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QSAR STUDY OF ASIATIC ACID AND ITS DERIVATIVES AS POTENTIAL INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) INHIBITORS

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ABSTRACT

Asiatic acid is a pentacyclic triterpenoid bioactive compound isolated from pegagan (*Centella asiatica*), which has been reported to show anti-inflammatory activities by inhibition of NO production. The aims of this study were to obtain model of Quantitative structure activity relationship (QSAR) of some triterpenoids suitable for prediction of asiatic acid and its derivatives as potential inhibitor of *inducible* nitric oxide synthase (iNOS). The method includes optimization of molecular geometry with semi-empirical method AM1 using hyperchem 8.01, selection and calculation of suitable descriptors using molecular operating environment (MOE 2009.10) and generating and validating of QSAR models. The QSAR models and their validation were performed by statistical application prrogram. The results revealed that the most suitable QSAR model is Log $1/C_{50}$ = -0.798 (± 0,207) AM1_HOMO + 0.515 (± 0.185) Log P – 0.937 (± 0.131) apol + 0.053 (± 0.015) vdw_area + 3.264 (± 0.678) mr – 1.299 (± 4.135) with r = 0.845, F = 16.451 and n = 43. The modified of hydroxy group, carboxylic group and rearrangemet of double bond of asiatic acid structures have more higher iNOS inhibitory activities than those of asiatic acid.

Key words: QSAR model, iNOS inhibitor, Asiatic acid and its derivatives.

INTRODUCTION

Asiatic acid (AA) is pentacyclic triterpenoid compound isolated from *Centella asiatica*, which structure is derived from ursane skeleton that has three hydroxyl at C(2), C(3) and C(23); it also has an olefin at C(12), and one carboxylic acid group function at

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C(28) (Fig. 1). Antiinflammatory activity of asiatic acid that the mechanism is suspected to inhibit NO production using *in vitro* as well as *in vivo* methods have been published before[1,2]. NO is a free radical with an unpaired electron. An enzymes which catalyzing nitric oxide (NO) formation from L-arginine is nitric oxide synthases (NOSs). iNOS (*inducible nitric oxide synthase*), one of three isozymes of NOS, is induced by microbial products, such as lipopolysaccharide (LPS) and inflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) and interferon-γ (INF-γ) in macrophages and some other cells[3]. NO, which is derived from iNOS enzyme have the importance rule of in inflammatory response.

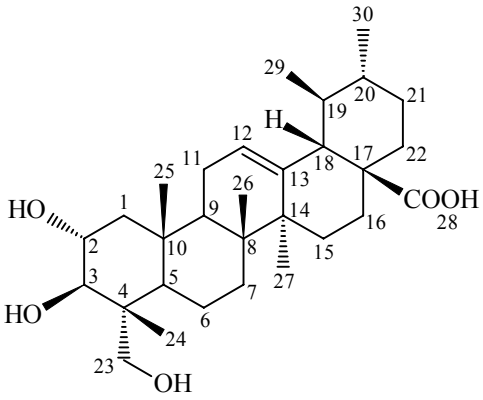


Fig. 1: Structure of asiatic acid

Asiatic acid is an ursane triterpenoid suitable to be selected as the lead compound for development of improved anti-inflammatory agent, because in addition to its anti-inflammatory effect, also shows others activity such as hepatoprotector[4], wound healing agent[5], and suppressed the UVA-induced reactive oxygen species production and lipid peroxidation[6]. The study of stucture-activity relationship of oleanane and ursane type triterpenoid derivatives has been reported as inhibitors of NO production in mouse macrophage[7,8].

Previously, we reported that the affinity of asiatic acid into iNOS is higher than COX-2 receptor using molecular docking method, and the important pharmacophore features are the hydroxyl (ring A) and carboxylic group acting as hydrogen bond acceptor (HBA), also olefin group at C(12) as a hydrophobic function[9] and prediction of the ADMET properties of asiatic acid modification structure and their affinity using molecular docking. Stucture modified was performed by adding of substituent of ring A (C2, C3 dan C23), oxidation of hydroxyl groups of ring A, rearrangement of double bond at C-12, and subtitute

methyl group at C-28[10]. This study to obtain the quantitative stucture activity relationship (QSAR) model of asiatic acid derivatives as an NO production inhibitor based on training set of triterpenoid pentacyclic compounds. The QSAR model was used to determine stucture activity relationship and molecular properties of asiatic acid derivatives, and to predict the activity of compounds as NO production inhibitor. This study was parts of several research efforts to explore a new selective inhibitor of NO production of asiatic acid derivatives.

EXPERIMENTAL

Material

Fourty eight compounds were used in the *training set* that have been reported by Honda et al.[7] and Sun et al.[8] consist of oleanane triterpenoid derivatives (40 compounds) (Fig. 2 and Table 1) and ursane triterpenoid derivatives (8 compounds) (Fig. 3 and Table 2) which has been known as NO inhibitors.

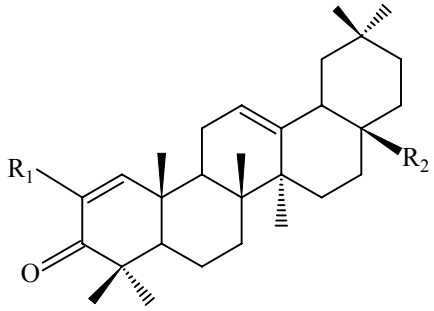


Fig. 2: Structure of oleanane

Table 1: Oleanane triterpenoid compounds[7] in the training set

No.	Compound	IC_{50} (μM)
1	5	37.1
2	6	40
3	7	6
4	8	30
5	9	26.5

Cont…

No.	Compound	IC_{50} (μM)
6	15	35.5
7	72	31
8	73	5.6
9	78	14
10	79	30
11	80	0.9
12	81	2.2
13	82	0.8
14	83	0.07
15	87	7.3
16	90	0.7
17	91	0.6
18	12	1.8
19	94	2.8
20	95	1.1
21	99	3
22	100	3.8
23	101	0.02
24	102	0.04
25	106	0.07
26	11	2.6
27	107	14
28	13	3.3
29	14	5.2

Cont…

No.	Compound	IC_{50} (µM)
30	16	8.5
31	111	9.7
32	113	0.7
33	114	0.2
34	115	0.0001
35	116	0.0002
36	118	0.1
37	119	0.0008
38	120	0.2
39	121	0.1
40	122	0.1

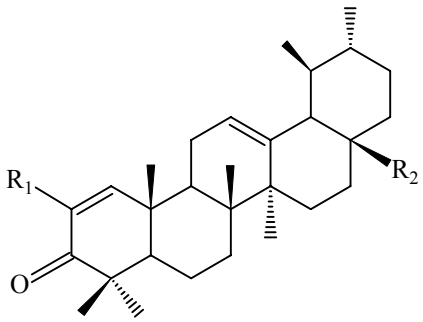


Fig. 3: Structure of ursane

Table 2: Ursane triterpenoid compounds[8] in the training set

No.	Compound	IC_{50} (µM)
1	4	17.6
2	76	13

Cont…

No.	Compound	IC_{50} (µM)
3	92	5.1
4	93	6.2
5	17	5.1
6	96	8.9
7	103	0.1
8	104	0.8

Modeling and molecular geometry optimization

Modeling and optimization of geometry molecular used semi-empirical AM1 method using Hyperchem 8.01 software.

Selection descriptor

Descriptors were calculated using Molecular operating environment (MOE 2009.10) software, which includes: AM1_HOMO (HOMO energy), Log P (o/w) (coefficient of partition), apol (sum of atomic polarizabilities), mr (molar refractivity), and vdw_area (Van Der Waals surface areas), log S (logarithm of solubility), LUMO (LUMO Energy), vdw_vol (Van Der Waals volume) descriptors.

QSAR Model calculation

The multilinier regression equation of 5 descriptors combination to the activity was calculated by statistical analisis using SPSS 21.0 software. QSAR model was generated using descriptors as independent variable (X), and an activity in log $1/IC_{50}$ as dependent variable (Y). Regression equation shows the quantitative relationship between the structure and activity of the test compounds. In this statistical analysis of IC_{50} values are expressed in the form of transformation log $1/IC_{50}$ to obtain a linear relationship between activity and physicochemical properties (descriptors). Based on the number of selected descriptors it will get a equation as much as 2^n-1 (n = number of descriptors)[11].

The QSAR models were evaluated by statistical parameters: correlation coefficient (r), regression coefficient (r^2), and the value of F (Fischer criterion)[12]. Model with the highest r value was obtained by determining the outlier compound. The outliers compounds determined by residual standard > 2[13].

Validation test of QSAR Model

The model has been validated by the LOO method (Leave One Out)[14]. This validation using the cross validation technique, where each compound in training set is eliminated in the calculation of linear regression analysis. The q^2 value was used as a validated parameter in the cross validation. Value of q^2 was calculated according to the following formula:

$$q^2 = 1 - \frac{\sum (yi - \hat{y}i)^2}{\sum (y-)^2} \quad \ldots(1)$$

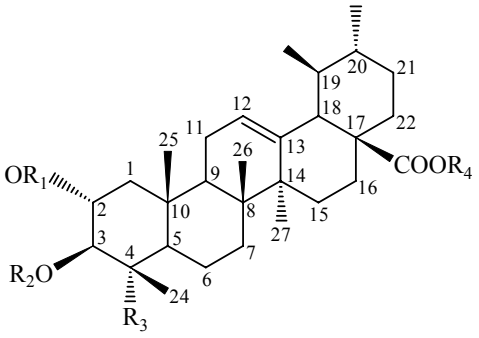
yi = observed activity

□i = average of observed activity

Yi = cross-validation prediction activities to compound-i

Based on QSAR model, therefore we predicted of asiatic acid and its derivatives that have been modified and published previously[10] (Table 3).

Table 3: The Functional groups modification of asiatic acid stucture



No.	Functional groups modification					
	R_1	R_2	R_3	C11	C13	R_4
1	H	H	CH_2OH	H	H	H
2	H	H	CH_2OH	H	H	CH_3

Cont…

No.	Functional groups modification					
	R_1	**R_2**	**R_3**	**C11**	**C13**	**R_4**
3	Ac	Ac	CH_2OAc	H	H	H
4	Ac	Ac	CH_2OAc	H	H	CH_3
5	Ac	Ac	CH_2OAc	Ene	Ene	CH_3
6	Oxo	Oxo	COOH	Ene	Ene	CH_3
7	Oxo	Oxo	COOH	Ene	Ene	H
8	Oxo	Oxo	COOH	H	H	CH_3
9	Oxo	Oxo	$COOCH_3$	Ene	Ene	CH_3
10	Oxo	Oxo	$COOCH_3$	Ene	H	CH_3
11	Oxo	OH	CH_2OH	H	H	CH_3
12	Oxo	Oxo	CH_3COOH	H	H	H
13	Oxo	OH	CH_2OH	H	H	H

RESULTS AND DISCUSSION

QSAR study was begun by determining the training set using selected group of pentacyclic triterpenoid compounds (oleanane and ursane type). The outlier compounds were determined by generating relationship between the value of the log $1/IC_{50}$ predicted vs log $1/IC_{50}$ observed on selected QSAR model. The result showed that the outlier were consist of five compounds with residual standard value > 2, are shown in Table 4. The compounds were removed from training set and the training set became 43 compounds.

Table 4: The value residual standard outlier compounds

No.	Compound	Residual standard
1	115	3.04
2	116	2.49
3	119	2.41
4	83	2.13
5	87	2.18

The fourty three compounds as a training set were used to determine the regression multilinier. Multilinear regression are then compiled and ranked based on the value of

statistical parameters, including the correlation coefficient (r), the regression coefficient (r^2, and the criteria Fischer (F). The model was performed by value of $r^2 \geq 0,8$ and the value of F indicates the significance level better than 95%. The value of r^2 and of F obtained from the analysis using SPSS software while F table value obtained using the formula:

$$F(1\text{-}P) = FINV\ (P,dfq,\ dfv) \quad \ldots(2)$$

P is the level of trust, dfq is k-1 and DFV is n-k, k is the number of variables and n is the number of compounds. The best of QSAR model was validated by q^2 (squared coefficient of cross validation Leave One Out as an indicator of the performance and stability of the model to all compounds of training set. The model equations must be fulfilled by the criteria, ie the value of $q^2 \geq 0,5$ (Table 5).

Table 5: The parameter value is based on the best equation statistics

No.	Descriptors	r^2	F_{calc}/F_{table}	q^2
1	AM1_HOMO	0.846	16.451	0.744
2	Log P(o/w)			
3	Apol			
4	Mr			
5	vdw_area			

The best QSAR model was generated based on the data of the five descriptor, as follows:

$$\text{Log } 1/C_{50} = -0.798\ (\pm\ 0.207)\ \text{AM1\_HOMO} + 0.515\ (\pm\ 0.185)\ \text{Log P} - 0.937$$

$$(\pm\ 0.131)\ \text{apol} + 0.053\ (\pm\ 0.015)\ \text{vdw\_area} + 3.264\ (\pm\ 0.678)$$

$$\text{mr} - 1.299\ (\pm\ 4.135) \quad \ldots(3)$$

According to QSAR model, it is known that the activity of the compounds is influenced by descriptor include HOMO energy, the partition coefficient, polarisability atoms, refractivity molar, and Van der Waals area (Table 5). Based on the results showed that descriptors of Log P, vdw_area, mr, AM1_HOMO must be increased to get higher activity, while apol descriptor must be decreased. The most influence descriptor was mr, because it has highest coefficient value.The relationship between the log $1/IC_{50}$ predicted and log $1/IC_{50}$ observed are shown in Table 6 and 7.

Table 6: IC_{50} observed and predicted of olean derivatives compounds in the training set

No.	Compd.	Log 1/IC_{50}		IC_{50} (µM)	
		Observed	Predicted	Observed	Predicted
1	5	-1.57	-2.13	37.15	134.90
2	6	-1.6	-1.15	39.81	14.13
3	7	-0.78	-1.18	6.03	15.14
4	8	-1.48	-0.95	30.20	8.91
5	9	-1.43	-0.98	26.92	9.55
6	11	-0.41	-0.2	2.57	1.58
7	12	-0.25	-0.08	1.78	1.20
8	13	-0.52	-0.62	3.31	4.17
9	14	-0.72	-0.62	5.25	4.17
10	15	-1.55	-1.52	35.48	33.11
11	16	-0.93	-1.02	8.51	10.47
12	72	-1.5	-1.19	31.62	15.49
13	73	-0.75	-1.18	5.62	15.14
14	78	-1.15	-1.02	14.13	10.47
15	79	-1.47	-1.00	29.51	10
16	80	0.04	-0.41	0.91	2.57
17	81	-0.34	-0.40	2.19	2.51
18	82	0.09	-0.51	0.81	3.24
19	90	0.15	0.04	0.71	0.91
20	91	0.22	0.06	0.60	0.87
21	94	-0.45	-0.02	2.82	1.05
22	95	-0.04	-0.08	1.10	1.20
23	99	-0.47	-1.02	2.95	10.47

Cont…

S. No.	Compd.	Log 1/IC_{50}		IC_{50} (µM)	
		Observed	Predicted	Observed	Predicted
24	100	-0.58	-0.14	3.80	1.38
25	101	1.7	1.26	0.02	0.05
26	102	1.4	1.22	0.04	0.06
27	106	1.15	0.06	0.07	0.87
28	107	-1.15	-0.59	14.13	3.89
29	111	-0.98	-1.02	9.55	10.47
30	113	0.15	0.17	0.71	0.68
31	114	0.7	0.14	0.20	0.72
32	118	1	0.85	0.10	0.14
33	120	0.7	0.81	0.20	0.15
34	121	1	0.94	0.10	0.11
35	122	1	0.72	0.10	0.19

Table 7: IC_{50} value observed and predicted of ursan derivative compounds in the training set

No.	Compd.	Log 1/IC_{50}		IC_{50} (µM)	
		Observed	Predicted	Observed	Predicted
36	4	-1.24	-1.46	17.38	28.84
37	17	-0.71	-0.47	5.13	2.95
38	76	-1.11	-1.61	12.88	40.74
39	92	-0.71	-0.49	5.13	3.09
40	93	-0.8	-0.42	6.31	2.63
41	96	-0.95	-0.49	8.91	3.09
42	103	1	0.77	0.10	0.17
43	104	0.09	0.77	0.81	0.17

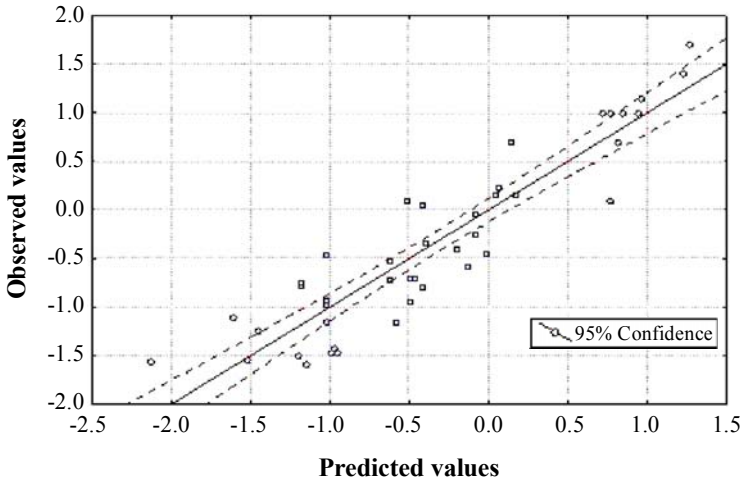


Fig. 4: The relationship between log 1/IC_{50} predicted (X) and log 1/IC_{50} observed (Y)

The relationship between log 1/IC_{50} obeserved and log 1/IC_{50} predicted has a linear relevancy with $r^2 = 0.845$.

The descriptors value of each compound were calculated, as well as the activity prediction (IC_{50}) using MOE 2009.10 software based on the best QSAR model (Table 8).

Table 8: The descriptors values and IC_{50} predicted of asiatic acid and its derivatives

Structure Number	Log P	vdw_ area	Mr	AM1_ Homo	Apol	Log 1/IC_{50} predicted	IC_{50} predicted (µM)
1	4.20	509.64	13.51	-9.20	88.82	-3.70	5004.62
2	4.47	531.85	14.00	-9.24	91.91	-3.62	4146.17
3	5.72	642.33	16.64	-9.11	105.78	-1.58	37.84
4	5.99	655.84	17.15	-9.14	108.88	-1.94	86.36
5	6.61	661.77	17.10	-8.86	107.54	-0.42	2.66
6	5.46	523.40	13.98	-9.01	87.38	0.41	0.39
7	5.19	501.19	13.48	-9.05	84.28	0.39	0.41
8	4.83	526.17	14.02	-9.40	88.71	-0.57	3.70

Cont…

No. of structures	Log P	vdw_ area	Mr	AM1_ Homo	Apol	Log 1/IC_{50} predicted	IC_{50} predicted (μM)
9	5.20	562.84	14.93	-8.96	93.56	-0.35	2.25
10	5.09	548.37	14.52	-9.33	91.80	-0.57	3.70
11	4.24	526.61	13.97	-9.20	90.58	-2.92	833.92
12	4.30	533.34	14.10	-9.50	90.04	-1.35	22.55
13	3.98	504.40	13.47	-9.35	87.48	-2.85	699.85

Based on the results showed that asiatic acid derivatives have a higher activity with IC_{50} predicted value more lower than asiatic acid. Based on the QSAR model showed that modified at hydroxy, carboxylic and olefin have more higher activity than thus lead compound. Compound of 3,4,5,6,7,8,9 and 10 have most highest activity. The modified of hydroxy group, carboxylic group and rearrangemet of double bond of asiatic acid structures have more higher iNOS inhibitory activities than those of asiatic acid. However, it must be considered the toxicity and ADME properties of the compounds**.**

The toxicity prediction of compound of 3 (triacethyl asiatic acid) has been reported that it has no hepatotoxicity properties, and a good absorption and distribution properties, as well as high affinity against iNOS receptor[15]. The compound of 3 has been synthesized and already published[4,10]. According the QSAR model showed that the compound has better activity than asiatic acid, with IC_{50} predicted of 37.84 μM.

CONCLUSION

QSAR equation model antiinflammatory by means of inhibition of NO production of the asiatic acid derivatives follows the equation: log $1/C_{50}$ = – 0.798 (± 0.207) AM1_ HOMO + 0.515 (± 0.185) log P – 0.937 (± 0.131) APOL + 0.053 (± 0.015) vdw_area + 3.264 (± 0.678) mr – 1.299 (± 4.135). The results of QSAR study showed that the designed structure of asiatic acid derivatives have higher activity than the asiatic acid, thus necessary for further synthesize the asiatic acid derivatives as potential iNOS inhibitors.

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