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Research Article

Pharmacokinetic properties and binding affinity prediction of leonurine and its derivatives design on phospodiesterease-5

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ABSTRACT

Leonurine is an alkaloid produced from the medicinal plant of deungdeureuman (*Leonurus artemisia* L.). It has been reported to show antiapoptotis, antihipertension, antiinflamation, antipiretic, and diuretic effect. In addition was also reported to show despile different meaning of aphrodisiac effect and evection, its interessting to study the possible influence of leonurine and its derivatives on erectile dysfunction condition. The cryatalographic structure of this enzyme is published and can be obtained from Brookhaven Data Bank with the code of PDB ID : 2H42 (*wang, et al., 2006*). Applying this PDB data, it is posible to perfom docking study of leonurine and its derivatives on PDE-5. As well as the pharmacokinetic properties of those compounds including oral absorption, distribution, metabolism, and toxicity (ADME/T) by means of *in silico* method. Thirteen Leonurine derivatives design which produced by stuctural modification of leonurine, especially on their carboxylic and methoxy group, were included in this research. The affinities of those compound designs were studied applying molecular docking using ArgusLab 4.0.1 2004 program, while those of pharmacokinetic properties were performed by PreADMET online free program. The result showed that ten leonurine derivatives designs have lower free energy binding (-9.460 kcal/mol) in comparison to leonurine (-7.397 kcal/mol). This compound has human intestial absorption (HIA), Cac0-2 cell permeability, and plasma protein binding values of 18.71%, 8.28 (nm/Sec), and 66.79 %, respectively, which are comparable to those of leonurine and other leonurine derivatives design. Based on overall results, it was conclude that *4-amino buthyl -4(-3amino-5-hydroxypentyl)guanidino)3,5-dimethoxybenzoat* was an leonurine derivative design with highest possibility to be further developed as a potential PDE5 inhibitor.

Keywords: Molecular Docking; Leonurine Derivatives; PDE5, ADMED/T

INTRODUCTION

In silico study an integral part in the discovery and development of drug because *In silico* approach successfully replied in the field of molecular design of a new drug candidate. Structure–based design techniques, including small-molecule docking and scoring, can provide structural and energetic information on ligan-protein binding and guide the design of more potent candidate molecules (Bottegoni *et. al*, 2012, Kitchen *et.al*, 2004).

Leonurine (4-guanidino-butyl ester-3.5-dimethoxy-4hydroxy-benzoic acid) of Figure 1. is an alkaloid produced from the medicinal plant (Leonurus Artemisia *sp*) that has been reported to show anti apoptotis, anti-hipertency, anti-inflamatory, antipiretic, dieretic (xinhua liu, *et. al*., 2011) and having aphrodisiac effects (Perry, L.M., 1994).

Leonurine have also been used recently for the treatment of blood hyperviscosity (Zou QZ., *et.al*, 1989). other pharmacological effect of leonurine include the uterotonic action(Kong YC., et.al., 1976), Sildenafil is a typical drug that selectively inhibits PDE-5, and is enzyme responsible for the enzimatic hydrolysis of cGMP, and hence maintaining erection of phenile tissue. Possible interaction of leonurine and its derivatives designs with PDE-5 as well as their pharmaocokinetic properties have been studied by means of in silico and computation methods. Leonurine and its derivatives designs were docked on PDE-5 applying ArgusLab version 4.0.1., while their pharmacokinetic properties, including permeability for Caco-2 cell, Human Intestinal Absroption (HIA) and Plasma protein binding were predicted PreADMET on line program.

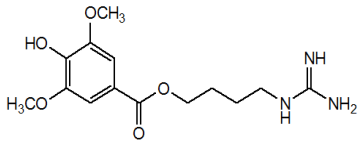

Figure 1: The Structure of Leonurine

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Table 1: Structure design of ligand

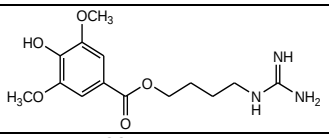
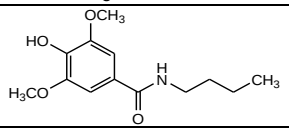
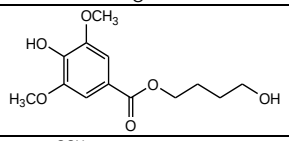
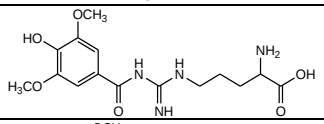
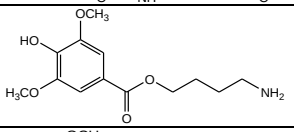
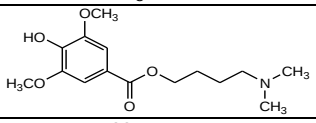
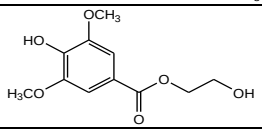
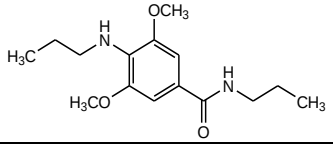
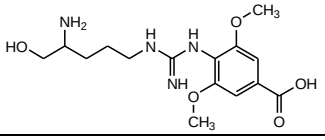
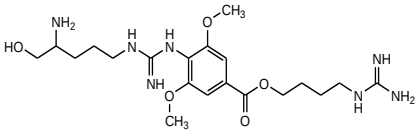
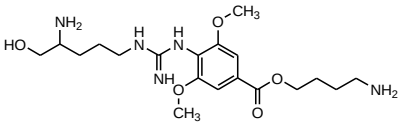
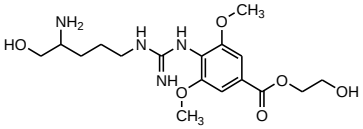
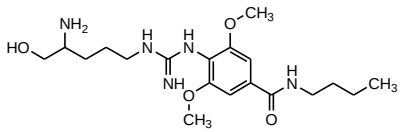
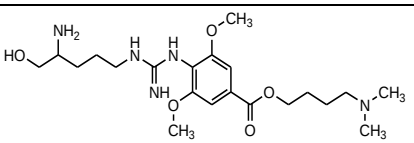
Code	Name of ligand	Chemical structure
L	Leonurine	
L-1	N-butyl-4-hydroxy-3,5-dimethoxybenzamide	
L-2	4-hydroxybutyl 4-hydroxy-3,5-dimethoxybenzoate	
L-3	2-amino-5-(3-(4-hydroxy-3,5-dimethoxybenzoyl)guanidino)pentanoic acid	
L-4	4-aminobutyl-4-hydroxy-3,5-dimethoxybenzoate	
L-5	4-(dimethylamino)butyl 4-hydroxy-3,5-dimethoxybenzoate	
L-6	2-hydroxyethyl 4-hydroxy-3,5-dimethoxybenzoate	
L-7	3,5-dimethoxy-N-propyl-4-(propylamino)benzamide	
L-8	4-(3-(4-amino-5-hydroxypentyl)guanidino)-3,5-dimethoxybenzoic acid	
L-9	4-guanidinobutyl 4-(3-(4-amino-5-hydroxypentyl)guanidino)-3,5-dimethoxybenzoate	
L-10	4-aminobutyl 4-(3-(4-amino-5-hydroxypentyl)guanidino)-3,5-dimethoxybenzoate	
L-11	2-hydroxyethyl 4-(3-(4-amino-5-hydroxypentyl)guanidino)-3,5-dimethoxybenzoate	
L-12	4-(3-(4-amino-5-hydroxypentyl)guanidino)-N-butyl-3,5-dimethoxybenzamide	
L-13	4-(dimethylamino)butyl 4-(3-(4-amino-5-hydroxypentyl)guanidino)-3,5-dimethoxybenzoate	

Table 2: Docking score of the leonurine and leonurine derivative with PDE-5

Code of Ligands	ΔG (kcal.mol^{-1})	Amino acid residu of PDE-5 binding site	Distance (Å)
L	-7.39773	783Ala (N) 816Met(O) 779Ala (O)	2.944 2,298 2.470
L-1	-7.254	817Gln(O) 612Tyr(O) 86HOH(O)	2.728 2.900 2.406
L-2	-7.71955	663SER(O) 779ALA(O) 783ALA(N) 663SER(O)	2.873 2.752 2.996 2.626
L-3	-7.8269	816MET(O) 802THR(O) 75HOH(O) 663SER(O) 663SER(O) 663SER(O)	2.586 2.872 2.595 2.428 2.267 2.999
L-4	-7.71635	663SER(O) 779ALA(O) 663SER(O)	2.852 2.564 2.887
L-5	-7.01506	817GLN(N) 783GLN(N) 783GLN(N) 779ALA(O)	2.998 2.971 2.959 2.248
L-6	-7.04699	820PHE(N) 816MET(O) 783ALA(N) 779ALA(O)	2.995 1.974 2.999 2.247
L-7	-8.08666	817GLN(O)	2.530
L-8	-8.22364	804LEU(O) 804LEU(O) 783ALA(O) 817GLN(O) 817GLN(O)	2.983 2.919 2.860 1.972 2.907
L-9	-8.54783	816MET(O) 86HOH(O) 765LEU(O) 768ILE(N)	2.566 2.529 2.899 2.594
L-10	-9.46015	775GLN(O) 775GLN(N) 802THR(O) 663SER(O) 663SER(O) 663SER(O)	2.800 2.612 2.976 2.804 2.475 2.881
L-11	-8.21297	663SER(O) 804LEU(O) 129HOH(O) 783ALA(N) 779ALA(O) 817GLN(O)	2.467 2.884 2.940 2.683 2.999 2.079
L-12	-8.84067	663SER(O) 75HOH(O) 663SER(O) 802THR(O) 804LEU(N)	2.429 2.734 2.869 2.900 2.566
L-13	-7.96422	45HOH(O) 661ASN(N) 661ASN(O) 613HIS(N)	2.999 2.998 2.901 2.811

Molecular doking is a tool in structural molecular biology and structural-based drug discovery. The goal of ligand-protein docking is to understand and predict molecular recognition, finding likely binding modes and predicting binding affinity (Morris and Lim-Wilby, 2008). ArgusLab 4.0.1. is a freely available docking software, which server two docking engine, i.e., GADock and ArgusDock (ArgusLab, 2004). These ingine are capable for binding free energy calculation betwen protein and ligands. Furthermore, ArgusLab is easy and inexpensive program useful for virtual screening (Oda and Takahashi, 2009).

Prediction of ADME (Absorption, distribution, metabolism and exretion) properties has been developed to reduce the probability of the failure at the development stage of drug candindates. PreADMET is a web-based application for predicting ADME data and building drug-likely library using in silico method. This programe is useful for the construction of drug absorption prediction system. In absorption, it provides prediction models for in vitro Caco-2–cell and MDCK cell (Madin-Darby canine kidney) assay as well as in silico HIA (Human intestinal absorption). In dsitribution, it provides prediction of plasma protein binding and BBB (blood brain barrier) penetration (Lee et al., 2003).

The target of this research are possible interaction of leonurine and its derivatives designs with PDE-5 as well as their pharmacokinetic properties have been studied by means of in silico and computation method.

MATERIAL AND METHODS

Research activities were conducted in Laboratory of Drug Design and High Computing, School of Pharmacy ITB, Indonesia.

Preparation of Protein Structure : Structure coordinat for PDE5 was obtained from the Protein Data Bank (PDBID: 2H42), in which phophodiesterease type-5 was cocristallized
with 5-{2-ethoxy-5-[(4-methylpiperazin-1-y) sulfonyl}-1-methyl-3-propyl-1H,6H,7H- pyrazo lo [4,3-D]pyrimidin-7-one as original ligand (*Wang, et al., 2006*), was used as a target for virtual screening using ArgusLab 4.0.1.

Preparation of Ligand designs : The 3D structure of leonurine and leonurine derivatives were design and drawn using Hyperchem 7, then were optimized using Austin Model (AM1). 200 maximum interaction, followed by conjugate gradient minimization to a Root Mean Squere (RMS) energy gradient of 0,01 kcal/(mol A) (ArgusLab 4.0.1, 2004). The resulting structure was then saved in mol file form for molecular docking studies. The structure design of ligands are displayed in the following table 1.

Molecular docking: Structures of Leonurine and leonurine derivatives (Table 1) were used as ligands for molecular docking to PDE-5 binding site. By using ArgusDock method for evaluated, the tirteen leonurine and leonurine derivatives were docked with PDE-5 and the binding affinity was characterized by binding energy (ΔG).. The validated method of docking calculation then used to perform the docking of leonurine and derivatives with PDE-5 binding site, the docking scores are presented in Table 2 . Binding affinity was characterized by binding energy value (ΔG) and hydrogen bond between ligands and the enzyme (Oda *et al*., 2007).

Visualisazation of enzyme-ligand complex interactions: Visulaisation of enzyme-ligand complexes was performed using MMV software for 3D visualization and MOE 2008.10 software for visualization of 2D form.

Predicting the absorption and distribution properties of Leonurine and its derivative using PreADMET: PreADMET program was accessed and compounds were drawn or uplouded from Molfile (*mol). The program automatically calculate the predictive adsorption and distribution parameters i.e., permeability for Caco-2 cell, HIA (human intestinal absorbption) and plasma protein binding (PreADMED, 2010). The ADME scores were presented in table 3.

RESULTS AND DISCUSSION

Validation of docking method

ArgusLab has two docking engine types, i.e., ArgusDock and Gadock. We compared the validation of ArgusDock and GADock for measuring the Root Mean Square Deviation (RMSD) of the caratesian coordinates of the atoms of the original ligand 5-{2-ethoxy-5-[(4-methylpiperazin-1-yl)sulfonyl]phenyl}-1-methyl-3-propyl-1H,6H,7H-pyrazolo[4,3-D]pyrimidin-7-one in the docked and crystallographic conformations. A docking method is generally regarded as successful if the RMSD value is less than 2Å (Morris an Lim-Wilby, 2008). In curent study, GADock engine was failed to perform a valid docking calculation, due to the RMSD value of 6.2236 Å. However, ArgusDok engine gave a better result. Figure. 2 shows the conformation superposition of 5-{2-ethoxy-5-[(4-methylpiperazin-1-yl) sulfonyl] phenyl}-1-methyl-3-propyl-1H,6H,7H-pyrazolo [4,3-D] pyrimidin-7-one from the X-ray crystal structure of 5-{2-ethoxy-5-[(4-methylpiperazin-1-yl)sulfonyl]phenyl}-1-methyl-3-propyl-1H,6H,7H-pyrazolo[4,3-D]pyrimidin-7-one-PDE5 complex and that from the docking by ArgusDok engine. RMSD value between the two conformation is only 1.924 Å, indicating that the parameter set for docking is capable of reproducing the x-ray structure. In addition both of ligand (original ligand and that from the docking simultation) interact to the same residu of PDE5 i.e Gln1134 and hend this engine was then used to perform the docking calculation of the leonurine and its derivatives.

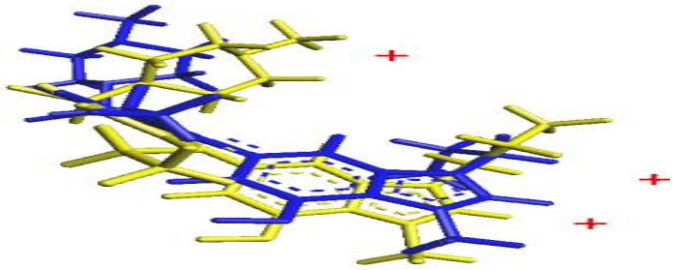


Figure 2: Conformation comparison of 5-{2-ethoxy-5-[(4-methylpiperazin-1- yl)sulfonyl]phenyl}-1-methyl-3-propyl-1H,6H,7H-pyrazolo[4,3-D]pyrimidin-7-one -PDE5 complex (blue) and that from the docking simultation using ArgusDock engine (yelow)

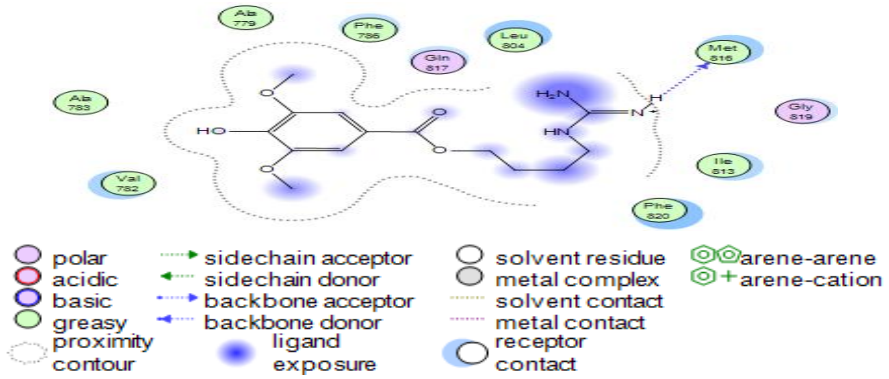


Figure 3: Two-dimensional (2D) of Leonurine (L)

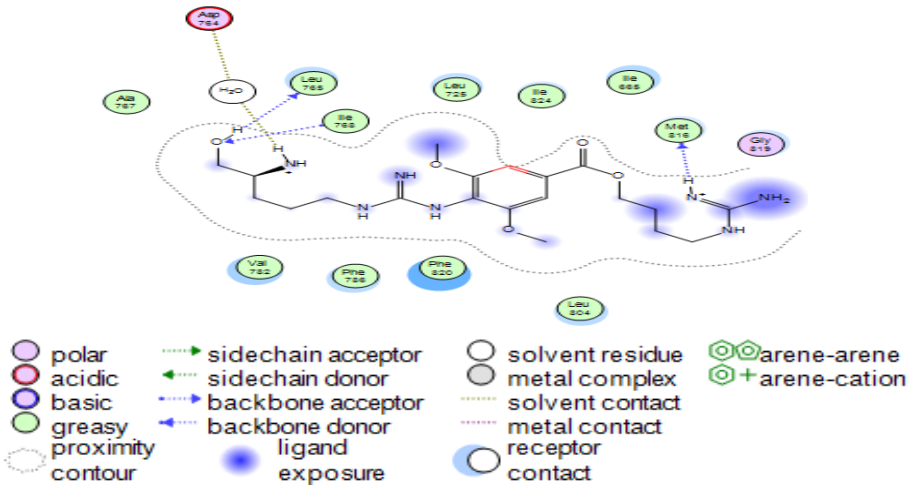


Figure 4: Two-dimensional (2D) of 4-aminobuthyl-4(-3(4-amino-5hydroxypenthyl) guanidino)3,5-dimethoxybenzoate

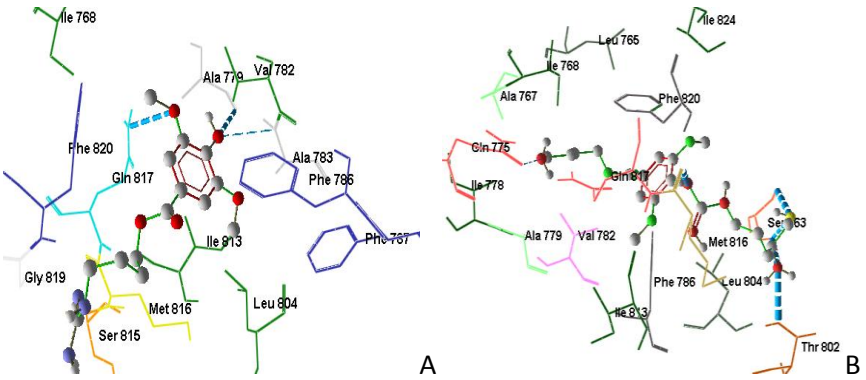


Figure 5: Interaction of Leonurine (A) and 4-aminobuthyl-4(-3(4-amino-5hydroxypenthyl) guanidino)3,5-dimethoxybenzoate (B) on PDE-5 binding site

Table 3: Predictive absorption and distribution properties of leonurine and leonurine derivatives

No.	Name Of Ligand	HIA (%)	Caco-2 cell (nm sec^{-1})	Distribution Plasma Protein binding (%)
1	Leonurine	40.105576	0.491978	33.470430
2	N-butyl-4-hydroxy-3,5-dimethoxybenzamide	90.172166	15.0056	74.802012
3	4-hydroxybutyl -4hydroxy-3,5-dimthoxybenzoate	88.47330	7.44945	74.476167
4	2-amino-5-(3-(4hydroxy-3,5-dimethoxybenzoyl)guanidino)pentanoic acid	12.821712	1.28604	42.544303
5	4-aminobutyl 4-hydroxy-3,5-dimethoxybenzoate	84.915400	9.12477	32.842798
6	4-(dimethylamino) buthyl 4-hydroxy-3,5-dimethoxybenzoate	94.767096	34.4322	40.859647
7	2-hydroxyethyl 4-hydroxy-3,5-dimethoxybenzoate	81.611024	21.1792	64.334506
8	3,5-dimethoxy-N-propyl-4-(propylamino)benzamide	91.76822	32.0557	81.817660
9	4-(3-(4amino-5hydroxypuntyl)guanidino)-3,5 dimethoxy benzoat acid	19.834600	18.3112	44.5107
10	4-guanidinobutyl 4-(3amino-5hydroxypentyl)guanidino-3,5-dimethoxybenzoate	0,000	9.116	69.388367
11	4-aminobutyl -4(-3(4 amino-5-hidroxypentyl)guanidino)-3,5-dimethoxybenzoat	18.718045	8.28372	66.791102
12	2-hydroxyethyl -4 (-3(4-amino-5hydroxypentyl)guanidino) 3,5-dimethoxy benzoat	14.351058	17.4861	36.894638
13	4 (4-amino-5-hydroxypentyl)guanidino) –N-buthyl-3,5 dimethoxybenzamide	41.3250132	8.52991	27.13073
14	4-(dimethylamino)buthyl 4-(3-(4-amino-5 hydroxypenthyl)guanidino)3,5dimethoxy benzoat	38.122524	8.7757	32.452363

Molecular Docking of Leonurine and its derivatives

Structure of leonurine and its derivatives were used as ligands for molecular docking on PDE5 (PDB: 2H42) binding site by using ArgusDock method. The ligand were docked with PDE-5 and binding affinitives were characterized by binding energy (ΔG). The following figures show the dock position of leonurine and (N-buthyl-4-hidroxy-3,5 dimethoxybenzamide) on PDE5 binding site

The above table shows that the L-10 has the lowest free energy with six contact amino acid residues (Gln-775, Gln- 775, Thr-802, Ser-663, Ser-663, Ser-663), indicating that the L-10 has the highest affinity on PDE-5

Some of the ligand interaction with PDE-5 in 2D using MOE 2009.10 software presented in figure 2-3, and visualisation in 3D using MMV 2.5 software presented in figure 3.

Table 3 : Presented the value of predicted absorption and distribution of leonurine and leonurine derivatives. Leonurine was predicted as moderately absorbed and low permeable compound, but weakly bound to plasma protein. These were not good properties for oral drug candidate. On the contrary as well as predictive absorption and distribution properties of some leonurine derivatives which predicted to have higher affinity on PDE-5 than leonurrrine, i.e. N-buthyl-4-hydroxy-3,5-dimethoxybenzamide, 4-hydroxybutyl-4-hydroxy-3,5-dimethoxybenzoat, 3,5-dimethoxy-N-propyl-4(propilamino)benzamide, generaly were better than those leonurine. N-buthyl-4-hydroxy-3,5-dimethoxybenzamide was predicted as well absorbed and weakly bound compounnnd. Then leonurine compound as well as leonurine derivatives were predicted as well absorbed and midle permeability compounds.

Absorption and distribution, the part of pharmacokinetics, were considered as important parameter to choose compounds as drug candidates. In our reserch, there parameters from PreADMET program were calculated for leonurine and leonurine derivatives. PreADMET featured predicted of absorption properties, including Caco-2-cell permeability as well as percent human intestinal absorption (%HIA). Caco-2 cell model is reliable in vitro models for the prediction of oral drug absorption, while HIA is the sum of bioavailability and absorption evaluated from ratio of exretion or cumulative excretion in urine, bile and feces. For distribution properties, we used the calculation of predictive plasma protein binding which available in PreADMET program. Only the unbound drug is available for diffusion or transport across cell membrane and also for intercation with a pharmacological target; therefore, plasma protein binding of a drug play an impotant role in drug's efficacy (Lee et all., 2003)

CONCLUSION

ArgusLab engine of ArgusDock method was more suitable to predict the PDE-5 inhibitory activities of

leonurine and leonurine derivatives. By using ArgusDock method, computational docking of 4-aminobutyl-4(-3(4amino-5-hidroxypentyl)guanidino)-3,5 dimethoxybenzoat (L-10) has best energy binding (-9.46 kcal/mol) than leonurine and derivatives so the L-10 can proposed to be more potential as PDE-5 inhibitor than leonurine.. The predictive absoption and distribution properties of these compound i.e. human instinal absorbtion, Caco-2 cell permeability and percent plasma protein binding were better than those leonurine.

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REFERENCES

Amir, A., M.A. Siddiqui, N. Kapoor, A. Arya and H. Kumar, 2010. In silico molecular docking of influenza virus (PB2) protein to chek the drug efficacy. Trend Bioinform., http://scialert.net/ abstract/doi=tb.0000. 19458.194558

ArgusLab 4.0.1., 2004. Planaria Software LLC.Seattle. (hhtp://www.planaria.software.com)

Battegoni, G. Protein-ligand docking. Front. Biosci. 2012, 17, 2289 – 2306.

Chang CF., at al, 1986., Experimental studies on the mechanism of anti-platelet aggregation action of motherwort. J. Inteng Chin Western Med 1986; 6(1):39-40

Chuanhua Liu, et al. 2011. Leonurine – Cystein Analog Conjugates as a new Class of Multifunctional anti-myocardial ischemia agent

Goodsell DS. & Olson Aj. Automated docking of substrates to protein by simulated anneling, Proteins : Str, Func, Genet. 1990(8): 195-202

Kong YC., et.al., 1976;. Isolation of the uterotonic principle from leonurus artemiasia, the Chinese mortherwort. Am J Chin Med 4(4):373-82

Lee, S.K., I.H. Lee, H.J. Kim, G.S. Chang, J.E. Chuang and K.T. No, 2003. The PreADMET Approach: Web-Based Program for Rapid Perdiction of Physico-Chemical, Drug Absorption and Drug-Like Properties. Blackwell Publishing, Massachusetts, pp: 418-420.

Morris, G.M. and M.Lim-Wilby, 2008. Molecular Dcking Method in Molecular Biology. In Molecular Modeling of proteins, Kukol, A (Ed). Humana Press, Totowa, New Jersey, pp: 365-382.

Morris, G.M., Goodsell, D.S., Halliday, R.S., Huey, R., Hart, W.E., Belew, R.K., Olson, A.J., J. Computational Chemistry, 1998, 19(14), 1639-1662

Oda, A, and O. Takahsihi, 2009. Validation of Arguslab efficiencies for binding free energy calculations. Chem-Bio. Informatics J., 9:52-61

Oda, A., M. Okayasu, Y. Kamiyama, T. Yoshida, O, Takahashi and H. Matsuzaki, 2007. Evaluation of docking accuracy and investigation of roles of parameters and each term in scoring fungtions for protein-loigand docking using ArgusLab software. Bull. Chem. Soc. Jap., 80 : 1920 – 1925.

Perry, L.M., 1994, Medicinal Plants of East And Southeast Asia : Attributed Properties and Uses. The MIT Press, Cambridge, 187

Wang, H., Liu, Y., Huai, Q., Cai, J., Zoraghi, R., Francis, S.H., Corbin, J.D., Robinson, H., Xin, Z., Lin, G., Ke, H, 2006. Multiple conformations of phosphodiesterase-5: implications for enzyme function and drug development. Journal. Biol.chem., 281:21468-21479

Xinhua Liua., et al. Leonurine Protects Against Tumor Necrosis Factor-α-mediated in Human Umbilical Vein Endothelial Cell. Organization agreement With Elsevier. 2011

Zou QZ., et.al., 1989. Effect of motherwort on blood hyperviscosity. Am J Chin Med. 1989; 17(1-2):65-70.