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In silico and *in vitro* anti-inflammatory evaluation of 2,6-bis-(3′-ethoxy, 4′-hydroxybenzylidene)-cyclohexanone, 2,6-bis-(3′-Bromo,4′-methoxybenzylidene)-cyclohexanone, and 2,6-bis- (3′,4′-dimethoxybenzylidene)-cyclohexanone

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ABSTRACT

The aim of this research is to design the new mono-carbonyl analogs of curcumin, synthesize the molecules, and determine its activity in cyclooxygenase inhibition *in vitro* and *in silico*. New design MACs were performed by the Quantitative Structure–Activity Relationship (QSAR) study using the BuildQSAR program. 2,6-bis-(3′-ethoxy, 4′-hydroxybenzylidene)-cyclohexanone, 2,6-bis-(3′-Bromo, 4′-methoxybenzylidene)-cyclohexanone, and 2,6-bis-(3′,4′-dimethoxybenzylidene)-cyclohexanone had been synthesized using aldol condensation reaction. The anti-inflammatory assay was performed to measure the level of malondialdehyde. *In silico* studies were carried out to evaluate the activity of cyclooxygenase inhibition in cyclooxygenase-1 and cyclooxygenase-2 specific proteins. Molecular operating environment program was used for protocol docking. The results of the QSAR study reveal the good relationship of anti-inflammatory activities. The *in vitro* anti-inflammatory activities of 6-bis-(3′-ethoxy, 4′-hydroxybenzylidene)-cyclohexanone, 2,6-bis-(3′-Bromo, 4′-methoxybenzylidene)-cyclohexanone, and 2,6-bis-(3′,4′-dimethoxybenzylidene)-cyclohexanone indicate the promising potential to inhibit cyclooxygenase enzyme with IC_{50} 13.53 µM, 11.56 µM, and 20.52 µM, respectively. The *in silico* evaluation showing that O atoms (47, from ketones) of 2,6-bis-(3′-Bromo, 4′-methoxybenzylidene)-cyclohexanone interact with ARG120 and TYR355 through H acceptor.

INTRODUCTION

For a decade, computational chemistry has significantly developed. Medicinal chemistry is one of the subjects that were affected by this phenomenon, especially in discovering new drugs. Quantitative Structure-Activity Relationship (QSAR) is one of the alternative approaches to find new drugs supposedly more effective and efficient (Arba *et al*., 2019). The study of QSAR assumes that there is a quantitative relationship between the molecular structure with its biological activity of a molecule (El-Hamamsy, 2017; Modi *et al*., 2018; Ujihara *et al*., 1988). The molecular structure is not only describing atoms and molecules bonding but also its physical and chemical properties (Desai *et al*., 2013).

Anti-inflammatory drugs are one of the agents that most widely used to treat the inflammation as a local protective response caused by damage in tissue. Physical trauma, damaging chemicals, or microbiology substances cause tissue damage. The inflammation process has its functions to destroy, reduce, or localize destructive agents and damaged tissue. Symptoms of inflammation were signed by swelling/edema, redness, heat, pain, and changes in its functions (Agustina *et al*., 2015).

Noted that mono-carbonyl analogs of curcumin (MACs) (Fig. 1) have antibacterial activity (Wijianto *et al*., 2019), anticancer and chemo-preventive effects on certain cancers with low toxicity (Anand *et al*., 2008; Kurnia *et al*., 2019), antioxidant (Selvam *et al*., 2005; Yuliarti and Nugroho, 2013), and anti-inflammatory by inhibit cyclooxygenase (Hayun *et al*., 2019; Yuliarti and Nugroho, 2013). Curcumin had much biological activity, but its therapeutic

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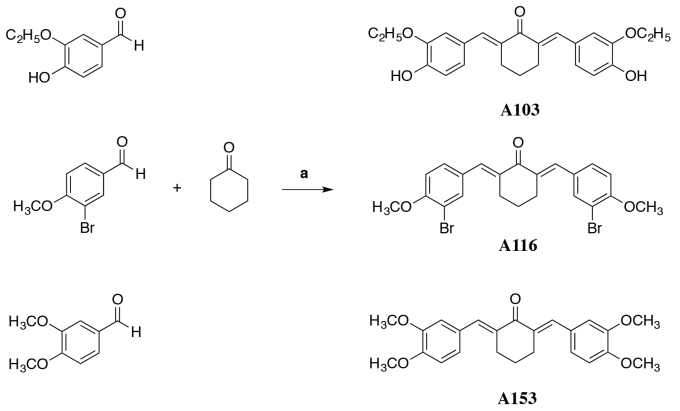


Scheme 1. Reagent and condition of synthesis: a) THF and HCl; 6-8 h and 60°C (Wijianto *et al*., 2019).

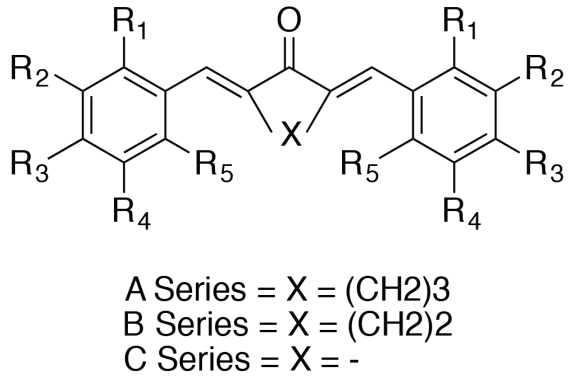


Figure 1. Structure of mono-carbonyl analogs of curcumin (Wijianto *et al*., 2019).

usage of curcumin limited by low water solubility, chemical, and metabolic stability and relatively weak *in vivo* bioavailability (Anand *et al*., 2008). The study of curcumin has modified to find that the analogs had better physical and chemical properties, better biological activity as well. Mono-carbonyl analogs of curcumin with cyclic as central can inhibit the cyclooxygenase enzyme (Sardjiman *et al*., 1997). Therefore, it is necessary to perform QSAR study for MACs to determine its anti-inflammatory activity leading to found a new anti-inflammatory drug. The aim of this research is to design the new MACs, synthesize the molecules, and assess its activity in cyclooxygenase inhibition for anti-inflammatory activity.

MATERIALS AND METHODS

Data set

The data set of structure and anti-inflammatory activity used in this study derived from MACs is shown in Table I. Anti-inflammatory activity was determined as pIC_{50} (negative log of the IC_{50} in Molar) explaining the ability of each compound in cyclooxygenase inhibition.

Chemical

3,4-dimethoxybenzaldehyde, 3-ethoxy-4-hydroxybenzaldehyde, 3-Bromo-4-methoxybenzaldehyde, cyclohexanone, tetrahydrofuran (THF), Hydrochloric acid, ethanol, ethyl acetate, sodium hydroxide, methanol, 2-hydroxyl acid), trichloroacetic acid, and arachidonic acid, all obtained from Sigma-Merck. Buffer Tris-HCl (pH 7.4) from Ultrol®. All material compounds used were analytical grade.

Table 1. Data sets of compounds with anti-inflammatory activity

Comp.	Substituent					pIC_{50} Cox Inh.
	R_1	R_2	R_3	R_4	R_5	
A1	-H	$-OCH_3$	-OH	-H	-H	5.10
A12	-H	$-C_2H_5$	-OH	$-C_2H_5$	-H	4.52
A15	-H	$-OCH_3$	-OH	$-OCH_3$	-H	4.11
B1	-H	$-OCH_3$	-OH	-H	-H	6.04
B12	-H	$-C_2H_5$	-OH	$-C_2H_5$	-H	4.81
B15	-H	$-OCH_3$	-OH	$-OCH_3$	-H	4.10
C1	-H	$-OCH_3$	-OH	-H	-H	4.87
C11	-H	$-CH_3$	-OH	$-CH_3$	-H	4.80

Instrument

Quantum mechanical calculations and descriptors were performed using the molecular operating environment (MOE) version 2018.0101 licensed. The QSAR model was calculated using the BuildQSAR open source program. BUCHI Melting Point B-540 with temperature gradient at 5°C/minute was used as a melting point test. The purity of compounds was measured using HPLC Elite La-Chrome®. JEOL® spectrophotometer of 500 MHz was used to measure ^{1}H-NMR Spectrum.

Procedure

Molecular modeling

Molecular modeling studies were carried out using the MOE 2018.01.01 software. All the structures of MACs were depicted in 2D in the "builder" menu of MOE. Optimization of the structure of compounds was performed using MOPAC (semi-empirical quantum chemistry algorithms) in MOE. Semi-empirical of the AM-1 method with gradient 0.01 was used to determine the quantum chemical algorithm.

Calculation of descriptors

The optimized structures were calculated using single-point calculations to obtain the value of descriptors. All descriptors were provided by all molecular descriptors that available in 2D and i3D MOE databases. Molecular descriptors data were used, such as highest occupied unoccupied molecular orbitals energy, lowest unoccupied molecular orbitals energy, hydration energy, partition coefficient, polarizability, molecular volume and mass, total energy, the heat of formation, molar refractivity, molecular surface area, amphiphilic, hydrophobic volume, globularity and dipole moment.

Statistical analysis of cyclooxygenase inhibition activity of test compounds (expressed as pIC_{50}) was performed using the multilinear regression (MLR) analysis and the GA-MLRA technique as applied in the BuildQSAR program. The best equation model obtained from the MLR analysis was used to predict the IC_{50} value. The best equation model was selected by considering the value of the statistical parameters. This approach allows to select the best models with several following characteristics, such as high regression coefficient (r), high Fisher coefficient (F), and low standard deviation (s) (Miladiyah *et al*., 2018; Sudarmanto and Oetari, 2007; Wijianto *et al*., 2019). The best equation model was validated using the leave-

one-out cross-validation to determine its ability and robustness in predicting, which is confirmed by the Q2 cross-validation coefficient and quadratic prediction errors (sPRESS).

Docking studies

In silico study was conducted to known anti-inflammatory activity through inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) using test proteins with ID.1EQH and 3PGH that available in the protein data bank. Protein validation and docking process were performed using the alpha PMI method on placement and induced fit on refinement scoring by GBVI/ WSA dG as protocols. Protein validated was selected based on the RMSD (Root Mean Square Deviation) value.

Synthesis of 2, 6-bis-(3'-ethoxy, 4'-hydroxybenzylidene)-cyclohexanone

About 6.018 mmol of 3-ethoxy-4-hydroxybenzaldehyde, 3.009 mmol of cyclohexanone, 2 ml of THF, and 0.2 ml of concentrated hydrochloric acid were mixed and stirred at room temperature for 2 hours. Then, the temperature was raised to 50°C–60°C, and stirring was continued for 8 hours until the reaction was completed. Crude products were washed out by adding ethanol and cold water with a ratio of 1:1. The mixture filtered then re-washed out by ethanol and cold water with ratio 3: 2 until pH 7–8 reached out. Powdery residue in Buchner dried in the oven. Recrystallization performed by dissolving the residue in ethanol, and then water was added until a precipitate was formed. Thin-layer chromatography (TLC) and melting point tests were conducted to determine the purity of the product qualitatively.

Synthesis of 2, 6-bis-(3'-Bromo, 4'-methoxybenzylidene)-cyclohexanone

About 4.650 mmol of 3-Bromo-4-methoxybenzaldehyde, 2.325 mmol of cyclohexanone, 2.0 mL of THF, and 0.25 ml of concentrated Hydrochloric acid were mixed and stirred up to 2 hours at room temperature. Then, the temperature was raised to 50–60°C, and stirring was continued for 8 hours until the reaction was completed. The crude product was washed out with ethanol and cold water (1:1) then filtered by Buchner. The second washed out with ethanol and cold water (3:2) up to pH 7–8 reached out, then filtered and dried in the oven. Recrystallization conducted by dissolving the residue in acetone, and then cold water was added until a precipitate was formed. TLC and melting point examination were performed to determine the purity of the product qualitatively.

Synthesis of 2, 6-bis-(3', 4'-dimethoxybenzyllidene)-cyclohexanone

About 6.018 mmol of 3, 4-dimethoxybenzaldehyde, 3.009 mmol of cyclohexanone, 2.0 ml of THF, and 0.25 ml of concentrated hydrochloric acid were mixed and stirred at room temperature for 2 hours. Then, the temperature was raised to 50°C–60°C, and stirring was continued for 8 hours until the reaction was completed. The crude product was washed out by ethanol: cold water (1:1) then filtered by Buchner. Second, washed out for residue was performed by different ratios of ethanol and cold water (3:2) until pH 7–8 reached out. The residue was filtered and dried in the oven. Recrystallization performed by dissolving the residue in acetone, and then cold water was added until a precipitate formed. TLC and melting point tests carried out to find out the purity of the product qualitatively.

Purity test by high-performance liquid chromatography (HPLC)

Analysis of purity was performed using HPLC Elite La-Chrome®, and for comparison, we used aldehydes as each starting material. Synthesis products and starting material were analyzed at 100 ppm concentration. Measurements were determined at UV wavelength of 350 nm and a mobile phase of 80:20 acetonitrile and water with a flow rate of 1 ml/ minute, 100 psi pressure, and 20 µl volume injection in column C_{18}.

From the results of the purity test conducted by HPLC, each compound can be separated appropriately using the system. The test results showed that the synthesized compound was pure, which was confirmed using the respective starting material as seen in Figure 2 and Table 2.

2, 6-bis-(3′-ethoxy, 4′-hydroxybenzylidene)- cyclohexanone (A103)

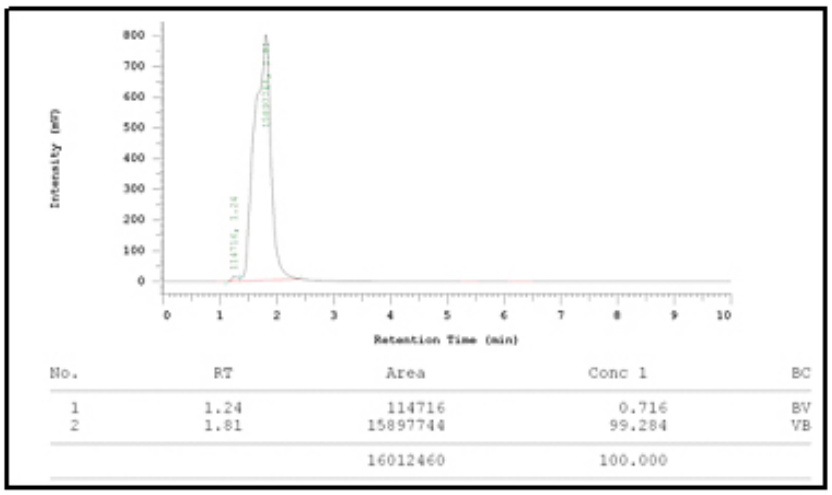
Greenish yellow powder, yield 57.84%, mp 156.1°C–157.8°C; Rf = 0.3, ethyl acetate:$CHCl_3$(1:20); IR γ (cm−1) (KBr): 3,368.86 (-OH hydrogen bonded); 2,979.35 (=C-H stretching aromatik); 2,881.23 (=C-H stretching alkena); 1,658.85 (C=C stretching alkena); 1595,93 (C=O stretching αβ,α'β'-unsat); 1,433.48 (C=C stretching aromatik); 1,399.68 (C-H bending alifatik); 1,288.36 (C-CO-C coupled stretching and bending); 1,213.24 (C-O-C stretching); 1,161.49 (C-OH stretching); MS (EI-MS, m/z)394 [M++2H]; 61 (base line); ^{1}H-NMR (500 MHz, ppm, DMSO-d6): δ 1.384 (3H, t, C9′, CH_3 aliphatic), δ 1.727 (1H, qui, H4, CH_2 cyclohexanone); δ 2.885 (2H, qui, H3,5, CH_2 cyclohexanone); δ 4.065 (2H, k, H8′, CH_2 aliphatic); δ 6.878 (1H, d, *J* = 8Hz H5′, Ar-CH); δ 7.040 (1H, dd, *J*1 = 10 Hz *J*2 = 1.5Hz, H6′, Ar-CH); δ 7.100 (1H, s, H2′, Ar-CH); δ 7.551 (1H, s, H7′, =C-H alkena); δ 9.437 (1H, s, Ar-OH).

2, 6-bis-(3′-bromo, 4′-methoxybenzylidene)-cyclohexanone (A116)

Yellow crystal, yield 62.76%, mp 182.1°C–182.5°C, Rf = 0.58, ethyl acetate : n-hexane (1:9); IR γ (cm−1) (KBr): 2,939.52 (=C-H *stretching* aromatik); 2,839.22 (-C-H *stretching* alkena); 1,658.78 (C=C *stretching* alkena); 1,589.34 (C=O *stretching* αβ,α′β′-unsat); 1,496.76 (C=C *stretching* aromatik); 1,288.45 (C-CO-C *coupled stretching and bending*); 1,157.29 (C-O-C *stretching*); 551.41 (C-Br *stretching*); MS (EI-MS, m/z): 492 [M+] (100%); ^{1}H-NMR (500 MHz, DMSO-d6): δ 1.726 (1H, *qui*, H_4, CH_2 cyclohexanone); δ 2.866 (2H, *t*, $H_{3,5}$, CH_2 cyclohexanone); δ 3.899 (3H, *s*, $H_{8'}$, O-CH_3); δ 7.200 (1H, *d*, *J* = 8.5 Hz $H_{5'}$, Ar-CH); δ 7.539 (1H, *s*, $H_{7'}$ =CH alkena); δ 7.577 (1H, *dd*, *J*1 = 9, *J*2 = 2.5 Hz, $H_{6'}$Ar-CH); δ 7.772 (1H, *d*, *J* = 2 Hz $C_{2'}$Ar-CH).

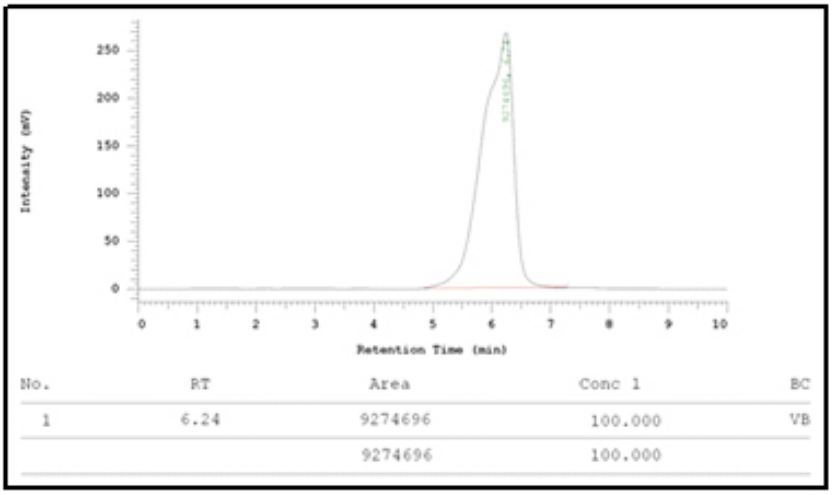
2, 6-bis-(3′, 4′-dimethoxybenzyllidene)-cyclohexanone (A153)

Yellow powder, yield 42.02%, mp 149.7°C–151.6°C, Rf = 0.23, DCM : n-hexane (2:1); IR γ (cm^{-1}) (KBr): 2,931.42 (=C-H *stretching* aromatik); 2,837.33 (-C-H *stretching* alkena); 1,650.70 (C=C *stretching* alkena); 1,595.85 (C=O *stretching* αβ,α′β′-unsat); 1,450.24 (C=C *stretching* aromatik); 1,334.92 (C-H *bending* alifatik); 1,285.56 (C-CO-C *coupled stretching and bending*); 1,139.51 (C-O-C *stretching*).; MS (EI-MS, m/z): 394 [M++2H]; 131 (base line); ^{1}H-NMR (500 MHz, DMSO-d6): δ 1.732 (1H, *qui*, H_4, CH_2 cyclohexanone); δ 2.912 (2H, *qui*, $H_{3,5}$, CH_2 cyclohexanone), δ 3.830 (6H, *s*, $H_{8',9'}$, O-CH_3); δ 7.043 (1H,



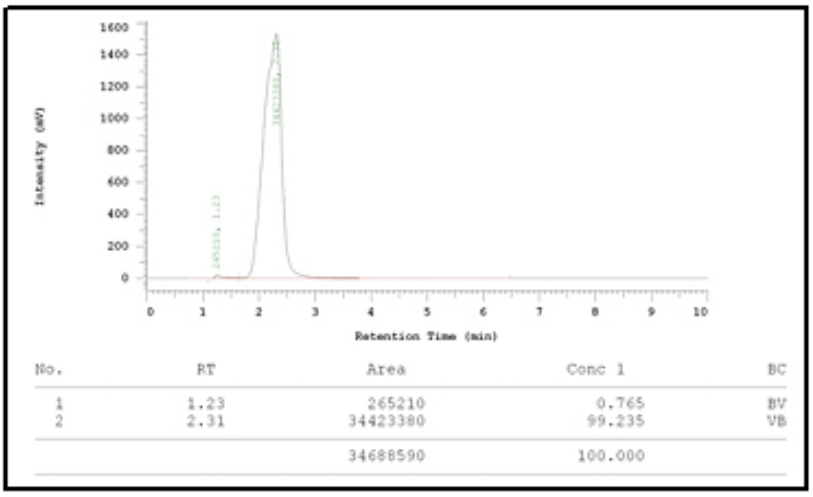
No.	RT	Area	Conc 1	BC
1	1.24	114716	0.716	BV
2	1.81	15897744	99.284	VB
		16012460	100.000	

A103



No.	RT	Area	Conc 1	BC
1	6.24	9274696	100.000	VB
		9274696	100.000	

A116



No.	RT	Area	Conc 1	BC
1	1.23	265210	0.765	BV
2	2.31	34423380	99.235	VB
		34688590	100.000	

A153

Figure 2. Chromatogram of the purity test results.

d, *J*=8.5 Hz, $H_{6'}$, Ar-CH); δ 7.139 (2H, *s*, $H_{2',5'}$, Ar-CH); δ 7.702 (1H, *s*, $H_{7'}$, =CH alkena).

In vitro anti-inflammatory assay

Several stages were performed for *in vitro* anti-inflammatory assay started by the platelet-rich plasma (PRP) preparation from fresh human blood. The non-enzymatic method was used in this study by measuring the level of malondialdehyde (MDA) in fluorescence spectrophotometer.

About 0.5 mL of plasma (PRP) and 5 µl of 3.13–50.0 µM sample [prepared by diluted in Dimethyl sulfoxide (DMSO)] are placed into Eppendorf tube then pre-incubated for 5 minutes at 37°C. About 100 µl of the fresh arachidonic acid solution was added then incubated for 30 minutes at 37°C. The reaction was stopped using 1 ml of TBA reagent in cold temperature. The mixture was heated at 80°C for 15 minutes and centrifuged at 3,000 rpm for 15 minutes. The absorbance was determined using fluorescence spectrophotometry in 553 nm wavelength for emission and 510 nm wavelength for excitation. DMSO was used as a control.

RESULTS AND DISCUSSION

QSAR study provides the best equation model to design new curcumin analogs of mono-ketone as an anti-inflammatory. QSAR studies were conducted several lead compounds that have reported having cyclooxygenase inhibitory activity (Sardjiman *et al*., 1997). As a result, 80 descriptors were selected that founded in 2D and i3D molecular database (Table 3).

Geometry optimization was performed toward optimized structure using the AM-1 semi-empirical method. Based on the previous study, the best analysis results on the QSAR series of curcumin analog compounds as antioxidants are shown in the AM-1 method compared to the PM-3 method (Sudarmanto and Oetari, 2007). In the previous study, AM-1 was used to predict curcumin compounds and their derivatives as class µ GST inhibitors as well (Istyastono *et al*., 2003).

Model 1 (Table 4) shows the best statistical parameter values. It shows the highest values of *r* and *F* by 0.999 and 501.319, respectively, and the smallest standard deviation (*s*) value of 0.042 compared to other models. The cross-validation value also shows a good statistical parameter, where the 0.991 Q2 value is the highest compared to other models and has the lowest sPRESS value of 0.077. Tables 5 and 6 show new MACs compounds with anti-inflammatory activity obtained from the best equation model.

Based on Table 5 results, we can observe that the equation we used is to provide good prediction value. Good prediction value determines by the small number of the residual value from each compound. Correlation analysis between observations over calculation value is shown in Figure 3. The best equation provided by model 1 is also used to predict three new MACs compounds.

In vitro evaluation of new mono-ketone curcumin analog compound

Three compounds, A103; A116; and A153, were used to determine their anti-inflammatory activity dissolved in DMSO. These compounds were obtained based on our QSAR studies, which have the best predictive values to be analyzed for *in vitro* anti-inflammatory assay. In this study, aspirin was used as a positive control.

Table 2. Area and retention time of HPLC purity test results.

Compound	Retention time (minute)	Area (%)
2,6-bis-(3'-ethoxy, 4'-hydroxybenzylidene)-cyclohexanone (A103)	1.81	99.284
3-ethoxy-4-hydroxybenzaldehyde (SM of A103)	1.36	100.00
2,6-bis-(3'-bromo,4'-methoxybenzylidene)-cyclohexanone (A116)	6.24	100.00
3-Bromo-4-methoxybenzaldehyde (SM of A116)	1.68	47.555
2,6-bis-(3',4'-dimethoxybenzylidene)-cyclohexanone (A153)	2.31	99.235
3,4-dimethoxybenzaldehyde (SM of A153)	2.18	79.230

Table 3. List of descriptors used in the QSAR study.

Symbol	Descriptor
Apol	Sum of Polarizability
logP(o/w)	Partition coefficient in octanol / water
Mr	Molar Refractivity
vdw-area	Van der Waals surface area
vdw-volum	Van der Waals volume
AM1_dipole	Dipole moment
AM1_E	Total Energy
AM1_Eele	Electronic energy
AM1_HF	Heat of formation
AM1_HOMO	Energy of the highest occupied molecular orbital
AM1_IP	Ionization potential
AM1_LUMO	Energy of the lowest unoccupied molecular orbital
ASA	Water accessible area
ASA_H	Total hydrophobic surface area
vsurf_A	Amphiphilic moment
vsurf_CP	Critical packing parameter
vsurf_CW	Capacity factor
vsurf_D	Hydrophobic volume
vsurf_EDmin	Lowest Hydrophobic volume
vsurf_EWmin	Lowest Hydrophilic energy
vsurf_G	Surface globularity
vsurf_HB	H-bond donor capacity
vsurf_HL	Hydrophilic-lipophilic balance
vsurf_ID	Hydrophobic integy moment
vsurf_IW1	Hydrophilic integy moment
vsurf_R	Surface rugosity
vsurf_S	Interaction field area
vsurf_V	Interaction field volume
vsurf_W	Hydrophilic volume
vsurf_Wp	Polar volume

As a standard gold method, anti-inflammatory assay with cyclooxygenase inhibition activity in human blood samples was used in our study. PRP isolated from human blood was used as a source of cyclooxygenase and arachidonic acid as the substrate was used to stimulate the inflammation. TBA was used to form Malondialdehyde-TBA (MDA-TBA) complex.

Five concentrations examined from each compound show inhibition activity of COX (Fig. 4). There was a correlation between increasing of concentration and the percentage of

Table 4. Parameter statistical of MLR result.

Model	*n*	*m*	Descriptor			Statistical value					
			*X*1	*X*2	*X*3	*r*	*s*	*F*	*Q*2	sPRESS	sDEP
1	8	3	vsurf_HB1	Vsurf_D7	Vsurf_ID8	0.999	0.042	501.319	0.991	0.077	0.058
2	8	2	vsurf_Wp3	vsurf_IW3		0.920	0.288	13.688	0.517	0.509	0.43
3	8	1	vsurf_D7			0.853	0.348	16.049	0.431	0.504	0.467

n = number of data, *m* = number of variables, *r* = coef. correlation, *s* = standard of error, *F* = coef. Fisher, SPress = Predictive Error Sum of Squares.

Table 5. The best equation model to calculated calculation-value of cyclooxygenase inhibition.

Equation:
pIC_{50} = + 0.0461 (± 0.0034) vsurf_D7 + 0.0110 (± 0.0023) vsurf_HB1 - 2.2761 (± 0.3235) vsurf_ID8 - 10.0719 (± 0.9317)

Comp.	Descriptor			pIC_{50} calc.	pIC_{50} obs.	Residual
	vsurf_D7	vsurf_HB1	vsurf_ID8			
A1	171.375	245.75	0.624	5.09	5.10	−0.01
A12	147.5	232.5	0.346	4.61	4.52	0.09
A15	126.5	288.875	0.372	4.05	4.11	−0.06
B1	171	273.375	0.343	6.03	6.04	−0.01
B12	147.5	247.125	0.263	4.71	4.81	−0.10
B15	127.25	304.75	0.444	4.15	4.10	0.05
C1	159.125	284.25	0.686	4.86	4.87	−0.01
C11	136.5	268.25	0.183	4.82	4.80	0.02

Table 6. Descriptor value of new design mono-carbonyl analogs of curcumin with anti-inflammatory activity.

Equation:
pIC_{50} = + 0.0461 (± 0.0034) vsurf_D7 + 0.0110 (± 0.0023) vsurf_HB1 - 2.2761 (± 0.3235) vsurf_ID8 - 10.0719 (± 0.9317)

Comp.	Descriptor			pIC_{50} calculation
	vsurf_D7	vsurf_HB1	vsurf_ID8	
A103	175.75	226.75	0.6233243	4.85
A116	189.375	232.625	0.9509437	4.88
A153	158.625	206.75	0.3499872	4.66

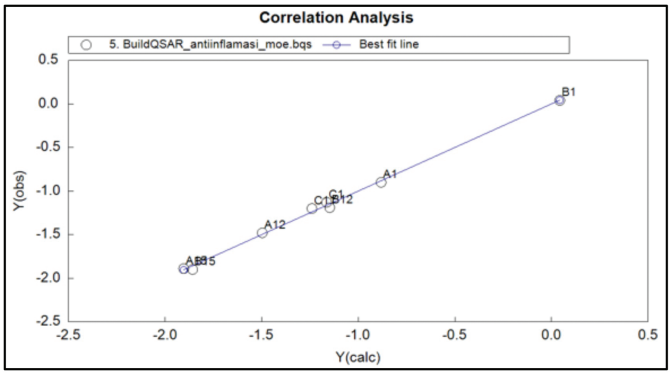


Figure 3. Correlation analysis between calculation and observation QSAR.

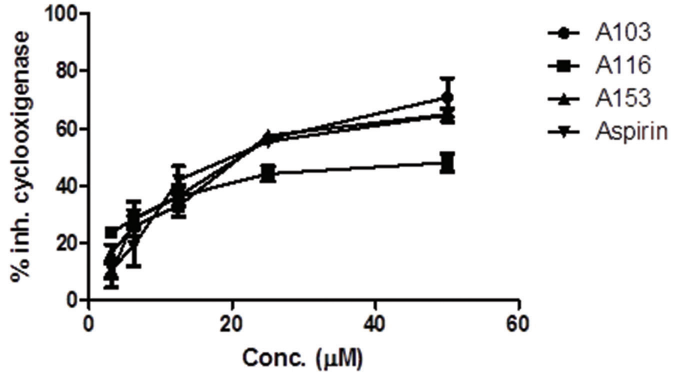


Figure 4. Percentage of cyclooxygenase inhibition.

cyclooxygenase inhibition. We have verified that the highest concentration will provide cyclooxygenase inhibition activity. We found that A116 at 3.125 µg/mL concentration has the best inhibition percentage values (23.77 ± 3.18). The ability of A116 to inhibit cyclooxygenase is higher than positive control aspirin as well (10.79 ± 5.34). The presence of halogen and methoxy groups on the benzene structure exerts a resonant and induction effect, which causes more potent cyclooxygenase inhibition activity.

The percentages of cyclooxygenase inhibition were used to determine the IC_{50} that indicates the ability of a compound to inhibit 50% cyclooxygenase. The smallest IC_{50} value indicates the best cyclooxygenase inhibition ability. IC_{50} values from each

compound are shown in Table 7. Efforts to compare the results of *in vitro* tests of new compounds with existing mono-carbonyl curcumin compounds were not carried out because they only used IC_{50} value of cyclooxygenase inhibition data in the process of designing compounds *in silico* can be seen in Table 7.

Result of study docking

In silico studies computationally were conducted over COX-1 and COX-2 receptor to determine the cyclooxygenase enzyme inhibition of new analogs of curcumin mono-ketone obtaining from QSAR study and those previously had been proven *in vitro* assay. MOE 2018.01.01 was used as a tool. All proteins downloaded then used in this docking study and then prepared in the 'sequence editor' menu of the software. The proteins are containing one strand of amino acid residue without a molecule of water. Autocorrect was performed after the protein was ready to be validated. The validated protein used in docking studies is shown in Figure 5.

The validity of protein was verified by the value of RMSD as a parameter used to evaluate the similarity of two structures. The similarity was measured based on the different distances of similar atoms. RMSD value < 2 indicates that the protein is valid and ready to use for further docking studies. The RMSD value of each protein is shown in Table 8. The results of the docking study show the interaction between the test compounds, whereas the protein target is shown in Figures 6 and 7.

ARG120 and TYR355 are the critical receptors necessary for the activity of cyclooxygenase-1 (COX-1) enzyme inhibition. It explains from the 2D and 3D interaction between native ligand (flurbiprofen) and proteins. For A103, O atom (55, from the ketone) interacts with N atom from ARG120 over H-acceptor. For A116, NH2 from ARG120 and OH groups from TYR355 interact through the H acceptor with O atoms (47, from ketones). For A153, C atom (11, from methoxy in the aromatic ring) interacts with 6-ring (benzene aromatic) of TYR385 over H-pi. For aspirin, O atom (6, from aldehydes) interacts with NH2 from ARG120 and OH from TYR355 over H-acceptor.

Table 7. IC_{50} values of COX inhibition.

Comp.	IC_{50} values (μM)
A103	13.53 ± 1.09
A116	11.56 ± 2.89
A153	20.52 ± 6.13
Aspirin	9.63 ± 3.77

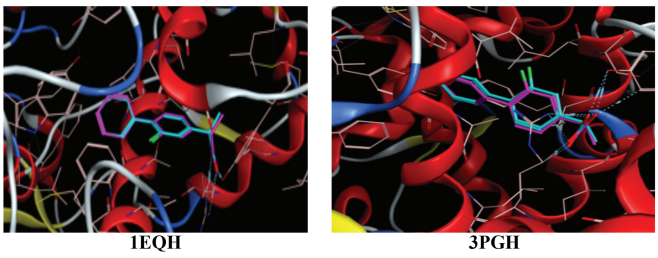


Figure 5. Appearance of validation proteins test.

Table 8. RMSD value of protein test.

ID. Protein	RMSD value	Result
1EQH	0.1695 < 2	Valid
3PGH	0.3109 < 2	Valid

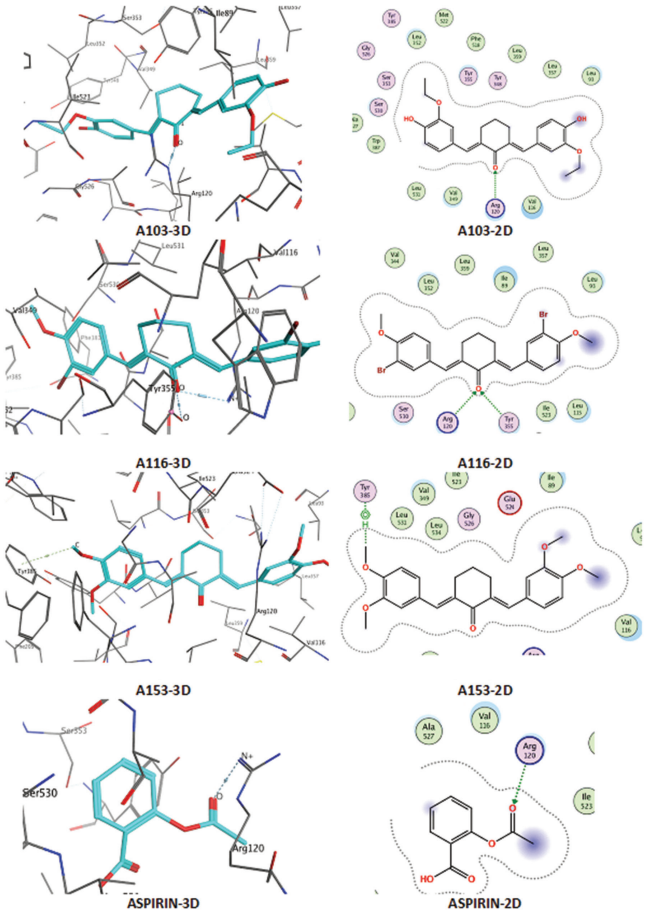


Figure 6. Interaction of test ligands on 1EQH protein.

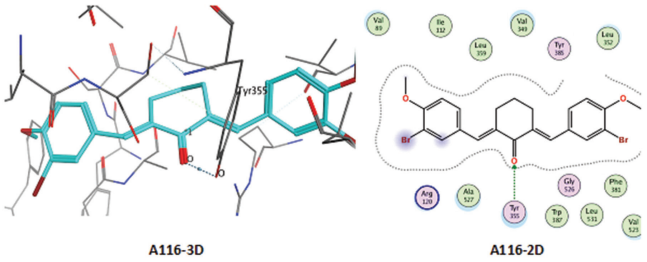


Figure 7. Interaction of test ligands on 3PGH protein.

Table 9. *s* value of new mono-carbonyl analogs of curcumin.

Comp.	*S* values	
	1EQH	3PGH
A103	−4.3012	-
A116	−5.7313	−5.4391
A153	−5.7654	-
Aspirin	−5.8078	-

The results of 2D and 3D interactions between native ligands (flurbiprofen) and proteins test known that ARG120 and TYR355 are critical receptors that play an essential role in the cyclooxygenase-2 (COX-2) inhibitory activity of 3PGH proteins. For A116, O atom (47, from ketone) interacts with OH group from TYR355 over H-acceptor. For A103 and A153, *in silico* study, results show no interaction between these two compounds and 3PGH protein. Thus, it can conclude that A103 and A153 do not have inhibition activity over the COX-2 enzyme. A similar pattern of results obtained for aspirin does not show interaction with the test protein as well. The findings are directly in line with previous findings that aspirin works to inhibit the COX-1 enzyme as irreversible (Kalantzi *et al*., 2012).

After the docking process, the *S* value (Table 9) of each test ligand was found, which described the strength of the bond affinity that occurs between the test ligand and the target protein. The smallest *S* value explains its more excellent affinity bond leading to the better for its biological activity.

CONCLUSION

The results show that the new curcumin analogs of mono-ketone compounds obtained from QSAR study using BuildQSAR have anti-inflammatory activity through their inhibition over cyclooxygenase enzyme, which confirmed by docking studies.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors confirm that this article content has no conflicts of interest.

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