G, H, I, J. Compound J showed a positive rerank score, these results indicated that there was no drug-receptor interaction formed on the cyclooxygenase target protein. -1 (COX-1), so it is predicted not to work on the receptor

Table 5. Docking result Novel Ketorolac with cyclooxygenase-2 (COX-2)

Protein Target	Compound	Rerank Score			Average (kkal/mol)
		Replication 1	Replication 2	Replication 3	
Cyclooxygenase-2 (1PXX)	Ketorolac	-95.6047	-95.6403	-95.9353	-95.7268
	A	-105.3800	-112.0930	-104.9530	-107.4653
	B	-91.8447	-98.9501	-54.7414	-81.8454
	C	-111.3180	-106.6890	-106.0330	-108.0133
	D	-100.2130	-111.6750	-97.1213	-103.0031
	E	-97.4249	-92.6281	-80.7686	-90.2739
	F	-115.9340	-122.1700	-121.5690	-119.8910
	G	-121.7170	-117.0900	-117.6590	-118.8220
	H	-89.9305	-98.0477	-112.605	-100.1944
	I	-84.8576	-105.7310	-55.9143	-50.4483
	J	-113.7530	-127.4480	-112.564	-117.9220

The results of docking with cyclooxygenase-2 (COX-2) receptors showed several compounds showing better affinity values than ketorolac as a comparison compound. Ketorolac itself has a rerank score of -95.7268, while compounds A, C, D, F, G, H and J show a lower rerank score than ketorolac. In contrast, compounds B, E, I showed a higher rerank score than ketorolac.

ADME and physicochemical properties Prediction

Furthermore, predictions were made on pharmacokinetic parameters, namely Absorption, Distribution, Metabolism, Excretion of ketorolac and 10 of its derivatives using the pkCSM online tool application. The compound SMILE code from ChembioDraw Ver.13. then the SMILE code is copied to the column listed on the web which will be processed. Based on table 6, it was found that all of the compounds and comparators can be absorbed in the gastrointestinal tract because they can be absorbed in the intestine very well because they have an absorption value of > 80%. Intestinal absorption is very good if >80% and not good if the value is <30% (Pires, Blundell and Ascher, 2015).

Table 6. ADME Prediction of ten Compound Novel Ketorolac

Compound	SMILES Code	Absorption (intestinal absorption in human)	Distribution (Volume Distribution in Human)	Metabolism (CYP1A2 inhibitior)	Excretion (Total Clearance)
Ketorolac	O=C(C1=CC=C2N1CC[C@@H]2C(O)=O)C3=CC=CC=C3	96.438%	-0.97 log L/kg	No	0.339 log ml/min/kg
A	O=C(C1=CC=C2N1CC[C@@H]2C(O)=O)C3=CC=C(CCCC(F)(F)F)C=C3	92.674%	-0.737 log L/kg	No	0.927 log ml/min/kg
B	O=C(C1=CC=C2N1CC[C@@H]2C(O)=O)C3=CC=C(CCOC)C=C3	97.112%	-0.851 log L/kg	No	0.493 log ml/min/kg
C	O=C(C1=CC=C2N1CC[C@@H]2C(O)=O)C3=	94.966%	-0.496 log L/kg	No	1.208 log ml/min/kg

	CC=C(CCCCC)C=C3				
D	O=C(C1=CC=C2N1CC[C@@H]2C(O)=O)C3=CC=C(CCCCN)C=C3	93.067%	0.307 log L/kg	No	0.949 log ml/min/kg
E	O=C(C1=CC=C2N1CC[C@@H]2C(O)=O)C3=CC=C(CCC(C)(C)C)C=C3	94.298%	-0.57 log L/kg	No	0.83 log ml/min/kg
F	O=C(C1=CC=C2N1CC[C@@H]2C(O)=O)C3=CC=C(CC(C4=CC=CC=C4)=O)C=C3	97.949 %	-1.03 log L/kg	No	0.399 log ml/min/kg
G	O=C(C1=CC=C2N1CC[C@@H]2C(O)=O)C3=CC=C(CC(C4=CC=C(Cl)C=C4)=O)C=C3	96.561 %	-0.988 log L/kg	No	-0.103 log ml/min/kg
H	O=C(C1=CC=C2N1CC[C@@H]2C(O)=O)C3=CC=C(CC(NC4=CC=C(F)C=C4)=O)C=C3	94.054%	-0.935 log L/kg	No	0.164 log ml/min/kg
I	O=C(C1=CC=C2N1CC[C@@H]2C(O)=O)C3=	94.601 %	-1.035 log L/kg	No	0.272 log ml/min/kg

	CC=C(CC(NC4 =CC=CC=C4)= O)C=C3				
J	O=C(C1=CC=C 2N1CC[C@@H]2C(O)=O)C3= CC=C(CC(NC4 =CC=C(CC(C)(C)C)C=C4)=O) C=C3	93.2 %	-0.85 log L/kg	No	0.073 log ml/min/kg

After absorption has passed, then the next is the distribution stage. The distribution prediction using the pkCSM tool predicted the VDSS value. The volume of distribution at steady state (VDSS) is the theoretical volume that the total dose of drug needs to be distributed evenly to provide the same concentration as in blood plasma. The higher the VDSS value, the more drug content is distributed to the tissues rather than the plasma. A compound is said to have a low Volume of Distribution if the Log VDSS value is <-0.15, and high if it is >0.45. The results of the Log VDSS value obtained were only 1 compound which was evenly distributed, namely compound D, while the other compounds, including the control compound, had low VDSS (Pires, Blundell and Ascher, 2015).

Metabolism is the process by which the liver converts drugs into their metabolites chemically in the body. CYP450 is a detoxification enzyme that is found in the liver. There are several isoforms of CYP450, one of which is CYPIA2. The prediction of the metabolic processes of CYPIA2 inhibitors is inhibition of CYPIA2 isoforms. Based on table 6 it was found that all compounds were not metabolized by CYPIA2 inhibitors (Pires, Blundell and Ascher, 2015).

Total and renal clearance of OCT2 is used to measure the excretion of a drug. Total clearance is a combination of hepatic and bile metabolism where excretion via the kidneys is both related to bioavailability and determines the dose level in a steady state state . The predicted total clearance of all compounds and controls is -0.103 log ml/min/kg to 1.208 log ml/min/kg (Pires, Blundell and Ascher, 2015).

Table 7. Physicochemical Properties of ten Compound Novel Ketorolac

Compound	Physicochemical Properties			
	Molecular Weight (g/mol)	LogP	HBA	HBD
Ketorolac	255.273	2.291	3	1
A	365.351	4.176	3	1
B	313.353	2.4799	4	1
C	325.408	4.0237	3	1
D	326.396	2.5724	4	2
E	339.435	4.2697	3	1
F	373.408	3.7164	4	1
G	407.853	4.3698	4	1
H	406.413	3.6113	4	2
I	388.423	3.4722	4	2
J	458.558	5.0608	4	2

An important parameter in drug development is the physicochemical prediction of a compound, where the prediction is based on Lipinski's five laws. Lipinski's fifth law states that a compound can be absorbed if it has physicochemical properties, namely molecular weight ≤500 g/mol, log P ≤5, number of donor H-bonds ≤5, H-bond acceptors ≤10(Kesuma *et al.*, 2018). Based on the results, it was found that almost all compounds including ketorolac as the comparator complied with Lipinski's 5th law (table 7) except for compound J which had a Log P value slightly higher than 5, namely 5.0608.

Table 8 Toxicity class based on Globally Harmonized System (GHS)

Class	Description	LD50 Dose (mg/Kg)
I	fatal if swallowed	LD50 ≤ 5
II	fatal if swallowed	5 < LD50 ≤ 50
III	toxic if swallowed	50 < LD50 ≤ 300
IV	harmful if swallowed	300 < LD50 ≤ 2000
V	may be harmful if swallowed	2000 < LD50 ≤ 5000

VI	non-toxic	LD50 > 5000

Toxic doses are often reviewed from the LD50 value in mg/kg body weight. LD50 is the average lethal dose which means at which dose with 50% of the test subjects died when exposed to a compound. Toxic class is determined according to a chemical labeling classification system based on Globally Harmonized System of Classification and Labeling of Chemical (GHS). All compounds including control compounds belong to toxicity class 3 (300<LD50≤2000) which according to the GHS classification are classified as drugs with moderate toxicity, dangerous if swallowed (Banerjee *et al.*, 2018), it can be seen at table 8 and table 9.

To determine the toxicity of a compound can be done with the Ames toxicity test, a method widely used to assess the mutagenic potential of compounds using bacteria. A positive test result indicates that the compound is mutagenic and therefore can act as a carcinogen. To find out the drug does not damage the liver using a hepatotoxicity test (Pires, Blundell and Ascher, 2015). Based on the prediction of toxicity, compound A showed the results of the Ames Toxicity test which was not mutagenic and not toxic to the liver. Meanwhile, compounds B to J showed the results of the Ames Toxicity test which were not mutagenic, but predicted to be toxic to the liver, it can be seen at table 9.

Table 9. Toxicity Prediction of ten Compound Novel Ketorolac

Compound	LD50 (mg/Kg)	Class	Ames Toxicity	Hepatotoxicity
Ketorolac	189	3	No	No
A	200	3	No	No
B	189	3	No	Yes
C	189	3	No	Yes
D	189	3	No	Yes
E	189	3	No	Yes
F	189	3	No	Yes
G	200	3	No	Yes
H	200	3	No	Yes
I	200	3	No	Yes
J	200	3	No	Yes

From all the data seen from the affinity for A, the compound that is selective for cyclooxygenase-2 (COX-2) and has a better affinity than ketorolac is compound J. However, compound J is predicted to be hepatotoxic, while compounds that are not hepatotoxic are compound A. However, compound A is not selective for cyclooxygenase-2 (COX-2) although it also shows a better affinity for ketorolac than ketorolac. Compound A also showed a better affinity for cyclooxygenase-1 (COX-1) than ketorolac. The good physicochemical properties of compound A also comply with Lipinski's 5th law which is predicted to be administered orally (Kesuma *et al.*, 2018). Compound J has a Log P value slightly higher than 5, which is 5.0608.

CONCLUSION

Based on the research data, the novel ketorolac can be further developed as a selective NSAID drug. The best compound is compound J because it does not show an affinity for cyclooxygenase-1 (COX-1), has a good ADME profile even though it is predicted to have hepatotoxic properties, so further in-depth research is needed to see how much hepatotoxicity it has and shows a log value. P is slightly above 5 which is not in accordance with Lipinski's 5th law. It can also be further developed from compound J which is a novel ketorolac to be modified so that it is not hepatotoxic.

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