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A combination of pharmacophore modeling, molecular docking and virtual screening for iNOS inhibitors from Chinese herbs

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Abstract. Inducible Nitric Oxide Synthase (iNOS) has been involved in a variety of diseases, and thus it is interesting to discover new iNOS inhibitors. This study was performed to identify natural iNOS inhibitors from traditional Chinese herbs through a combination of pharmacophore modeling, molecular docking and virtual screening. First, the pharmacophore models were generated though six known iNOS inhibitors and validated by a test database. The pharmacophore model_017 showed good performance in external validation and was employed to screen Traditional Chinese Medicine Database (Version 2009), which resulting in a hit list of 498 compounds with matching score (QFIT) above 40. Then, the hits were subjected to molecular docking for further refinement. An empirical scoring function was used to evaluate the affinity of the compounds and the target protein. Parts of compounds with high docking scores have been reported to have the related pharmacological activity from the literatures. The results provide a set of useful guidelines for the rational discovery of natural iNOS inhibitors from Chinese herbs.

Keywords: Inducible Nitric Oxide Synthase, Virtual screening, pharmacophore, Traditional Chinese Medicine, active natural ingredients identification

1. Introduction

The key physiologic mediator, nitric oxide (NO), which plays an important role in the regulation of various biological processes, is produced in mammalian cells by three distinct nitric oxide synthases (NOSs): neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) [1-2]. The three isoforms share significant sequence homology (~50%) and catalyze the oxidation of L-arginine to NO [3]. The low levels of NO production are found to protect the body organ, such as the liver from ischemic damage [4]. The action of NO is based on its concentration in the body, e.g., the low concentration produces the key signaling molecule for vasodilatation and neurotransmission while the high concentration leads to the defensive cytotoxin [5]. Inducible NOS was first described in macrophages as a mechanism of macrophage cytotoxicity, and previous studies reveal that it is expressed and activated during inflammatory events [6-8]. Generally, this isoform is not expressed in healthy quiescent

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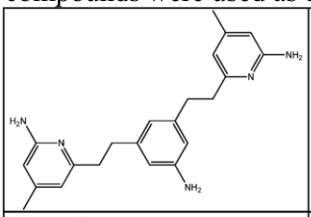
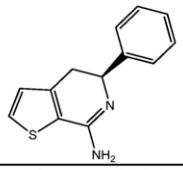
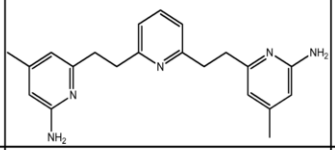
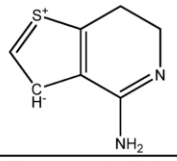
cells and NO is sustained at a low level. However, when iNOS is stimulated by various inflammatory stimuli, a high level of NO is produced and it induces tissue injury at the inflammatory site [9]. Considerable evidence has shown that overproduction of NO induced by iNOS has been implicated in various pathological diseases including septic shock, tissue damage, and rheumatoid arthritis (RA) [10]. Therefore, iNOS has become a potential target for drug development in the treatment of inflammatory diseases.

The work from this paper is to discover the potential natural ingredients which can inhibit the activity of iNOS. A combinational approach based on pharmacophore and molecular docking was employed to screen the chemical database of Traditional Chinese medicine for potential biological active ingredients.

2. Materials and methods

2.1. Data Sets and Tools

The studies were implemented on a series of iNOS inhibitors reported by literature [11-15]. The structures of iNOS inhibitors are listed in Figure 1. The chemical structures were drawn in ISIS-Draw software and converted to 3D structures by SYBYL X-1.2 software. Considering the distribution of structural diversity, six compounds were selected to generate the pharmacophore model and the other compounds were used as test set to validate the model.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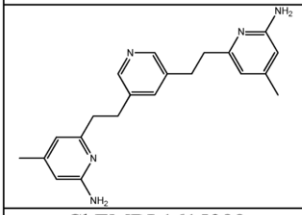	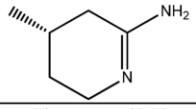	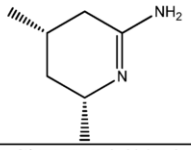	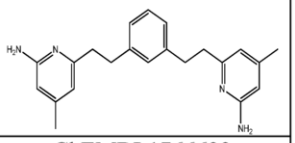
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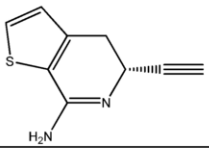	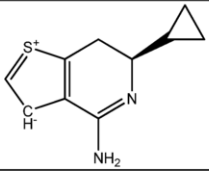	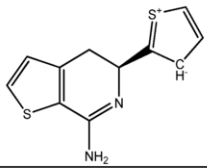	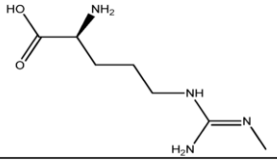
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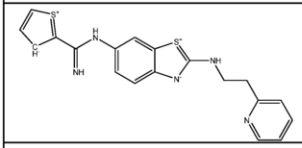	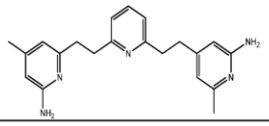	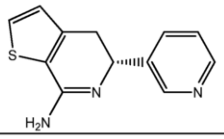	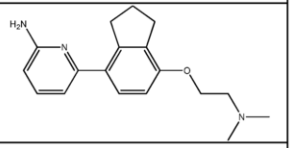
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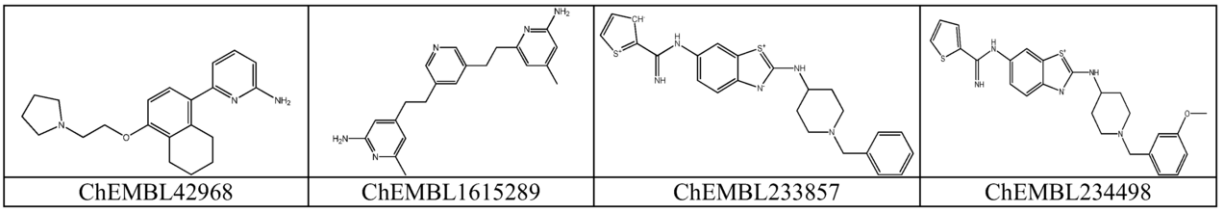

Fig. 1. Chemical structure of iNOS inhibitors

The calculation was conducted on a Dell Red Hat Linux workstation through the programs SYBYL X-1.2 package (Tripos Inc., USA) with default values except those especially referred to. The GALAHAD module was used to generate the pharmacophore model of iNOS inhibitors, the 3D UNITY module was used to perform a flex search for the potential antagonists based on the pharmacophore model and the Surflex-Dock (SFXC) module was used to perform molecular docking.

2.2. Pharmacophore studies

A series of compounds reported in the literature can be divided into three subtypes according to their scaffold, but alignment of all subtypes is difficult. Under such conditions, ChEMBL1615292, ChEMBL1615290, ChEMBL1615288, ChEMBL1766633, ChEMBL1615291 and ChEMBL1615289, with the same scaffold, were chosen as the data source to generate the pharmacophore model. GALAHAD is a proprietary pharmacophore module from Tripos Ldt, which generates pharmacophore models and alignments from sets of compounds (Tripos International, St. Louis). The structures of all compounds were checked for bond orders, hydrogen atoms were added and a minimization procedure was implemented using the MMFF94 force-field. GALAHAD was run for 80 generations with a population size of 60. The generated models were evaluated by a test database which was composed 80 of experimentally known iNOS inhibitors and 320 non-active compounds picked out from MDL Drug Data Report (MDDR, Version 200712) database.

2.3. Model evaluation and virtual screening

The pharmacophore models were generated by GALAHAD and validated by the test database. Several parameters, A%, Y%, N and CAI, introduced from our previous work [16], were employed for model evaluation. The model with the highest value of CAI was considered to be the best model and used to screen Traditional Chinese Medicine Database (TCMD, version 2009), which contains 23033 natural compounds from 6735 medicinal plants.

2.4. Molecular Docking

The X-ray crystal structure of iNOS (PDB code: 2ORO) was selected as the docking template [17]. The ligand N-[2-(1,3-BENZODIOXOL-5-YL)ETHYL]-1-[2-(1H-IMIDAZOL-1-YL)-6-METHYLPYRIMIDIN-4-YL]-D- PROLINAMIDE (Ligand 228) was extracted and treated flexibly while the protein was held rigidly in the docking procedure. The crystallographic water molecules in the structure were removed and hydrogen atoms of modeled structure were added to define the correct configuration and tautomeric states. With the standard parameters, the modeled structure was of minimized energy- using AMBER7 F99 force field. After extracting the binding ligand, the structure of iNOS was used for re-docking with Ligand 228, and the RMSD was calculated to check the accuracy

of the Surflex-Dock program. The compounds hit by the pharmacophore generated were automatically docked into the binding site of iNOS successively. A protomol-based method and an empirically derived scoring function were used to calculate the interaction of the ligands and iNOS.

3. Results and discussion

3.1. Pharmacophore modeling

Twenty GALAHAD models were derived from the training sets. Model 7, 11~15 and 18 had high energy (SE > 1.0×10^6), which is considered to be due to steric clashes. Those models were excluded from the analysis. The other 13 models were evaluated successively by the test database constructed previously. Table 1 shows the validation results. Ht is the total number of hits while Ha is the number of active hits. A% represents the ability to identify active compounds from the test database. Y represents the proportion of active compounds in total hits. N represents the ability to identify active compounds from non-active compounds. CAI was proposed to evaluate of the models comprehensively [16]. D is the total number of compounds in the test database and A is the number of active compounds. The parameters were calculated as follows:

$$A\% = \frac{Ha}{A} \times 100\% \; ; \; Y\% = \frac{Ha}{Ht} \times 100\% \; ; \; N = \frac{Ha \times D}{Ht \times A} \; ; \; CAI = \mathrm{N} \times A\%$$

Table 1

The validation results for each pharmacophore model

Model	Ht	Ha	A%	Y	N	CAI
1	59	37	46.25	0.63	3.14	1.45
2	75	33	41.25	0.44	2.20	0.91
3	78	17	21.25	0.22	1.09	0.23
4	97	15	18.75	0.15	0.77	0.14
5	65	28	35.00	0.43	2.15	0.75
6	39	11	13.75	0.28	1.41	0.19
8	12	6	7.50	0.50	2.50	0.19
9	105	45	56.25	0.43	2.14	1.21
10	124	55	68.75	0.44	2.22	1.52
16	78	48	60.00	0.62	3.08	1.85
17	54	42	52.50	0.78	3.89	2.04
19	51	31	38.75	0.61	3.04	1.18
20	33	19	23.75	0.58	2.88	0.68

Model_017, with the highest value of CAI, was considered to be the best model. The pharmacophore features were displayed in Figure 2, where cyan, green and magenta spheres indicate hydrophobes, HB acceptors and HB donors, respectively. Model_017 includes six pharmacophore features: three hydrophobes, two HB donors and one HB acceptor.

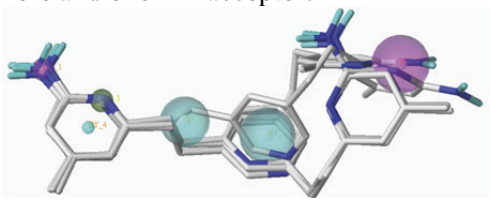

Fig. 2. Pharmacophore Model_017 and molecular alignment of the compounds.

3.2. Virtual Screening

The query of Model_017 was used to perform virtual screening experiment by using the Unity 3D database search protocol with all options set to default. A query fit (QFIT) value was computed for each hit to rank the matching rate of its required structural features on the pharmacophoric query. Virtual screening based on pharmacophore was performed resulting in a hit list of 498 compounds, which were then subjected to molecular docking for further refinement.

3.3. Molecular Docking

The low root mean-square deviation (RMSD) of 2.31 Å between the docked and the crystal conformation indicated the high reliability of Surflex-dock in reproducing the experimentally observed binding mode for iNOS inhibitor. All the 498 compounds were docked into the active pocket of iNOS, resulting in a hit list of 207 compounds. The top 20 compounds with high docking scores were shown in Table 2. The docking schematic diagram of preferred ligands with target was shown in in Figure 3.

Table 2

The docking results of the natural compounds with iNOS

Compound ID	Score	Name	source plant
1	7.43	2,3-Dihydro-7-methoxy-2S*,3R*-dimethyl-2-[4-methyl-5-(4-methyl-2-furyl)-3(E)-pentenyl]-furo[3,2-c]coumarin	*Ferula ferulioides*
2	7.24	Saucernetin 7	*Saururus chinensis*
3	7.16	Annocatacin B	*Annona muricata*
4	6.99	α-D-(6-O-4-Methyl-3,5-dimethoxycinnamoyl)-glucopyranosyl-(1→2)-β-D-(3-O-sinapoyl)-fructofuranose	*Polygala tenuifolia*
5	6.57	Saucernetin 8	*Saururus chinensis*
6	6.29	3,6-Di-O-caffeoyl-(α/β)-glucose	*Rubus sanctus*
7	6.17	Portulenol	*Portulaca grandiflora*
8	6.01	Fukanefuromarin E	*Ferula fukanensis.*
9	5.65	Rhododendrin	*Rhododendron chrysanthum*
10	5.62	Fukanemarin B	*Ferula fukanensis*
11	5.49	Panduratin A	*Kaempferia pandurata*
12	5.48	Silybin	*Silybum marianum*
13	5.29	Myrsinionoside D	*Myrsine seguinii*
14	5.27	3β-cis-p-Coumaroyloxy-2α,23-dihydroxyolean-12-en-28-oic acid	*Eugenia sandwicensis*
15	4.88	Tanshinone IIA	*Salvia miltiorrhiza*
16	4.74	Asiaticoside	*Centella asiatica*
17	4.59	Tryptanthrine	*Isatis indigotica*
18	4.43	Bazzanin Q	*Lepidozia incurvata*
19	4.05	2α,3β,13(S),16α-Tetrahydroxystemodane	*Rhizopus oryzae*
20	4.04	Bazzanin R	*Lepidozia incurvata*

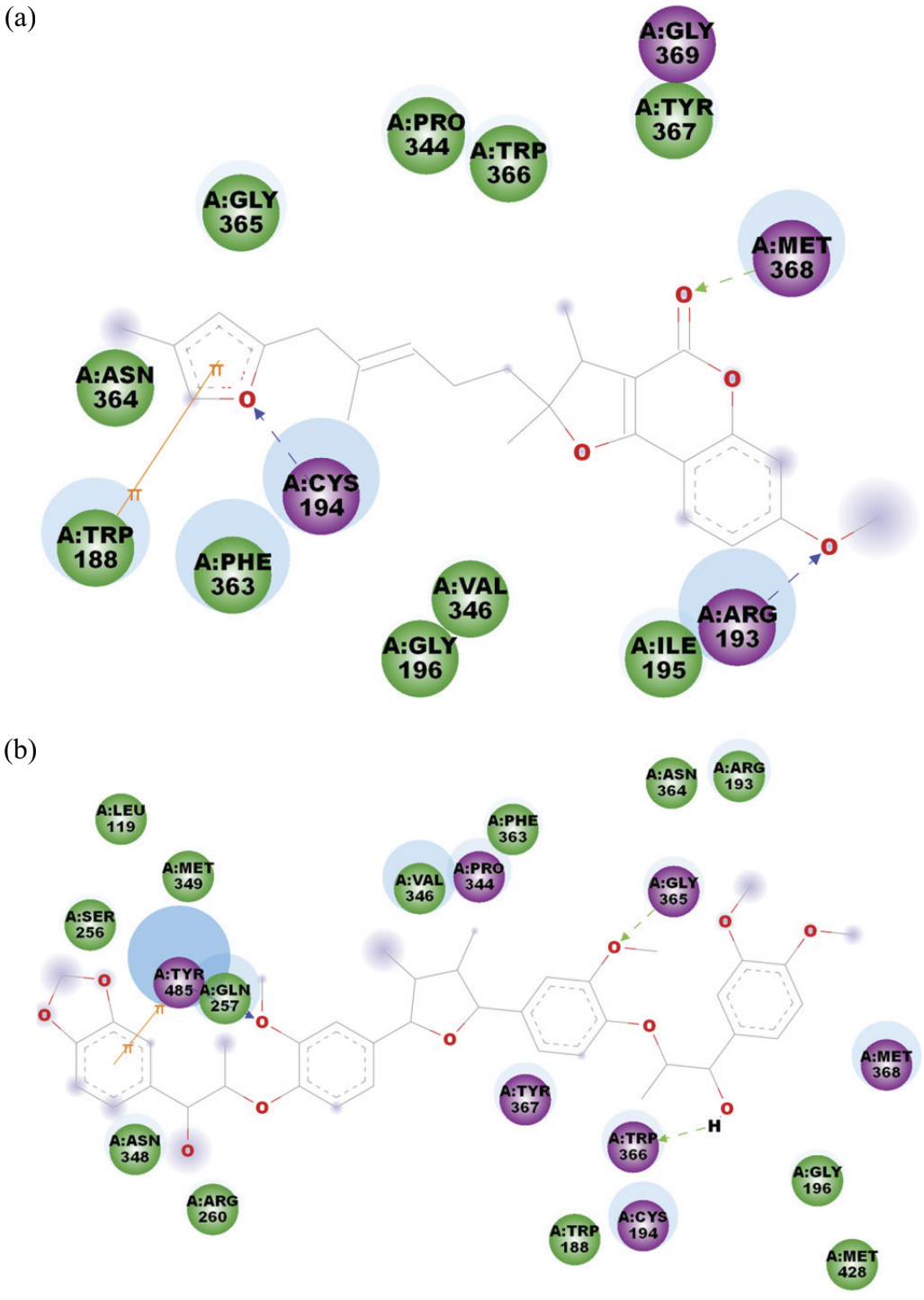

Fig. 3. The interactions between (a) compound 1 or (b) compound 2 and iNOS

3.4. Pharmacological evidence

Several related literature has reported that natural compounds with specific structure have the activities in decreasing the activity or production of NO. Jang [18] found that Tanshinone IIA isolated from *Salvia miltiorrhiza* could markedly inhibit the production of NO through suppressing the expression of iNOS in a dose-dependent manner. Choi [19] reported that four tanshinones isolated from *Salvia miltiorrhiza* demonstrated significant inhibition of the LPS-induced nitric oxide production in RAW 264.7 cells. Guo [20] investigated that *Centella asiatica* water extract and asiaticoside can inhibit the activity of iNOS, which could interpret the anti-inflammatory property of *Centella asiatica.* Silibinin (silybin), a flavonoid derived from the herb milk thistle, has potent anti-inflammatory and antioxidant activities. Lu [21] examined the effect of silibinin on the fear-conditioning memory deficits, inflammatory response, and oxidative stress induced by the intracerebroventricular injection of Aβ peptide25~35 (Aβ25~35) in mice. He found that silibinin can inhibit the overexpression of iNOS and TNF-α mRNA in the hippocampus and amygdala. To a certain extent, the modeling approach in this study may shed light on the mechanism of the molecular recognition of iNOS inhibitors from natural compound database.

4. Conclusion

In summary, a combined computational approach was applied to give insight into the pharmacodynamic characteristics of iNOS inhibitors and screen the potential bioactive components from natural herbals. Meanwhile, the hits through pharmacophore-based virtual screening were further refined by molecular docking procedure. Some of the hit compounds with high score were reported to have the activities of inhibiting iNOS or down-regulation of the expression of iNOS gene. The positive results indicated that the modeling strategies in the present study are most likely to be an encouraging way forward for the rational discovery of natural iNOS inhibitors. Combining with the biological experiments, this approach is recommended to be applied in the research of active ingredients and pharmacological mechanisms from traditional Chinese medicine.

5. Acknowledgement

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References

[1] P. VALLANCE, J. LEIPER, Blocking NO synthesis: how, where and why?, Nat Rev Drug Discov 1 (2002), 939-950.
[2] D. J. STUEHR, Enzymes of the L-arginine to nitric oxide pathway, J Nutr 134 (2004), 2748S-2751S; discussion 2765S-2767S.
[3] H. HUANG, H. JI, H. LI, Q. JING, K. J. LABBY, P. MARTASEK, L. J. ROMAN, T. L. POULOS, R. B. SILVERMAN, Selective monocationic inhibitors of neuronal nitric oxide synthase. Binding mode insights from molecular dynamics simulations, J Am Chem Soc 134 (2012), 11559-11572.
[4] Y. C. HOU, A. JANCZUK, P. G. WANG, Current trends in the development of nitric oxide donors, Curr Pharm Des 5 (1999), 417-441.

[5] C. FENG, Mechanism of Nitric Oxide Synthase Regulation: Electron Transfer and Interdomain Interactions, Coord Chem Rev 256 (2012), 393-411.
[6] J. B. HIBBS, JR., R. R. TAINTOR, Z. VAVRIN, E. M. RACHLIN, Nitric oxide: a cytotoxic activated macrophage effector molecule, Biochem Biophys Res Commun 157 (1988), 87-94.
[7] C. BOGDAN, Nitric oxide and the immune response, Nat Immunol 2 (2001), 907-916.
[8] G. CIRINO, E. DISTRUTTI, J. L. WALLACE, Nitric oxide and inflammation, Inflamm Allergy Drug Targets 5 (2006), 115-119.
[9] M. A. MARLETTA, Nitric oxide synthase: aspects concerning structure and catalysis, Cell 78 (1994), 927-930.
[10] G. NAGY, J. M. CLARK, E. I. BUZAS, C. L. GORMAN, A. P. COPE, Nitric oxide, chronic inflammation and autoimmunity, Immunol Lett 111 (2007), 1-5.
[11] S. L. DELKER, F. XUE, H. LI, J. JAMAL, R. B. SILVERMAN, T. L. POULOS, Role of zinc in isoform-selective inhibitor binding to neuronal nitric oxide synthase, Biochemistry-Us 49 (2010), 10803-10810.
[12] R. K. WEBBER, S. METZ, W. M. MOORE, J. R. CONNOR, M. G. CURRIE, K. F. FOK, T. J. HAGEN, D. W. HANSEN, JR., G. M. JEROME, P. T. MANNING, B. S. PITZELE, M. V. TOTH, M. TRIVEDI, M. E. ZUPEC, F. S. TJOENG, Substituted 2-iminopiperidines as inhibitors of human nitric oxide synthase isoforms, J Med Chem 41 (1998), 96-101.
[13] H. BEATON, N. BOUGHTON-SMITH, P. HAMLEY, A. GHELANI, D. J. NICHOLLS, A. C. TINKER, A. V. WALLACE, Thienopyridines: nitric oxide synthase inhibitors with potent in vivo activity, Bioorg Med Chem Lett 11 (2001), 1027-1030.
[14] J. A. LOWE, 3RD, W. QIAN, S. E. DROZDA, R. A. VOLKMANN, D. NASON, R. B. NELSON, C. NOLAN, D. LISTON, K. WARD, S. FARACI, K. VERDRIES, P. SEYMOUR, M. MAJCHRZAK, A. VILLALOBOS, W. F. WHITE, Structure-activity relationships of potent, selective inhibitors of neuronal nitric oxide synthase based on the 6-phenyl-2-aminopyridine structure, J Med Chem 47 (2004), 1575-1586.
[15] J. PATMAN, N. BHARDWAJ, J. RAMNAUTH, S. C. ANNEDI, P. RENTON, S. P. MADDAFORD, S. RAKHIT, J. S. ANDREWS, Novel 2-aminobenzothiazoles as selective neuronal nitric oxide synthase inhibitors, Bioorg Med Chem Lett 17 (2007), 2540-2544.
[16] W. XING, X. YUHONG, R. ZHENZHEN, Z. YANLING, Q. YANJIANG, Rational questing for inhibitors of endothelin converting enzyme-1 from Salvia miltiorrhiza by combining ligand- and structure-based virtual screening, Can J Chemistry 91 (2013), 1-9.
[17] D. D. DAVEY, M. ADLER, D. ARNAIZ, K. EAGEN, S. ERICKSON, W. GUILFORD, M. KENRICK, M. M. MORRISSEY, M. OHLMEYER, G. PAN, V. M. PARADKAR, J. PARKINSON, M. POLOKOFF, K. SAIONZ, C. SANTOS, B. SUBRAMANYAM, R. VERGONA, R. G. WEI, M. WHITLOW, B. YE, Z. S. ZHAO, J. J. DEVLIN, G. PHILLIPS, Design, synthesis, and activity of 2-imidazol-1-ylpyrimidine derived inducible nitric oxide synthase dimerization inhibitors, J Med Chem 50 (2007), 1146-1157.
[18] S. I. JANG, S. I. JEONG, K. J. KIM, H. J. KIM, H. H. YU, R. PARK, H. M. KIM, Y. O. YOU, Tanshinone IIA from Salvia miltiorrhiza inhibits inducible nitric oxide synthase expression and production of TNF-alpha, IL-1beta and IL-6 in activated RAW 264.7 cells, Planta Med 69 (2003), 1057-1059.
[19] H. S. CHOI, D. I. CHO, H. K. CHOI, S. Y. IM, S. Y. RYU, K. M. KIM, Molecular mechanisms of inhibitory activities of tanshinones on lipopolysaccharide-induced nitric oxide generation in RAW 264.7 cells, Arch Pharm Res 27 (2004), 1233-1237.
[20] G. YUNQI, G. YUNLAN, L. XIHUI, P. LIHONG, Inhibitory Effect of Silybin-Phosphatidylcholine Compound on Production of Nitric Oxide and Reactive Oxygen Species in Mouse Macrophage, Journal of Applied Clinical Pediatrics 23 1691-1692.
[21] P. Lu, T. Mamiya, L. L. Lu, A. Mouri, M. Niwa, M. Hiramatsu, L. B. Zou, T. Nagai, T. Ikejima, T. Nabeshima, Silibinin attenuates amyloid beta(25-35) peptide-induced memory impairments: implication of inducible nitric-oxide synthase and tumor necrosis factor-alpha in mice, J Pharmacol Exp Ther 331 (2009), 319-326.