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Binding affinity of asiatic acid derivatives design against *Inducible Nitric Oxide Synthase* and ADMET Prediction

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ABSTRACT

Asiatic acid (AA) is a pentacyclic triterpenoid compound isolated from pegagan (*Centella asiatica)* and is reported to show anti-inflammatory activities by inhibiting *inducible nitric oxide synthase* (*i*NOS), an isoenzyme responsible for the catalysis of nitric oxide formation. The aim of this study was to obtain information regarding binding affinity of some potential asiatic acid derivatives to *iNOS* as well as pharmacokinetic properties including oral absorption, distribution, metabolism, and toxicity (ADME/T) using *in silico* methods. Twelve AA derivatives that were produced by modeling of AA on A- or C-ring or its carboxylic acid group, were included in this study. The affinities of these compounds were studied using molecular docking methods, while pharmacokinetic properties were studied using the PreADMET online program. The results showed that eight AA derivative designs have lower free energy binding (FEB) in comparison to AA (–9.79 kcal/mol), while four of the compound designs showed higher FEB than AA. *2,3-dioxo-11,13 diene-23-carboxy asiatic acid* (7) showed the lowest FEB of -11.33 kcal/mol. This compound has the human intestinal absorption (HIA), Caco-2 cell permeability, and plasma protein binding values of 96.62%, 20.90 (nm/Sec.), and 98.46%, respectively, which are comparable to those of AA and other AA derivatives. It is concluded that 2,*3-dioxo-11,13 diene-23-carboxy asiatic acid* (7) is an AA derivative with potential to be developed as a potential iNOS inhibitor.

INTRODUCTION

Nitric oxide synthases (NOS) is an enzyme that catalyzes the formation of nitric oxide (NO) from L-arginine. 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 (Calixto *et al.*, 2003). NO is a free radical with an unpaired electron and is an important cellular signaling molecule that has a role in septic shock and autoimmune diseases. The high levels of NO formation by iNOS have an important role in the inflammatory response, and it can destroy functional normal tissues during acute and chronic inflammation. There have been several research efforts to identify a selective iNOS inhibitor. Compounds that inhibit expression or activity of iNOS are proposed to be potential anti-inflammatory agents. Anti-inflammatory activities of asiatic acid (AA), including acting as an iNOS inhibitor, have been reported using *in vitro and in vivo* methods, and it is more active against iNOS than COX-2 (Huang *et al.*, 2001; Yun *et al.*, 2008). AA is a pentacyclic triterpenoid compound isolated from *Centella asiatica*, and its structure is derived from an 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 C(28). The structure-activity relationship of ursolic and oleanolic acid derivatives that have an ursane structure show a modification of the A ring, C ring, and carboxyl group of the structure, which is important for their significant activity as inhibitors of nitric oxide production in mouse macophages (Honda *et al.*, 1997, Honda *et al.*, 2000). Previously, we reported that the affinity of asiatic acid to iNOS is higher than the COX-2 receptor, and the important pharmacophore features are the hydroxyl (ring A) and carboxylic group acting as hydrogen bond acceptor (HBA), and also the olefin group at C(12) as a hydrophobic function (Musfiroh *et al.*, 2013). In the field of molecular modelling, molecular docking is method to explore the interaction between the ligand and receptor, and it can be used to predict the binding affinity. The principle of docking involves docking the ligand into the binding site of receptors based on its form-similarity and characteristics

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including its electrostatic nature (Goodsell *et al.,* 1990). In the drug discovery and development process, the discovery of drugs not only has good activity and bind selectively to target but also have the appropriate physico-chemical properties such as absorption and distribution properties (Zhao *et al.,* 2001), as well as the toxicity to reach the target site when delivered orally.

Computational methods in the early discovery were conducted to reduce the probability of failure at the development stage of drug candidates. As mentioned above, the structure modification of asiatic acid functional groups might provide important information of the structure-activity relationships for the development of novel anti-inflammatory agents. Asiatic acid is a triterpenoid suitable to be selected as the lead compound for development of an improved anti-inflammatory agent, because in addition to its anti-inflammatory effect, it also shows other activities such as being hepatoprotective (Zhao *et al.,* 2007), a wound-healing agent (Jeong *et al.,* 2006), and it suppresses tumor promotion.

Here, we studied the binding affinity and inhibitory parameters of some asiatic acid derivatives against the iNOS enzyme that were better than the lead (AA) by the molecular docking method (structure based) using autodock v.3.05 software (Goodsell *et al.,* 1990), and to predict their ADMET properties using Pre-ADMET software (Lee *et al.,* 2003). The toxicity prediction was generated using Toxtree software.

MATERIAL AND METHODS

The protein crystal structure of iNOS complexed with Arginine (Arg) was downloaded from the RSCB protein Data Bank (PDB ID: 1NSI) (Li *et al.,* 1999). The 3D structure of iNOS was reported by Li *et al.* (1999) using X-ray diffraction technique with a resolution of 2.55 Å. Twelve structures of asiatic acid derivatives were constructed using Chemdraw, then were optimized using Moleculer Mechanic (MM+), 3000 maximum cycles, followed by conjugate gradient minimization to a Root Mean Square (RMS) energy gradient of 0.01 kcal/(mol Å).

Molecular Docking Simulation Parameters

Molecular docking simulations were performed using the AutoDock 3.0.5. All water molecules and hetero-atoms attached to the proteins were removed. Hydrogen atoms were removed from the protein structure but later added only for polar hydrogen atoms using the programed protonation and charges assigned using the kollua.amber option of AutoDock 3.0.5. The grid box of 40×40×40 points with a spacing of 0.375 Å were set for AutoGrid computation with grid centered at (x) 9.740; (y) 64.640; (z) 15.986 to cover important residues in the binding site (Musfiroh *et al.,* 2013). Molecular docking was performed employing the Lamarckian genetic algorithm (LGA) with pseudo-Solis and Wets local search and with the following standard parameters: population size of 150 and a total of 50 docking runs for each ligand. The other parameter was default. The docking results from each of the calculations were clustered based on RMSD and on FEB (Morris, *et al*., 1998).

Predicting the absorption, distribution and toxicity properties

The PreADMET program was accessed at http://preadmet.bmdrc.org/. Asiatic acid, twelve structure modifications, and its glycoside were used in this study. The structure of all compounds were converted into molfile (*.mol). The program automatically calculated the predictive absorption for Caco-2 cell, HIA (human intestinal absorption), and plasma protein binding (Lee *et al.,* 2003). Predicting the toxicity properties was done using Toxtree free software and using Benigni/Bossa rule-base methods (for mutagenicity and carcinogenicity) (Begini *et al*.,2008).

RESULTS AND DISCUSSIONS

Predicting the affinities of asiatic acid derivatives to iNOS

The modification of asiatic acid derivative structures was done on the A ring (C2, C3, C23), C Ring (C11), and carboxyl groups (C28) and produced twelve derivative structures. The interactions of these compounds against the iNOS enzyme were compared to that of asiatic acid. The results showed that the compounds of (3), (6), (7), (8), (9), (11), (12), and (13) (Fig.1) had the free energy binding (FEB) values of -10.17 kcal/mol, -11.30 kcal/mol, -11.33 kcal/mol, -10.93 kcal/mol, -11.08 kcal/mol, -10.97 kcal/mol, -10.13 kcal/mol, and -10.18 kcal/mol respectively. These affinity values were lower than that of AA (-9.79 kcal/mol) and showed that these compounds were suggested to be more active as an iNOS inhibitor.

The results revealed some interesting structure/activity relationships: the acetylation in the A ring modification (C2, C3, C23) resulted in much higher affinity than asiatic acid. In the C-ring modification, a substitution and rearrangement of a double bond at the position of C11 and C13 was important to enhance the affinity into the binding site of iNOS, and the carboxyl group at C28 without a methyl group (e.g., 7) gave much higher affinity than the methylesther group (e.g., 8). Hydrophilic groups appeared to have higher affinity against the iNOS binding site than hydrophobic groups of asiatic acid derivatives.

Previously, the study of interactions against iNOS showed that asiatic acid formed hydrogen bonds from Gln 263 and Trp 372 of iNOS to the carboxyl group of C28 and the hydroxyl of the A Ring (Musfiroh *et al.,* 2013). In this study, the results showed that there are different amino acid residues of iNOS taking part in the interaction between asiatic acid and its derivatives; however they have more ability to compete against iNOS to replace the role of L-Arg than AA (Table 1). These derivative compounds form hydrogen bonds with various amino acid residues in the iNOS binding site (Table 2).

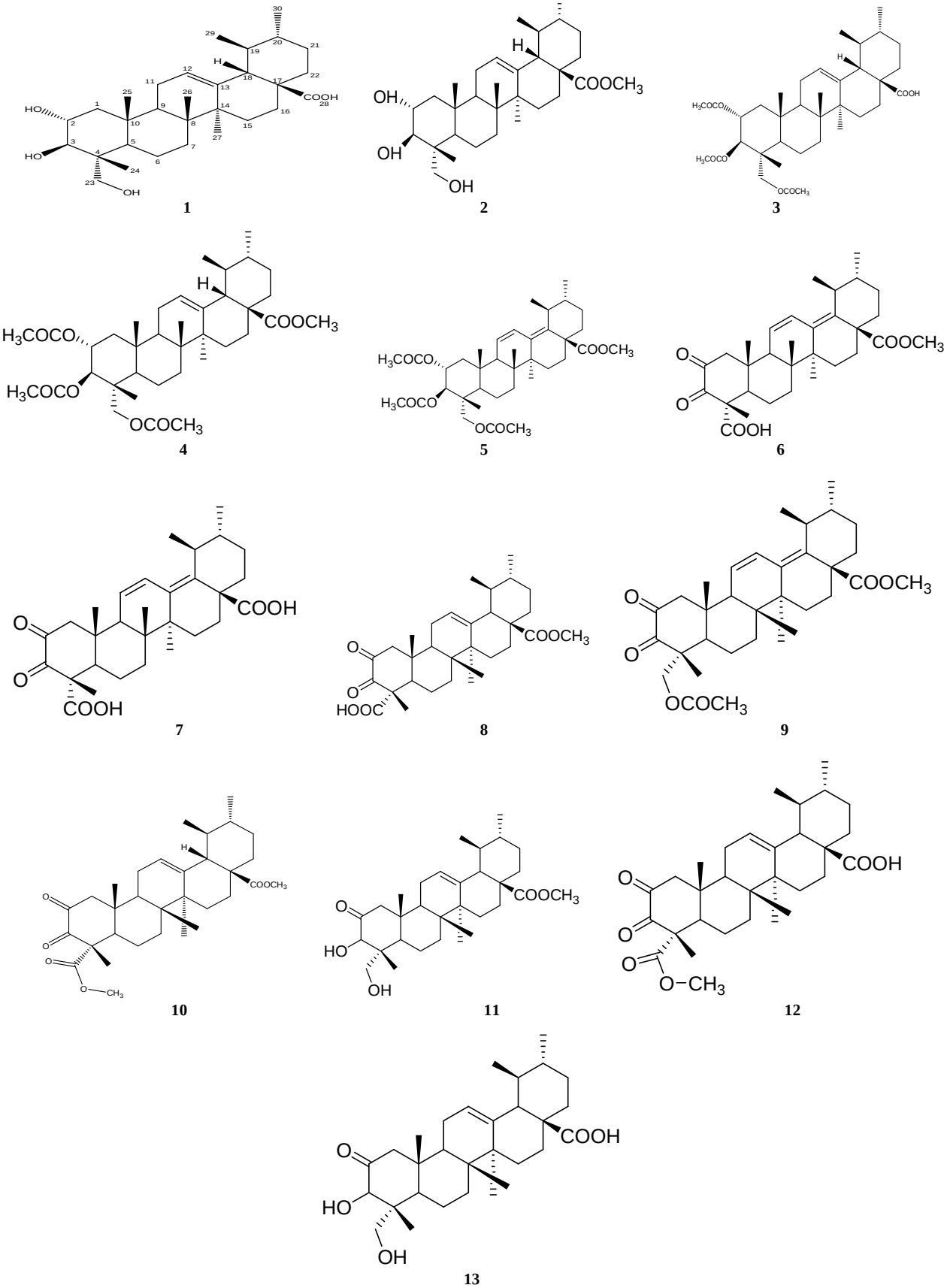

Fig. 1: The structure of asiatic acid and its modifications.

Table. 1: The substituent of asiatic acid derivatives, their binding energies, and predicted inhibitory activities to iNOS.

Number of structures	Substituents						$\Delta G_{calculated}$ (kcal/mol)	$Ki_{predicted}$ (M)
	C2	C3	C23	C11	C13	C28		
1.	OH	OH	CH_2OH	H	H	H	-9,79	6.42×10^{-8}
2.	OH	OH	CH_2OH	H	H	CH_3	-9,33	1.46×10^{-7}
3.	OAc	OAc	CH2OAc	H	H	H	-10.17	3.52×10^{-8}
4.	OAc	OAc	CH_2OAc	H	H	CH_3	-9.78	6.74×10^{-8}
5.	OAc	OAc	CH_2OAc	Ene	ene	CH_3	-9.31	1.51×10^{-7}
6.	Oxo	Oxo	COOH	Ene	ene	CH_3	-11.30	5.18×10^{-9}
7.	Oxo	Oxo	COOH	Ene	ene	H	-11.33	4.98×10^{-9}
8.	Oxo	Oxo	COOH	H	H	CH_3	-10.93	9.73×10^{-9}
9.	Oxo	Oxo	$COOCH_3$	Ene	ene	CH_3	-11.08	7.55×10^{-9}
10.	Oxo	Oxo	COOCH3	Ene	H	CH_3	-9.54	10×10^{-8}
11.	Oxo	OH	CH2OH	H	H	CH_3	-10.97	1×10^{-8}
12.	Oxo	Oxo	CH3COOH	H	H	H	-10.13	3.77×10^{-8}
13.	Oxo	OH	CH_2OH	H	H	H	-10.18	3.44×10^{-8}

Table. 2: The amino acid residues form hydrogen bonds between AA derivatives in the binding site of iNOS.

Compounds	Amino acid residues	Ligand atoms	Binding distance (A°)
1	Gln 263	O of COOH	2.75
	Trp 372	O of OH	3.09 (Musfiroh *et al*, 2013)
2	Gln 263	$COOCH_3$ (C28)	2.81
3	Val 352 (NH)	CH_2OAc (C23)	1.84
4	Tyr 491 (OH)	CH_2OAc (C23)	2.31
5	Val 352 (NH)	Oxo (C2)	2.03
	Cys 200 (SH)	CH_2OAc (C23)	2.89
	Glu 377	CH_3	
6	Gly 371 (NH)	Oxo (C3)	2.70
7	Val 352 (NH)	Oxo (C2)	2.16
	Asn 370	Oxo (C3)	3.04
	Cys 200	COOH (C23)	3.15
	Glu 377	CH_3	2.99
			2.958
8	Trp372 (NH)	Oxo (C2)	2.98
	Gly 371(NH)	COOH (C23)	1.99
	COOCH3 (C28)	Arg 199 (O)	2.01
9	Cys 200 (S)	COO (C23)	2.89
	Val 352 (NH)	Oxo (C2)	2.03
	Glu 377 (COOH)	CH_3 (C27)	2.90
			2.93
10	Gln 263 (NH)	COOCH3	2.34
	Glu 377 (COO)	CH3 (27)	2.62
			3.35
	Trp 372 (NH)	COOCH3 (C23)	1.84
			2.32
11	Cys 200 (S)	COO (C23)	3.04
			2.92
12	Gln 263 (NH)	COOH (C23)	1.90
	Trp 372 (NH)	COOH (23)	1.99
			2.23
	Glu 377 (COOH)	CH_3(C27)	2.59
			3.37
13	Arg 388 (NH)	Oxo (C_2)	2.13
	Arg (NH)	OH (C_3)	2.03
			2.41
	Glu 377 (COOH)	CH_3 (C27)	3.49
			2.61

Prediction of absorption, distribution, and toxicity properties

The pharmacokinetic parameters, absorption and distribution, were considered for selection of compounds as drug candidates. In this study, the PreADMET program was used to predict ADME of asiatic acid and its derivatives. The aspect prediction of absorption properties included percentage human intestinal absorption (% HIA) and Caco-2 cell permeability. Caco-2 cells are derived from colon adenocarcinoma and possess multiple drug transport cycles through the intestinal epithelium. The Caco-2 cell model is reliable in vitro model for prediction of oral drug absorption, while HIA is the sum of bioavailability and absorption evaluated from the ratio of excretion or cumulative excretion in urine, bile, and feces. The distribution properties were calculated using predictive plasma protein

Table 3. Predictive absorption, distribution, toxicity, and some physicochemical properties of asiatic acid derivatives.

No. of compounds	Absorption		Distribution	Toxicity risk parameters		Log P
	HIA (%)	Caco-2 cell (nm sec^{-1})	In vitro plasma protein binding (%)	Mutagenicity	Carcinogenicity	
Asiaticoside	1.81	19.56	38.68	No risk	No risk	1.73
1	91.23	20.97[b]	96,45[a]	No risk	No risk	5.32
2	91.94	21.27[b]	96,41[a]	No risk	No risk	5.58
3	98.62	22.24[b]	91.55[a]	No risk	No risk	6.01
4	99.74	24.98[b]	90.90[a]	No risk	No risk	5.86
5	99.74	24.98[b]	90.90[a]	No risk	No risk	5.80
6	99.08	21.12[b]	95.88[a]	No risk	No risk	5.23
7	96.62	20.90[b]	98.46[a]	No risk	No risk	4.97
8	98.73	21.12[b]	98.32[a]	No risk	No risk	5.71
9	96.62	20.90[b]	98.46[a]	No risk	No risk	5.23
10	99.30	21.76[b]	95.11[a]	No risk	No risk	5.97
11	94.52	21.41	96.78	No risk	No risk	5.24
12	98.74	21.23	96.35	No risk	No risk	5.71
13	94.79	21.01	96.82	No risk	No risk	4.97
Classification	Well absorbed (70-100%)	a. low permeability b. Middle permeability	a : strongly bound b : weakly bound			

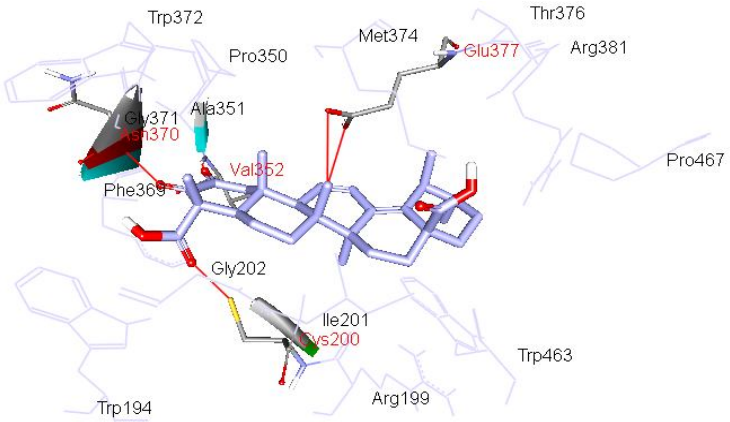

Fig. 2: The interaction of asiatic acid derivatives (7) in the iNOS binding site (red line is hydrogen bond).

binding, which is available in PreADMET software also. It was used because the degree of plasma protein binding of a drug has an important role on its disposition and the drug`s efficacy (Lee *et al*., 2003). The study of the pharmacokinetic profile of asiatic acid and asiaticoside showed that asiatic acid is well absorbed in human volunteers after oral administration (Grimaldi *et al*., 1990), while the bioavailability of asiaticoside is very low for intragastric administration (Liu *et al*., 2010). Our prediction value of HIA showed similar results with that study: AA and asiaticoside have HIA values of 91.23% (well-absorbed categories) and 1.81% (low absorbed), respectively. There is different lipophilicity between asiatic acid (Log P: 5.32) and its glycoside (log P: 1.73). Prediction HIA value of AA derivatives are comparable to those of AA, and those compounds have a similar level of lipophilicity with AA. However, the plasma protein binding of asiatic acid and its derivatives were very strongly bound. The toxicity risk parameters such as mutagenicity and carcinogenicity of the AA and its derivatives have no risk probability (Table 3). The results showed that compounds (3), (5), (6), (7), (8), (9), (11), (12), and (13) had a higher affinity than the asiatic acid, as well as good affinity absorption properties. Based on the lipophilicity properties (Log P < 5), compounds 7 and 13 are more likely to be developed further as iNOS inhibitors for oral administration (Lipinski, 2001).

However, the structure of 7 (*2,3-dioxo-11,13 diene-23-carboxy asiatic acid*) shows the highest affinity against iNOS. The interaction between compound 7 and iNOS formed hydrogen bonds with Val 352 (NH) (2.16 Å), Asn 370 (3.04 Å), Cys 200 (S) (3.15 Å), Glu 377 (COOH) (2.99 Å, 2.96 Å) residues (Fig. 2).

CONCLUSION

The affinity prediction of asiatic acid structure modification indicated that the structures (3), (6), (7), (8), (9), (11), (12), and (13) (Fig.1) have lower FEB values than that of AA; however, the structure of (7) shows the lowest FEB. The HIA, Caco-2 cell permeability, and plasma protein binding values are comparable to those of AA and other AA derivatives. Based on the overall results, it is concluded that the structure of 7 (*2,3-dioxo-11,13 diene-23-carboxy asiatic acid*) was an AA derivative structure with the highest possibility of being developed as a potential iNOS inhibitor.

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REFERENCES

Calixto JB, Otuki MF, Santos ARS, Anti-Inflammatory Compounds of Plant Origin. Part I. Action on Arachidonic Acid Pathway, Nitric Oxide and Nuclear Faktor kB (NF-kB), *Planta med*., 2003; 69: 973-983.

Benigni,R. Bossa C., Jeliazkova N, Netzeva T., and Worth A., The Benigni/Bossa rulebase for mutagenicity and carcinogenicity–a module of Toxtree", European Commission Reports, 2008, EUR 23241 EN.

Grimaldi R., De Ponti F., D`Angelo L., et al., Pharmacokinetics of The Total Triterpenic Fraction of *Centella asiatica* after Single and Multiple Administrations to Healthy Volunteers, A New Assay for Asiatic Acid, *J. Ethnopharmacol*, 1990; 28: 235-241.

Goodsell DS. & Olson AJ, Automated Docking of Substrates to Proteins by Simulated Annealing, *Proteins: Str. Func. Genet.* 1990; 8: 195-202.

Honda T., Finlay HJ., Gribble GW., Suh N., Sporn MB., New Enon Derivatives of Oleanolic Acid and ursolic Acid as Inhibitors of Nitric Oxide Production in Mouse Macrophages, *Bio. & Med. Chem Letters*,1997, 7(13):1623-1628.

Honda T., Rounds BV., Bore L., *et al.*, Synthetic Oleanane and Ursane Triterpenoids with Modified Rings A and C: A Series of Highly Active Inhibitors of Nitric Oxide Production in Mouse Macrophages, *J.Med. Chem.*, 2000, 43: 4233-4246.

Huang SS, CS Chiu, HJ Chen, WC Hou, MJ Sheu, YC Lin, PH Shie, GJ Huang, Antinociceptive Activities and the Mechanisms of Anti-Inflammatory of Asiatic Acid in Mice, *Evidence-Based Complementary and Alternative Medicine*, Volume 2011; 895857: 1-10.

Jeong BS, Asiatic Acid Derivatives for New Wound Healing Agent, *Archives of Pharmacal Research*, 2006; 29(7): 556-562.

Lee SK, Lee IH, Kim HJ, Chang GS, Chung JE, The PreADME Approach: Web-based Program for Rapid Prediction of Physico-Chemical, Drug Absorption and Drug-Like Properties. Blackwell Publishing Massachusetts, 2003, pp. 418-420.

Li H, Raman CS, Glaser CB, Blasko E, Young TA, Parkinson JF, Whitlow M, Poulos TL, Crystal structures of zinc-free and -bound heme domain of human inducible nitric-oxide synthase. Implications for dimer stability and comparison with endothelial nitric-oxide synthase, *J Biol Chem*. 1999; 274(30): 21276-84.

Lipinski CA., Lomardo F., Dominy BW., Feeney PJ., Experimental and computational approaches to estimate

solubility and permeability in drug discovery and development settings, *Adv. Drug Deliv. Rev*., 2001, 46: 3–26.

Liu SJ, Liu ZX, Ju WZ, Chen ZY, Xiong NN, Tan HS, Determination of Asiaticoside in Rat Plasma by LC-MS-MS and its Application to Pharmakokinetics Studies, *Chromatographia*, 2010; 72(9/10): 821-825.

Musfiroh I, Nursamsiar, A Muhtadi, RE Kartasasmita, Daryono HT, S Ibrahim, In Silico Study of Asiatic Acid Interaction with Inducible Nitric Oxide Synthase (iNOS) and Cyclooxygenase (COX-2), *Int J Pharm Pharm Sci*, 2013, 5(1): 204-207

Morris GM., Goodsell DS., Halliday RS., Huey R., Hart WE. Belew RK., *et al.*, Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, *Journal of Computational Chemistry*. 1998; 19(14): 1639-62.

Yun KJ, Kim JY, Kim JB, Lee KW, Jeong SY, Park HJ, Jung HJ *et a*l., Inhibition of LPS-induced NO and PGE2 Production by Asiatic Acid via NF-κB Inactivation in RAW 264.7 Macrophages: Possible Involvement of the IKK and MAPK Pathways, *Int. Immunopharmacol*, 2008; 8: 431–441.

Zhao YH., Le J, Abraham MH, Hersey A., *et al*., Evaluation of human intestinal absorption data and subsequent derivation of a quantitative structure–activity relationship (QSAR) with the Abraham descriptors, J. Pharm. Sci., 2001, 90(6):749.

Zhao LX., Park Hg., Jew Ss., *et al.*, Modification of C11, C28, C2,3 or C2, 23, 28 Functional Groups on Asiatic Acid and Evaluation of Hepatoprotective Effects, Bull. Korean Chem. Soc., 2007, 28(6);970.

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