Flavonoids can be Potent Inhibitors of Human Phenylethanolamine *N*-Methyltransferase (hPNMT)

Jee-Young Lee, Ki-Woong Jeong, and Yangmee Kim*

*Department of Bioscience and Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Korea. *E-mail: ymkim@konkuk.ac.kr.*
Received June 17, 2009, Accepted June 26, 2009

Inhibition of human phenylethanolamine *N*-methyltransferase (hPNMT) has been proposed as a method for the treatment of several mental processes which related on adrenaline metabolism. We performed *in silico* screening to identify flavonoid inhibitors of hPNMT using automated docking method and selected 9 inhibitor candidates based on ligand score (LigScore) and binding free energy (ΔG_{bind}) estimation. Among 9 flavonoid candidates, 7 flavonoids belong to flavones while the rest of them belong to flavanone. All candidates have common chemical features; two hydrogen bond interactions with side chain of Lys75 and backbone carbonyl oxygen of Asn39, and two hydrophobic interactions. One hydrophobic site is formed by Val53, Leu262, and Met258 and the other is made up of Phe182, Ala186, Tyr222, and Val269. This study can be helpful to understand the structural features for inhibition of PNMT and showed flavonoids as promising inhibitor candidates for hPNMT.

Key Words: PNMT, Mental disease, Flavonoid, Docking study, *In silico* screening

Introduction

N-Methylation is a prominent pathway for the metabolism of several endogenous hormones and neurotransmitters. This reaction occurs via transfer of a methyl group from S-adenosyl-L-methionine (SAM) to nucleophilic amino groups, leading to the production of *N*-methylated metabolites and *S*-adenosylhomocysteine (SAH).[1] Adrenaline (or epinephrine) accounts for 5 - 10% of total catecholamines in the central nervous system (CNS).[2] Adrenaline is synthesized *in vivo* from noradrenaline in a reaction catalyzed by phenylethanolamine *N*-methyltransferase (PNMT), a 30 kDa enzyme that utilizes the cofactor SAM to methylate the amine of noradrenaline.[3] PNMT is employed as a catecholamine biosynthetic marker, and the presence of PNMT-containing neurons in the brain suggests that CNS adrenaline is involved in the central control of blood pressure, respiration, and pituitary hormone secretion.[3] It has been implicated in the effects of ethanol intoxication and neural degeneration observed in Alzheimer's disease.[4,5] There have been efforts to develop potent PNMT inhibitors as angina pectoris, myocardial infarction and anxiety neuroses agents.[6,7]

Flavonoids have various biological activities such as antioxidant, antibacterial, and anticancer effect.[8,9] It has been reported that flavonoids play roles in inhibition of catecholamine-metabolizing enzymes.[10] Therefore flavonoids may act as inhibitors of PNMT. In this study, we performed high-throughput *in silico* screening with automated docking and proposed a binding model between hPNMT and flavonoid. Finally, we suggested important features to design of potent flavonoid inhibitors of hPNMT.

Methods

***In silico* screening with automated docking.** To find potent flavonoid inhibitors of hPNMT, docking study was performed using AutoDock (version 4.2)[11,12] based on X-ray complex structure of hPNMT and inhibitor SK&F 29661 (1HNN.pdb).[3] The Lamarckian genetic algorithm (LGA) was used for ligand conformational searching and the default parameters are used for docking. Flexibilities of protein and ligand were incorporated in simulations and the binding free energies (ΔG_{bind}) were estimated for the docked flavonoids.[11,13] The docking parameters included a trials of 100 dockings, random starting position and conformation, translation step ranges of 2 Å, rotation step ranges of 30°, mutation rate of 0.02, crossover rate of 0.8, 15 million energy evaluations, and a maximum 20,000 generation. More than 500 flavonoids from Indofine Chemical Company (Belle Mead, NJ) were included in our database. We selected potential inhibitor candidates of hPNMT from the calculation of LigScore and ΔG_{bind}.[14,15]

Results and Discussion

A known inhibitor SK&F 29661 and several inhibitors of PNMT accept hydrogen bonds directly with Lys57, Glu219, and Asp267 of hPNMT.[16] Among them, a hydrogen bond between Lys57 and inhibitor is critical for inhibitory activity against hPNMT.[17] We defined the active site of hPNMT around the center of inhibitor. Docking study was performed with consideration of flexibility of compound and protein. Since the structural flexibility of side chain of Lys75 has been studied extensively,[17] we assigned Lys57 as flexible residue in our docking study.

45 flavonoids were fit to the active site of hPNMT and finally we selected 9 flavonoids as candidates from the estimation of LigScore and G_{bind}. The LigScore is a scoring function that predicts the affinity of ligand-receptor binding.[14] One of the advantages of AutoDock allows a calculation of ΔG_{bind} and prediction of binding constants for docked igands.[11,13] ΔG_{bind} of the protein-ligand complex is simply calculated by

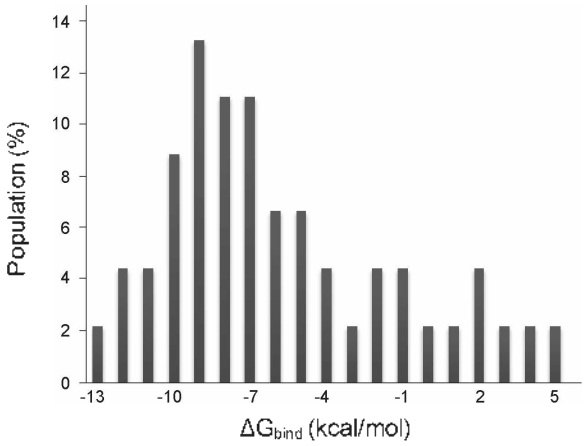

Figure 1. Population of binding free energy (ΔG_{bind}) of 45 initial hit flavonoids.

Table 1. LigScore and binding free energy (G_{bind}) of selected flavonoids

	Compound	Name	Lig-Score	ΔG_{bind} (kcal/mol)
	SK&F 29661	-	6.92	-13.3
Flavone	1	2'-hydroxyflavone	5.78	-10.6
	2	2',3'-hydroxyflavone	5.64	-10.4
	3	7,2'-dimethoxy-3-hydroxyflavone	5.11	-11.2
	4	3-hydroxy-6-methoxyflavone	6.33	-12.2
	5	5-hydroxy-7-methoxyflavone	6.70	-13.1
	6	3-hydroxy-6-methylflavone	6.14	-12.5
	7	2'-hydroxy-β-naphthoflavone	5.55	-11.5
Flavanone	8	2'-hydroxyflavanone	5.16	-10.4
	9	3'-hydroxyflavanone	5.31	-10.5

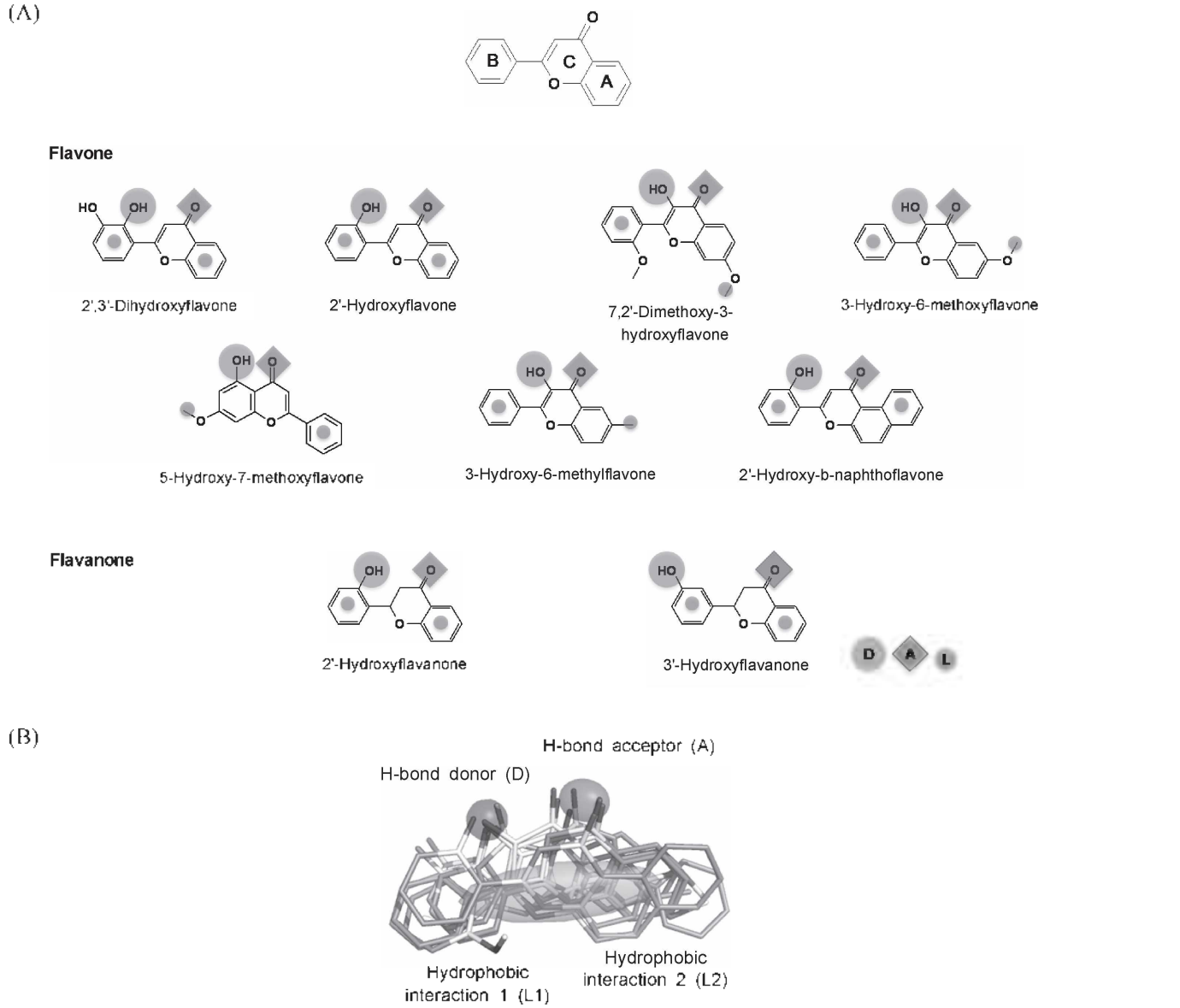

Figure 2. (A) 2D structures of 9 hit flavonoids and (B) Common pharmacophore of hits. (D is hydrogen bonding donor, A is hydrogen bonding acceptor, and L is hydrophobic interaction).

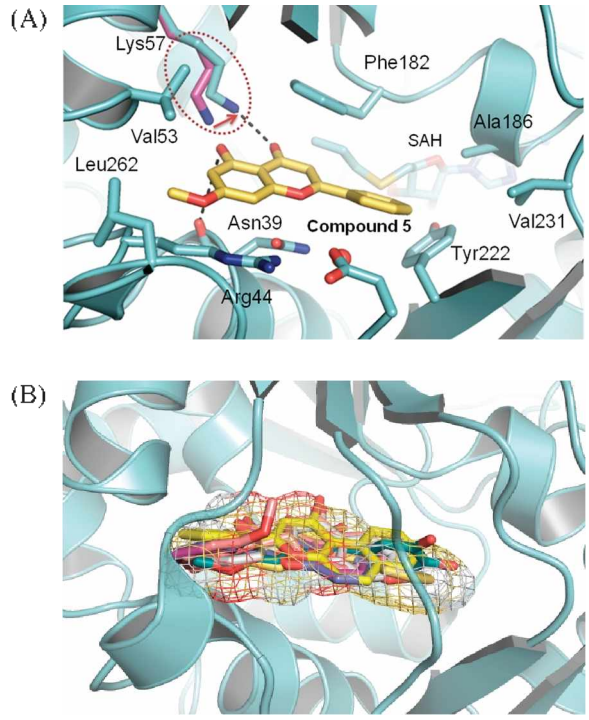

Figure 3. Hit models of (A) compound5 and hPNMT. The red dotted circle represented the movement of Lys57 side chain. The original conformation of Lys57 is depicted in magenta and the red arrow represented the movement of side chain. Hydrogen bonds between compound 5 and hPNMT are represented by the black dashed line. (B) All 9 hit flavonoids and hPNMT.

the following equation (1).[18]

$$\Delta G_{bind} = \Delta G_{complex} - (\Delta G_{protein} - \Delta G_{ligand}) \quad (1)$$

Here, $\Delta G_{complex}$ is the free energy of protein-ligand complex. $\Delta G_{protein}$ is the free energy of protein while ΔG_{ligand} is the free energy of ligand. The low ΔG_{bind} implies a good binding affinity. Distribution (population %) of ΔG_{bind} of 45 initial hit flavonoids was depicted in Figure 1 and ΔG_{bind} of 45 flavonoids ranged from -13.3 to + 5.75 kcal/mol.

For final selection of candidates, we collected the structural information of 10 known PNMT inhibitors from world patent and calculated the LigScore and ΔG_{bind} of these 10 known inhibitors and SK&F 29661.[19-21] LigScore of all 11 PNMT inhibitors were in the range of 4.81 to 7.24 and ΔG_{bind} were in the range of -10.2 to -13.3 kcal/mol. Experimental binding constants (K_i) of these inhibitors ranged from 0.26 to 0.97 μM. The typical inhibitor SK&F 29661 show 6.92 of LigScore and -13.3 of ΔG_{bind}. Therefore, we selected flavonoids with LigScore > 5 and ΔG_{bind} < -10 kcal/mol. LigScore and ΔG_{bind} of selected 9 flavonoids are listed in Table 1.

Among 9 flavonoids, seven belongs to flavones and the rest of them are classified as flavanone. Structures of hits are shown in Figure 2. Flavones are classes of flavonoids with the backbone of 2-phenylchromen-4-one and flavanones are based on the structure of 2-phenylchroman-4-one which has one less double bond in C-ring.

All hit flavonoids have two hydrogen bonds and two hydrophobic interactions with hPNMT. Carbonyl oxygen of flavonoid C-ring formed a hydrogen bond with NH of Lys57 side chain. OH group of C- or B-ring participated in the hydrogen bonding interaction with backbone carbonyl oxygen of Asn39. Two hydrophobic interactions are formed. First interaction site is formed by Leu262, Val53, and Arg44, and second site is near the Phe182, Ala186, Tyr222, and Val231. As compared with the crystal structure of PNMT, inhibitor of SK&F 29661 forms several hydrogen bonding interactions with Glu219, Lys57, and water-mediated hydrogen bonds, but it forms only one hydrophobic interaction with Phe182, Asn39, and Val269 which correspond to the first hydrophobic site at the interaction model between flavonoids and hPNMT. In Figure 2, we depicted the 2D structures and common pharmacophore of 9 hit flavonoids. The docking models in Figure 3 showed that side chain of Lys57 was adapted to the docking complex resulting in movement of side chain about 1.2 Å. Original conformation of Lys57 was depicted in magenta and the side chain of Lys57 in docking structure was shown in cyan. Movement of Lys57 side chain represented by the red arrow allowed flavonoids to fit into the active site of PNMT.

Among the 9 flavonoids which can be possible inhibitors of hPNMT, compound 5 was the most potent candidate because it has the highest LigScore (6.70) as well as the lowest ΔG_{bind} (-13.1). Compound 4 and 6 also can be potential candidates of PNMT inhibitors but their scoring values were slightly less than compound 5. This result can be explained by the difference of hydrophobic interaction between hPNMT and flavonoids. Hydroxyl group of A-ring and carboxyl group of C-ring of compound 5 participated in hydrogen bond with side chain of Lys57 and backbone of Asn39, respectively. The methoxy group of A-ring formed a hydrophobic interaction with first phobic site including aliphatic hydrophobic residues such as Leu262 and Val53 (Figure 3). B-ring of compound 5 participated in the hydrophobic interaction with the second phobic site. In the case of compound 4 and 6, the hydrogen bonding interactions were identical with those of compound 5 but the hydrophobic interactions were different from those of compound 5. The first hydrophobic site was occupied by a ring (B-ring) and second phobic site was grasped by methoxy group of 4 and 6 instead of aromatic ring. As shown in Figure 3, although the second hydrophobic site included two aromatic hydrophobic residues (Phe182 and Tyr222) and two aliphatic residues (Ala186 and Val231), the aromatic stacking interaction would be more favorable than aliphatic hydrophobic interaction for their inhibitory activity against hPNMT.

Automated docking process for hPNMT and flavonoid was successfully performed. From the docking model we proposed here the important key features for interactions between inhibitor and hPNMT and that hit flavonoids can be potent inhibitors of hPNMT. We will investigate the binding properties of these hit flavonoids further.

Conclusion

We performed *in silico* screening using automated docking to find flavonoids as inhibitor candidates for hPNMT. Among

500 flavonoids, only 45 flavonoids were fit to the active site of hPNMT and final 9 flavonoids were chosen by two scoring function, LigScore and binding free energy (ΔG_{bind}). 9 flavonoids formed two hydrogen bonds with side chain amide proton of Lys57 and backbone carbonyl oxygen of Asn39. Furthermore, they showed two important hydrophobic interactions with hPNMT. First one is aliphatic hydrophobic interaction with Arg44, Val53 and Leu262, and the second site is formed with Phe182, Ala186, Tyr222, and Val231. These results implied that flavonoids may be potent inhibitors of hPNMT as therapeutic agents and this study may offer a helpful strategy to design flavonoid inhibitors of hPNMT.

Acknowledgments. This work was supported by the Research Program for New Drug Target Discovery (M106010-00153-07N0100-15310) grant from the Ministry of Science & Technology, South Korea and by the Korea Research Foundation (KRF-2006-005-J03402). Ki-Woong Jung is supported, in part, by the second BK21 (MOE).

References

1. Matsubara, K.; Aoyama, K.; Suno, M.; Awaya, T. *Neurotoxicol. Teratol.* **2002**, *24*, 593.
2. Berecek, K. H.; Brody, M. J. *Am. J. Physiol.* **1982**, *242*, H593.
3. Martin, J. L.; Begun, J.; McLeish, M. J.; Caine, J. M.; Grunewald, G. L. *Structure* **2001**, *9*, 977.
4. Mefford, I. N.; Lister, R. G.; Ota, M.; Linnoila, M. *Alcohol. Clin. Exp. Res.* **1990**, *14*, 53.
5. Kennedy, B. P.; Bottiglieri, T.; Arning, E.; Ziegler, M. G.; Hansen, L. A.; Masliah, E. *J. Neural. Transm.* **2004**, *111*, 547.
6. Krizanova, O.; Myslivecek, J.; Tillinger, A.; Jurkovicova, D.; Kubovcakova, L. *Stress* **2007**, *10*, 173.
7. Comings, D. E. *Ann. N Y Acad. Sci.* **2001**, *931*, 50.
8. Cazarolli, L. H.; Zanatta, L.; Alberton, E. H.; Figueiredo, M. S.; Folador, P.; Damazio, R. G.; Pizzolatti, M. G.; Silva, F. R. *Mini Rev. Med. Chem.* **2008**, *8*, 1429.
9. Rajnaryana, K.; Sripalreddy, M.; Chalavadi, M. R.; Krishna, D. R. *Indian J. Pharmacol.* **2001**, *33*, 2.
10. Saraf, S.; Ashawat, M. S.; Saraf, S. *Phcog. Rev.* **2007**, *1*, 30.
11. 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.
12. Lee, J. Y.; Jung, K. W.; Kim, Y. *Bull. Korean Chem. Soc.* **2008**, *29*, 1717.
13. Healy, E. F.; Sanders, J.; King, P. J.; Robinson, W. E., Jr. *J. Mol. Graph. Model.* **2009**, *27*, 584.
14. Krammer, A.; Kirchhoff, P. D.; Venkatachalam, X. J. C. M.; Waldman, M. *J. Mol. Graph. Model.* **2005**, *23*, 395.
15. Lee, J. Y.; Jung, K. W.; Woo, E. R.; Kim, Y. *Bull. Korean Chem. Soc.* **2008**, *29*, 1479.
16. Gee, C. L.; Tyndall, J. D.; Grunewald, G. L.; Wu, Q.; McLeish, M. J.; Martin, J. L. *Biochemistry* **2005**, *44*, 16875.
17. Gee, C. L.; Drinkwater, N.; Tyndall, J. D.; Grunewald, G. L.; Wu, Q.; McLeish, M. J.; Martin, J. L. *J. Med. Chem.* **2007**, *50*, 4845.
18. Raha, K.; Merz, K. M., Jr. *Calculating Binding Free Energy in Protein-ligand Interaction, in Annual Reports in Computational Chemistry*; Spellmeyer, D., Ed.; Elsevier: Amsterdam, 2005; Vol. 1, p 115.
19. Grunewald, G. L.; Romero, F. A.; Criscione, K. R. *J. Med. Chem.* **2005**, *48*, 134.
20. Wu, Q.; Criscione, K. R.; Grunewald, G. L.; McLeish, M. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4217.
21. Coen, C. W.; Gallo, R. V. *J. Endocrinol.* **1986**, *111*, 51.