Docking Study of Biflavonoids, Allosteric Inhibitors of Protein Tyrosine Phosphatase 1B

Jee-Young Lee, Ki-Woong Jung, Eun-Rhan Woo,† and Yangmee Kim*

Department of Bioscience and Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Korea
**E-mail: ymkim@konkuk.ac.kr*
†*College of Pharmacy, Chosun University, Gwangju 501-759, Korea*
Received June 13, 2008

Protein tyrosine phosphatase (PTP) 1B is the superfamily of PTPs and a negative regulator of multiple receptor tyrosine kinases (RTKs). Inhibition of protein tyrosine phosphatase 1B (PTP1B) has been proposed as a strategy for the treatment of type 2 diabetes and obesity. Recently, it has been reported that amentoflavone, a biflavonoid extracted from *Selaginella tamariscina*, inhibited PTP1B. In the present study, docking model between amentoflavone and PTP1B was determined using automated docking study. Based on this docking model and the interactions between the known inhibitors and PTP1B, we determined multiple pharmacophore maps which consisted of five features, two hydrogen bonding acceptors, two hydrogen bonding donors, and one lipophilic. Using receptor-oriented pharmacophore-based *in silico* screening, we searched the biflavonoid database including 40 naturally occurring biflavonoids. From these results, it can be proposed that two biflavonoids, sumaflavone and tetrahydroamentoflavone can be potent allosteric inhibitors, and the linkage at 5',8''-position of two flavones and a hydroxyl group at 4'-position are the critical factors for their allosteric inhibition. This study will be helpful to understand the mechanism of allosteric inhibition of PTP1B by biflavonoids and give insights to develop potent inhibitors of PTP1B.

Key Words : PTP1B, Biflavonoid, Docking study, *In silico* screening, Allosteric site

Introduction

Protein tyrosine phosphatase (PTP) 1B is the superfamily of PTPs and a negative regulator of multiple receptor tyrosine kinases (RTKs).[1,2] Also PTP1B is involved in the down-regulation of insulin and leptin signaling. Thus, inhibitors of PTP1B have potential as therapeutics for treating Type 2 diabetes and obesity.[2,3] Recently, it has been reported that PTP1B inhibition may lead to increased oncogenic signaling.[4-6] Therefore, PTP1B is increasingly drawing attention as an attractive target for anticancer as well as diabetes and obesity.

In the strategy of design of PTP1B inhibitors, selectivity between PTPs is very important factor. The catalytic site of PTPs is highly conserved and has the intractability to small molecule drug discovery, thus the investigation of other mechanisms of inhibition is prompted. At 2004, a novel allosteric site in PTP1B was discovered by Hansen group.[7] Since this site is not well conserved among phosphatases, it afforded an opportunity to outwit the problems associated with inhibition of catalytic site. Allosteric inhibition is a promising strategy for targeting PTP1B and constitutes a mechanism that may be applicable to other tyrosine phosphatases.[7]

According to the recent report, amentoflavone, a naturally occurring biflavonoids derived from *Selaginella tamariscina*, inhibited activity of PTP1B by allosteric inhibition.[8] Biflavonoids are the dimer of flavonoids (homo or hetero) connected with a C-C or C-O-C bond.[9] Many different combinations of flavonoids are possible and may result in various chemical structures. For example, flavanone-flavone, flavone-flavone, and flavanone-flavonol are the most common biflavonoids with connecting linkages at diverse positions. In natural biflavonoids, hydroxyl/methoxy groups are substituted at different positions. Even though numerous biflavonoids with various combinations of flavonoids can be possible, plants that contain biflavonoids as major constituents are not widely distributed.[9,10] More than 100 biflavonoids have been identified from plants and a variety of biological activities of biflavonoids have been published, including anti-inflammatory, antimicrobial, antioxidant activities.[11-13] A symmetric biflavone, amentoflavone is a homo-dimer of apigenins and has several known pharmacological activities such as anti-inflammatory, antioxidative, and anticancer effects.[14,15]

Here, automated docking study for PTP1B was performed and a docking model between PTP1B and amentoflavone at the allosteric site of PTP1B was proposed. Furthermore, we determined a pharmacophore map by receptor-oriented pharmacophore-based *in silico* screening using structure-based focusing (SBF) module of Cerius2[16-20] and screened potent biflavonoid inhibitors.

Methods

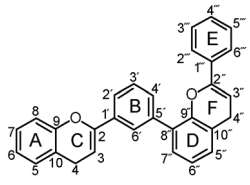
Docking study. Docking study was performed between amentoflavone and PTP1B in order to find specific binding model using AutoDock[21,22] based on x-ray structure of PTP1B (1T49.pdb and 1T4J.pdb). The Lamarckian Genetic Algorithm (LGA) of the AutoDock 3.05 was used for

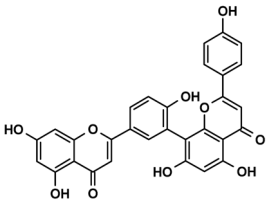
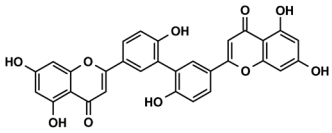
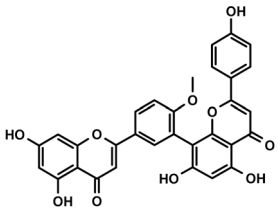
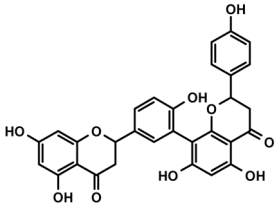
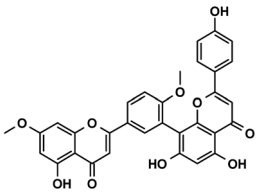
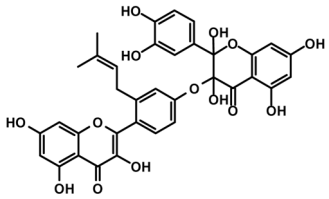
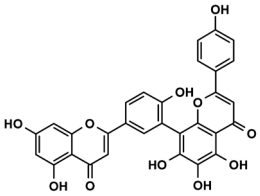
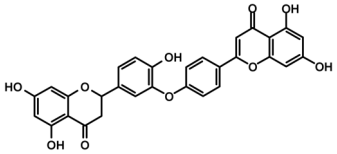
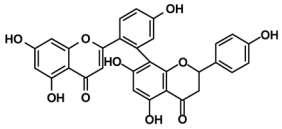
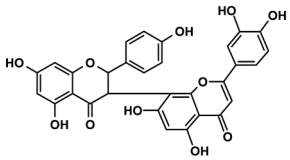
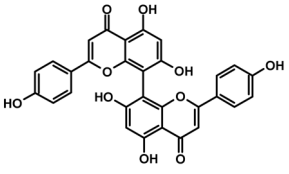
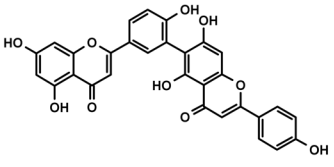
docking experiments. Distance-dependent function of the dielectric constant was used for the calculation of the energetic maps. MD simulations were performed for the final docking structures in the canonical ensemble (NVT) at 300K using the program InsightII/Discover. All atoms of the system were considered explicitly, and their interactions were computed using the consistent valence force field. A distance cutoff of 10 Å was used for van der Waals interactions and electrostatic interactions. The time step in the MD simulations was 1 fs and MD simulation was performed for 2 ns. Coordinates were saved every 1 ps.

Receptor-oriented Pharmacophore-based *in silico* Screening. The interaction model used for this process is a list of features, such as hydrogen bonds and lipophilic interactions. These features include hydrogen bond donors (HBDs), hydrogen bond acceptors (HBAs) and lipophilicity (Lipo).

We defined the active site of PTP1B using the center and the radius of the docked inhibitor and amentoflavone. An interaction model was generated within 10 Å of the center of

Table 1. Chemical structures of representative biflavonoids in our database



Name	Structure	Name	Structure
Amentoflavone (5',8"-biflavone)		(5',5'"-biflavone)	
Bilobetin (5',8"-biflavone)		Tetrahydro-amentoflavone (5',8"-biflavanone)	
Ginkgetin (5',8"-biflavone)		Prodoverine B [4',3"-biflavonyl (flavanone-flavonol) ether]	
Sumaflavone (5',8"-biflavone)		Ochnaflavone [5',4"-biflavonyl (flavanone-flavone) ether)	
6',8"-biapigenin		Morelloflavone [3,8"-(flavanone-flavone)]	
Cupressuflavone (8,8"-biapigenin)		Robustaflavone (5',6"-biflavone)	

the active site. Multiple pharmacophore maps were determined with five features for each map and the exclusion volume. The exclusion volume was built from the heavy atoms within 10 Å of the center of the active site. Then, pharmacophore map which reproduced the docking model of PTP1B and amentoflavone was selected. We built a database of 40 biflavonoids with different combinations of flavonoids as listed in Table 1, such as flavanone-flavone, flavone-flavone, flavanone-flavonol.[23-41] This database was browsed using selected pharmacophore map. The scoring functions (LigScore2, PLP1, and PLP2) were calculated for hits to establish a relationship between this pharmacophore and the set of inhibitors using Cerius2.[42,43]

Results and Discussion

Analysis of x-ray structure of PTP1B and allosteric inhibitor. The catalytic site of PTP1B is centered at Cys 215 residue and included WPD loop, which closure is essential for the catalytic mechanism of PTP1B. Allosteric site of PTP1B is located ~20 Å away from Cys 215 at the catalytic active site.[7] The interactions between PTP1B and allosteric inhibitors have been identified from the X-ray crystal structures and we utilized this information to analyze the docking model of PTP1B and amentoflavone. Two active sites of PTP1B, catalytic site and allosteric site, are represented in Figure 1. X-ray structures of PTP1B showed that allosteric inhibitors (inhibitor 2 and 3) formed several important hydrogen bonds with PTP1B.[7] Hydrogen bonding interactions are observed with the side chain of Asn193 and the carboxyl oxygen of Glu276. In addition, one water-mediated hydrogen bonding between hydroxyl group of inhibitor and the main chain carbonyl of Phe196 is formed. Two allosteric inhibitors particularly wrap around Phe280 and these hydrophobic interactions, combined with the hydrogen bonding with Glu276, probably increase their potency.[7] In the case of inhibitor 1, this molecule was not wrapping the hydrophobic residue Phe280 and did not formed a hydrogen bond with Glu276. This is a reason for the low inhibitory activity of inhibitor 1 on PTP1B. The binding model between inhibitor 2 and PTP1B is shown in Figure 1.

Docking study between PTP1B and amentoflavone. Docking model of amentoflavone and PTP1B was similar to x-ray structures of PTP1B complex with known inhibitors, and several hydrogen bonds were also found in our docking model. 4-carboxyl group of amentoflavone and side chain of Asn193 formed a hydrogen bonding, and 4'-hydroxyl group and side chain carboxyl oxygen of Glu276 also participated in hydrogen bonding interaction. It is already known that the hydrogen bonding between inhibitor and Glu276 has significant effect to increase the inhibitory activity. 7-hydroxyl group of amentoflavone is participated in water mediated hydrogen bond interaction. In addition, hydroxyl group at 7"'-position of amentoflavone formed a hydrogen bonding with backbone oxygen of Phe280 and this hydrogen bonding was not found in the x-ray structures of PTP1B complex with three inhibitors. Thus, this is a unique feature of biflavonoids inhibitors of PTP1B. Amentoflavone also wrapped around Phe280 and formed hydrophobic interaction with Phe196 and Leu 192. The interaction model between amentoflavone and PTP1B is shown in Figure 2A.

Receptor-oriented pharamcophore-based *in silico* screening. Based on docking structure, we generated a receptor-oriented interaction model for the active site of PTP1B and five phamrcophore maps were determined based on the hydrogen bonding interactions and hydrophobic interaction between PTP1B and known allosteric inhibitors. Five maps consisted of five features, two HBA including a water mediated hydrogen bonding with Phe 196 and a hydrogen bonding with Asn193, two HBD including a hydrogen bonding with Glu276 and that with backbone carbonyl of Phe280, and one Lipo including hydrophobic interaction with Leu192 and Phe280. Among five features in pharmacophore map, three features including two HBA and one HBD were satisfied with the interactions between PTP1B and known inhibitors. To define a proper Lipo site, we determined five multiple maps which have a Lipo site at different coordinate in allosteric site. Among these five maps, only Map 3 reproduced the binding model of amentoflavone and PTP1B properly and Map 3 is shown in Figure 2B.

Structural data of 40 known biflavonoids were collected

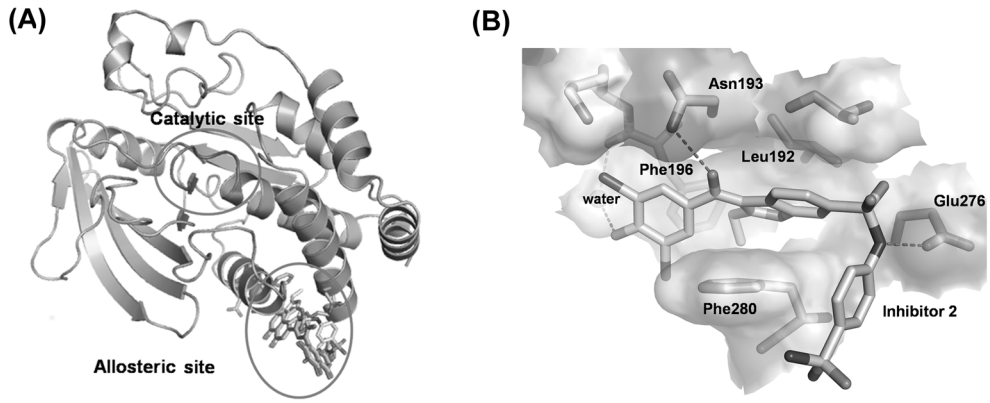


Figure 1. (A) Crystal structure showing the catalytic site and allosteric site of PTP1B. (B) Binding structure of PTP1B and inhibitor 2 in allosteric site.[7]

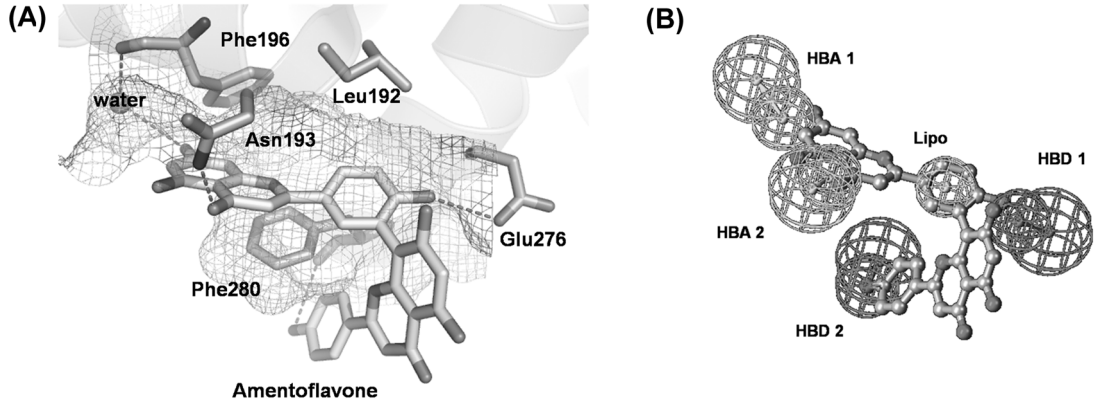


Figure 2. (A) Docking model of PTP1B and amentoflavone. Side chain of Phe196, Leu192, and Phe280 participated in hydrophobic interactions with amentoflavone. (B) Optimal pharmacophore map, Map 3.

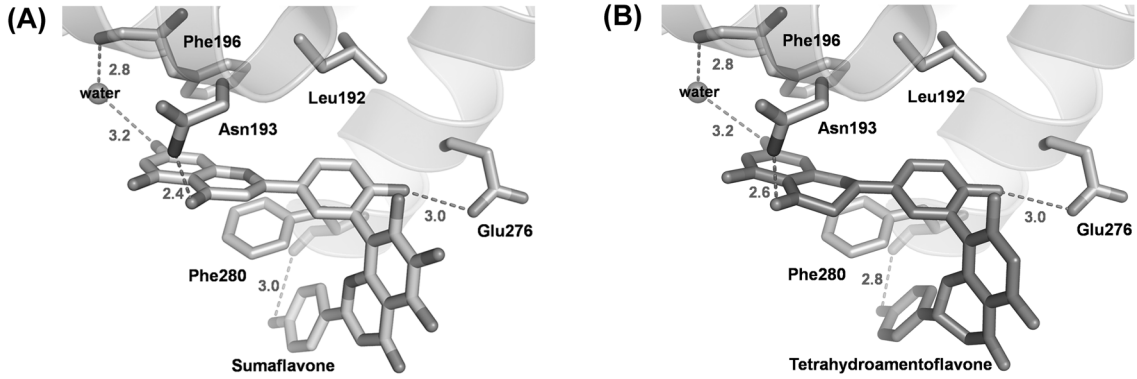


Figure 3. Hit models of biflavonoids and PTP1B. (A) Sumaflavone (B) Tetrahydro-amentoflavone

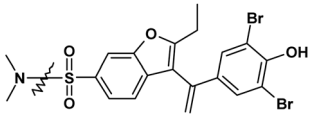
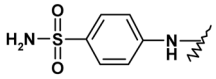
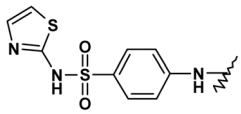
from several articles and biflavonoid database.[23-41] These are naturally occurring biflavonoids and they clustered by combinations of flavanone-flavone, flavone-flavone, and flavanone-flavonol with various linkages. Chemical structures of representative 12 biflavonoids are depicted in Table 1. We built bioflavonoid database and then searched it with Map 3. Among 40 biflavonoids, only two, sumaflavone and tetrahydroamentoflavone, matched well with the pharmacophore map. Sumaflavone is an asymmetric biflavones isolated from plants such as *Selaginella tamariscina*.[23] Tetrahydroamentoflavone (THF) is one of the major flavonoids extracted from *Semecarpus anacardium* Linn. (Anacardiaceae).[24] These two biflavonoids have potent anticancer activity.[23,24] Hit models of two biflavonoids satisfied each features of phamracophore maps with proper configurations. Interactions between PTP1B and these two biflavonoids and the distances between the atoms participated in hydrogen bonding interactions are shown in Figure 3.

Amentoflavone, sumaflavone, and THF are biflavone linked at 5',8" position. Amentoflavone is 5',8"-biapigenin and sumaflavone is constructed of one apigenin and 4',5,6,7-tetrahydroxy flavones linked at 5',8" position. The chemical name of THF is 5',8"-binaringenin. Other biflavonoids which do not have 5',8" linkage could not bind suitably at the allosteric site of PTP1B because of the steric hindrance and could not wrap Phe280 properly. Even though ginkgetin is a biflavonoid with 5',8" linkage, methylation of 4'-OH disrupted the hydrogen bond with Glu276 and the water mediated hydrogen bond is missed by methylation of 5-OH. Bilobetin also missed the hydrogen bonding interaction with Glu276 because of methylation of 4'-position.

From these results, linkage type of biflavonoids is the critical factor for the inhibitor to wrap Phe280 properly. A hydroxyl group on 4'-position of biflavonoids provides the stable hydrogen bonding with Glu276 which is the essential feature for allosteric inhibition. Additionally, 4'"-OH of these biflavonoids form an extra hydrogen bonding with the backbone carbonyl of Phe280 and increase the inhibitory activity of them.

Evaluation of docking study between PTP1B and biflavonoids. To verify docking study, we calculated scoring functions; LigScore2, PLP1, and PLP2. The LigScore2 is a scoring function that possesses high predictive accuracy of affinity of ligand-receptor binding as well as pK_i values.[42] The PLP (Piecewise Linear Potential) is an empirical scoring function with two types, PLP1 and PLP2. In the PLP1 function, each non-hydrogen ligand or non-hydrogen receptor atom is assigned as PLP atom types. All hydrogen atoms are excluded from the PLP function. In PLP2, PLP atom type remains the same as in PLP1, but a PLP atomic radius is assigned to each atom except for hydrogen, thus atomic interactions represent the hydrogen bonding, repulsion, and dispersion. In both PLP functions, the higher PLP score indicates the stronger binding affinity with the receptor.[43]

Table 2. The scoring function LigScore2 and PLP of known inhibitors and biflavonoids

Compound	IC50 (μM)	LigScore2	PLP1	PLP2
 Inhibitor 1[a]	350[a]	5.58	74.0	73.3
 Inhibitor 2[a]	22[a]	7.30	132.7	122.0
 Inhibitor 3[a]	8[a]	7.33	134.3	129.7
Amentoflavone	7.3 ± 0.5[b]	7.69	136.4	131.5
Sumaflavone	–	7.51	130.4	128.6
Tetrahydroamentoflavone	–	7.64	135.5	134.4

[a]Christian, W. *et al. Nat. Struc. Mol. Biol.* 2004. [b]Na, M. K. *et al. Biol. Pharm. Bull.* 2007.

Experimental IC_{50} value of allosteric inhibitors and amentoflavone reported previously,[7,8] and the calculated scoring functions (LigScore2, PLP1, and PLP2) of known inhibitors and hit biflavonoids are compared in Table 2.

All three scoring functions of amentoflavone and inhibitor 3 which have the best IC_{50} values are highly ranked than the rest two inhibitors. The least active inhibitor (inhibitor 1) has the lowest scores as listed in Table 2. Scoring functions, especially PLP 2, proved that automated docking process for amentoflavone and PTP1B was well performed and the docking model represented well the critical features for inhibitors of PTP1B. It can be proposed that hit biflavonoids, sumaflavone and THF, in this study can be potent allosteric inhibitors of PTP1B.

Conclusion

In this study, we determined the docking model between amentoflavone and PTP1B using automated docking study and we confirmed that amentoflavone was well docked in the allosteric site of PTP1B. Based on docking model, we determined multiple pharmacophore maps using by receptor-oriented pharmacophore-based *in silico* screening and determined optimal map which consisted of five features; two HBA, two HBD, and one Lipo. We searched biflavonoid database including 40 naturally occurring biflavonoids with this map. Docking study and receptor-oriented pharmacophore based *in silico* screening, it was proposed that two biflavonoids, sumaflavone and tetrahydrowamentoflavone can be potent allosteric inhibitors of PTP1B, where two flavones are linked at 5',8"-position and have a hydroxyl group at 4'-position. These results designated that certain biflavonoids can affect cancer disease, suggesting therapeutic potential against cancer. Further study, we will investigate further anticancer activity of these biflavonoids for several anticancer targeted proteins, protein tyrosine phosphatases such as PTP1B and PRL-3. This study may provide a strategy for the development of novel PTP1B allosteric inhibitors.

Acknowledgments. This work was supported by the Research Program for New Drug Target Discovery (M10601000153-07N0100-15310) grant from the Ministry of Science & Technology, South Korea and by Bio/Molecular Informatics Center of Konkuk University (KRF-2006-005-J03402). Ki-Woong Jung is supported, in part, by the second BK21 (MOE).

References

1. Östman, A.; Böhmer, F. *Trends Cell. Biol.* **2001**, *11*, 258.
2. Li, S.; Depetris, R. S.; Barford, D.; Chernoff, J.; Hubbard, S. R. *Structure* **2005**, *13*, 1643.
3. Dixit, M.; Tripathi, B. K.; Tamrakar, A. K.; Srivastava, A. K.; Kumar, B.; Goel, A. *Bioorg. Med. Chem.* **2001**, *15*, 727.
4. Yi, T.; Lindner, D. *Curr. Onco. Report* **2008**, *10*, 114.
5. Dubé, N.; Cheng, A.; Tremblay, M. L. *PNAS* **2004**, *101*, 1834.
6. Lyon, M. A.; Ducruet, A. P.; Wipf, P.; Lazo, J. S. *Nat. Rev.* **2002**, *1*, 961.
7. Wiesmann, C.; Barr, K. J.; Kung, J.; Zhu, J.; Erlanson, D. A.; Shen, W.; Fahr, B. J.; Zhong, M.; Taylor, L.; Randal, M.; McDowell, R. S.; Hansen, S. K. *Nat. Struc. Mol. Biol.* **2004**, *11*, 730.
8. Na, M.; Kim, K. A.; Oh, H.; Kim, O. Y.; Oh, W. K.; Ahn, J. S. *Biol. Pharm. Bull.* **2007**, *30*, 379.
9. Chen, J.; Chang, H. W.; Kim, H. P.; Park, H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2373.
10. Kim, H. P.; Park, H.; Son, K. H.; Chang, H. W.; Kang, S. S. *Arch. Pharm. Res.* **2008**, *31*, 265.
11. Lin, Y.; Flavin, M. T.; Cassidy, C. S.; Mar, A.; Chen, F. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2101.
12. Blazso, G.; Gabor, M.; Rohdewald, P. *Pharmazie.* **1997**, *52*, 380.
13. Ursini, F.; Rapuzzi, I.; Toniolo, R.; Tubaro, F.; Bontempelli, G. *Methods Enzymol.* **2001**, *335*, 338.
14. Woo, E. R.; Lee, J. Y.; Chom, I. J.; Kim, S. G.; Kang, K. W. *Pharmaco. Res.* **2005**, *51*, 539.
15. Pan, X.; Tan, N.; Zeng, G.; Zhang, Y.; Jia, R. *Bioorg. Med. Chem.* **2005**, *13*, 5819.
16. Paul, D. K.; Rob, B.; Scott, K.; Marvin, W.; Venkatachalam, C. M. *J. of Comp. Chem.* **2001**, *22*, 993.

17. Hoffrén, A. M.; Murray, C. M.; Hoffmann, R. D. *Curr. Pharm. Des.* **2001**, *7*, 547.
18. Luke, S. F.; Osman, F. G. J. *Braz. Chem. Soc.* **2002**, *13*, 777.
19. Pickett, S. D.; Mason, J. S.; McLay, I. M. *J. Chem. Inf. Comput. Sci.* **1996**, *36*, 1214.
20. Lee, J. Y.; Baek, S.; Kim, Y. *Bull. Korean Chem. Soc.* **2007**, *28*, 379.
21. 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*, 1639.
22. Lee, J. Y.; Lee, S. A.; Kim, Y. *Bull. Korean Chem. Soc.* **2007**, *28*, 941.
23. Yang, S. F.; Chu, S. C.; Liu, S. J.; Chen, Y. C.; Chang, Y. Z.; Hsieh, Y. S. *J. Ethnopharmaco* **2007**, *110*, 483.
24. Gil, R. R.; Lin, L.; Cordell, G. A.; Kumar, M. R.; Ramesh, M.; Reddy, B. M.; Mohan, G. K.; Rao, A. V. N. A. *Phytochem* **1995**, *39*, 405.
25. Selvam, C.; Sanjay, M. J. *J. Ethnopharmaco* **2004**, *95*, 209.
26. Li, X.; Hoshi, A. S.; Tan, B.; ElSohly, H. N.; Walker, L. A.; Zjawiony, J. K.; Ferreira, D. *Tetrahedron* **2002**, *58*, 8709.
27. Bekker, R.; Ferreira, D.; Swart, K. J.; Brandt, E. V. *Tetrahedron* **2000**, *56*, 5297.
28. Hyun, S. K.; Kang, S. S.; Son, K. H.; Chung, H. Y.; Choi, J. S. *Chem. Pharm. Bull.* **2005**, *53*, 1200.
29. Yamaguchi, L. F.; Vassao, D. G.; Kato, M. J.; Mascio, P. D. *Phytochem.* **2005**, *66*, 2238.
30. Das, B.; Mahender, G.; Rao, Y. K.; Prabhakar, A.; Jagadeesh, B. *Chem. Pharm. Bull.* **2005**, *53*, 135.
31. Kumar, N.; Singh, B.; Bhandarim, P.; Gupta, A. P.; Uniyal, S. K.; Kaul, V. K. *Phytochem.* **2005**, *66*, 2740.
32. Choi, S. K.; Oh, H. M.; Lee, S. K.; Jeong, D. G.; Ryu, S. E.; Son, K. H.; Han, D. C.; Sung, N. D.; Baek, N. I.; Kown, B. M. *Nat. Product. Res.* **2006**, *20*, 341.
33. Lee, C. W.; Choi, H. J.; Kim, H. S.; Kim, D. H.; Chang, I. S.; Moon, H. T.; Lee, S. Y.; Oh, W. K.; Woo, E. R. *Bioorg. Med. Chem.* **2008**, *16*, 732.
34. Slade, D.; Ferreira, D.; Marais, J. P. J. *Phytochem.* **2005**, *66*, 2177.
35. Weniger, B.; Vonthron-Sénécheau, C.; Kaiser, M.; Brun, R.; Anton, R. *Phytomedicine* **2006**, *13*, 176.
36. Ariyasena, J.; Baek, S. H.; Perry, N. B.; Weavers, R. T. *J. Nat. Prod.* **2004**, *67*, 693.
37. Mayer, R. *Phytochem.* **2004**, *65*, 593.
38. Innocenti, M.; Michelozzi, M.; Giaccherini, C.; Ieri, F.; Vincieri, F. F.; Mulinacci, N. *J. Agric. Food Chem.* **2007**, *55*, 6596.
39. Likhitwitayawuid, K.; Kaewamatawong, R.; Ruangrungsi, N. *Biochem. Systemat. Ecology* **2005**, *33*, 527.
40. Rampendahl, C.; Seeger, T.; Geiger, H.; Zinsmeister, H. D. *Phytochem.* **1996**, *41*, 1621.
41. Howell, H.; Malan, E.; Brand, D. J.; Kamara, B. I.; Bezuidenhoudt, B. C. B.; Marais, C.; Steenkamp, J. A. *Chemistry of Natural Compounds* **2007**, *43*, 533.
42. Krammer, A.; Kirchhoff, P. D.; Venkatachalam, X. J. C. M.; Waldman, M. *J. Mol. Graph. Model.* **2005**, *23*, 395.
43. Verkhivker, G. M.; Bouzida, D.; Gehlhaar, D. K.; Rejto, P. A.; Arthurs, S.; Colson, A. B.; Freer, S. T.; Larson, V.; Lutyi, B. A.; Marrone, T.; Rose, P. W. *Journal of Computer-Aided Molecular Design* **2000**, *14*, 731.