Discovery of Monoamine Oxidase A Inhibitors Derived from *in silico* Docking

Geunhyeong Jo, Su Hyun Sung, Younggiu Lee, Bong-Gyu Kim, JunWie Yoon, Hye Ok Lee,
Sang Yun Ji,[†] Dongsoo Koh,[‡] Joong-Hoon Ahn, and Yoongho Lim[*]

Division of Bioscience and Biotechnology, BMIC, Konkuk University, Seoul 143-701, Korea. [*]E-mail: yoongho@konkuk.ac.kr
[†]National Institute of Animal Science, Rural Development Administration, Suwon 441-706, Korea
[‡]Department of Applied Chemistry, Dongduk Women's University, Seoul 136-714, Korea
Received July 10, 2012, Accepted August 6, 2012

Although more than 20,000 papers concerning monoamine oxidase (MAO) have been reported in PubMed since the 1950s, over 300 papers are still being published every year on this topic. This indicates the importance of MAO, which was discovered by Mary Hare-Bernheim in 1928.[1] Two isoforms of MAO have been identified based on the selectivity to their substrates and inhibitors; they are called MAOA and MAOB. While the former shows a high affinity for serotonin and norepinephrine, the latter has a high affinity for β-phenylethylamine. Likewise, MAOA is selectively inhibited by clorgyline and MAOB by selegiline.[2] MAOA and MAOB are encoded from different genes that are located in the human X chromosome.[3] They are activated by different transcription factors;[4] thus, the expressions of the two proteins are localized: the ratio of MAOA to MAOB is 1:3 in the human brain, 1:1 in the liver, and 4:1 in the intestine.[5,6] MAO acts on monoamines, but it does not metabolize diamines. It metabolizes monoamine to aldehyde, which is then oxidized to carboxylic acid by aldehyde dehydrogenase. During this oxidation, MAO requires flavin adenine dinucleotide (FAD), which becomes $FADH_2$. The oxidation of $FADH_2$ is achieved by the production of hydrogen peroxide from oxygen and protons. Consequently, catabolism of MAO produces hydrogen peroxide. Especially, MAOA oxidizes and degrades serotonin which modulates neuropsychiatric function and dysfunction.[7,8] In addition, MAOA has effects on several psychiatric conditions such as anxiety, mood, and impulsivity, and is implicated in several psychiatric illnesses and elevated in depressive disorder.[9,10] Therefore, MAOA could be a potent drug target for depression. MAOA inhibitors prevent the breakdown of serotonin as well as norepinephrine and dopamine, and MAOA knockout mice showed reduced immobility, acoustic startle, and perception of external stress, so that they can be used for the treatment of depression.[11-14]

MAOA inhibitors (MAOAIs) have been used as antidepressants for over forty years. Iproniazid was introduced in 1957, but it was withdrawn because of hepatotoxicity.[15] Tranylcypromine was developed in the mid-1960s, withdrawn from the market because of problems related to hypertension, then reintroduced for limited usage.[16] Many MAOAIs have been developed and used for treating atypical depression after the failure of other classes of antidepressant drugs such as selective serotonin reuptake inhibitors and tricyclic antidepressants.[6] While iproniazid and tranylcypromine were nonselective MAOAIs, a selective MAOAI, clorgyline, was introduced in the latter half of the 1960s. Recently, more selective and safe MAOAIs, namely moclobemide, toloxatone, and tetrindol, were launched.[17,18] However, their side effects and activities need further improvement. Therefore, we have made efforts to discover new MAOAIs.

The three-dimensional (3D) structure of human MAOB was determined in 2002 and that of human MAOA in 2005.[19,20] While human MAOB has a dimeric structure, human MAOA is monomeric. The 3D structure of rat MAOA is dimeric. Because the 3D structure of human MAOA is known, structure-based drug design is possible. Therefore, we selected compounds from a chemical library that showed high binding affinity to human MAOA by *in silico* docking. Based on their structural features, we designed compounds, performed *in silico* docking, and synthesized them. Their inhibitory effects on MAOA were tested using high-performance liquid chromatography (HPLC). We report here a potent MAOA inhibitor.

Two structures of MAOA were found in a protein data bank. 2BXS.pdb was determined at 3.1 Å resolution and 2Z5X.pdb at 2.2 Å resolution; we used 2Z5X.pdb for our *in silico* docking experiments.[20,21] The apo-protein without substrates was subjected to energy minimization on an Intel Core 2 Quad Q6600 (2.4 GHz) Linux PC with Sybyl 7.3 software (Tripos, St. Louis, MO).

The chemical library provided by ChemDiv (San Diego, CA) was used for *in silico* docking. The DC04_100000 library was selected, which includes compounds coded as C260-2636 and D223-0320 where 100,000 druggable compounds are contained. The library contains only two dimensional (2D) structures; thus, all the compounds were converted into their 3D structures using the 3D-converter module of the Sybyl program. Energy minimization was run over 1,000 iterations. The final structures from the *in silico* docking experiments were saved as sdf files.

All the compounds obtained above were docked into MAOA using the FlexX Single Receptor Module in Sybyl. Tyr69, Asn181, Phe208, Val210, Gln215, Cys323, Ile325, Ile335, Leu337, Phe352, Tyr407, and Tyr444 were assigned as the binding sites for docking. The selection radius was 6.5

Å. The docking process was iterated 30 times for each compound.

Because MAOA (2Z5X.pdb) contains harmine as an inhibitor, harmine was docked into apo-protein MAOA obtained from energy minimization using the FlexX Single Receptor Module. The residues surrounding harmine were analyzed using LigPlot provided by the European Bioinformatics Institute.[22] Harmine docked well into the apo-protein. Nine residues had hydrophobic interactions with harmine: Ile180, Asn181, Phe208, Gln215, Ile335, Leu337, Phe352, Tyr407, and Tyr444. Most of the residues (except Tyr69, Ile325, Ile335) agreed with those previously reported (Supporting information Figs. S1a and S1b).[20,21] This indicated that our *in silico* docking system was working well. Among the compounds docked into MAOA from the chemical library, those 30 compounds that showed the best docking scores were selected for further study (Supporting information Fig. S2). Their docking scores ranged between –42.9 and –37.4 kcal/mol, where a more negative docking score indicated higher affinity. All 30 compounds selected from the chemical library have common features: all compounds contain at least more than two aromatic rings, and rings containing nitrogen are involved, as marked as bold lines in Figure S2, and especially, phenylpyrazole moiety is found in several compounds such as C548-2263, D086-0417, C791-0992, D039-0063, C794-0509, D086-0478, D003-0050, D003-1075, D086-0420, and C270-0359. Therefore, we tried to design the compounds containing aromatic rings or phenylpyrazole moiety. To avoid toxicity problem, the compounds were derived from natural products. Among various natural products, polyphenols consist of aromatic rings. Fortunately, polyphenols such as isoflavone, flavone, and coumarin in particular have behavior as MAOA inhibitors.[23-25] However, above polyphenols are famous compounds showing various biological activities, so that we started designing with benzoflavanone because it was not known well. Flavanone is composed of C6-C3-C6 three ring skeleton. Benzoflavanone has naphthalene instead of the first C6 ring. 3-(4-Methoxyphenyl)-2,3-dihydro-1*H*-benzo[*f*]chromen-1-one (DK382, Fig. 1) was designed and prepared for this experiment. To obtain compounds satisfying another common feature, phenylpyrazole moiety, C3 ring of benzoflavanone was replaced with pyrazole group. As a result, 2-(5-(3,5-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-1-ol (DK264, Fig. 1) was prepared. In addition to this compound, another compound with chlorophenyl group found in C289-0373 and C800-0715, two of 30 compounds selected from the chemical library, 1-(1-(4-chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-2-ol (DK96, Fig. 1), was prepared.

In order to conform whether the compounds designed here bind to MAOA *in silico*, they were docked into apo-protein MAOA using the FlexX Single Receptor Module (Supporting information data 1). Comparing the docking pose of harmine in MAOA to the poses of the three compounds, all docked well into MAOA. The 3D structural models of the harmine-MAOA complex and the DK382-MAOA complex
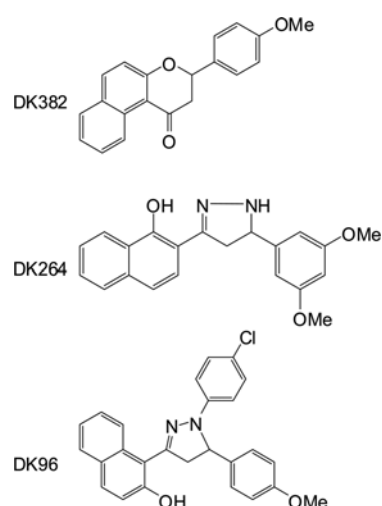

Figure 1. Structures of compounds designed from *in silico* docking. DK382, 3-(4-methoxyphenyl)-2,3-dihydro-1*H*-benzo[*f*]chromen-1-one; DK264, 2-(5-(3,5-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-1-ol; DK96, 1-(1-(4-chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-2-ol.

are shown in Figures 2(a) and 2(b), and the DK264-MAOA complex and the DK96-MAOA complex are in Supporting information Figures S3(a) and S3(b), respectively.

Harmine and the three compounds docked into MAOA were analyzed using LigPlot software. Four more residues, Gly67, Tyr69, Met350, and Gly443, were observed in the complex of DK382 with MAOA (Supporting information Fig. S4(a)). The Tyr69 residue disappeared in the complex of MAOA with harmine but it was shown in the complex with DK382. While 26 hydrophobic interactions between harmine and MAOA were observed, 31 hydrophobic interactions were observed with DK382. Eight residues matched between harmine and DK264, and one hydrogen bond (H-bond) was observed between Tyr69 and DK264 (Supporting information Fig. S4(b)). Because LigPlot was run under the same conditions, the H-bond observed in DK264 implied a difference in the surrounding residues even though DK264 docked well into MAOA. In addition, four more residues, Gly66, Gly67, Ala68, and Gly443, had hydrophobic interactions with DK264. From the Ligplot analysis for DK96, only one residue, Phe208, in the complex of DK96 with MAOA was the same as that with harmine (Supporting information Fig. S4(c)). Two residues, Ser94 and Val210, displayed H-bonds. Although LigPlot analysis demonstrated that DK96 docked well into MAOA, it was clear that its binding condition was different than that for harmine. *In silico* experiments with the three compounds designed using the chemical library indicated good binding into MAOA. Based on their binding conditions, DK382 was expected to be the best inhibitor.

Because MAOA metabolizes monoamines, the catecholamine releasing agent tyramine (4-hydroxyphenethylamine) was selected to test the activity of MAOA.[26] It was analyzed using HPLC (Agilent 1100 series, Santa Clara, CA). The HPLC retention times of tyramine and 2-(4-hydroxyphenyl)-acetic acid (HPA) were 3.1 and 16.2 min, respectively (Sup-

Notes

Bull. Korean Chem. Soc. **2012**, Vol. 33, No. 11   3843
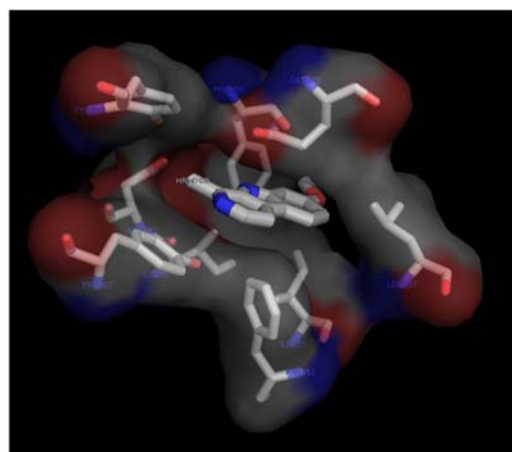
(a)
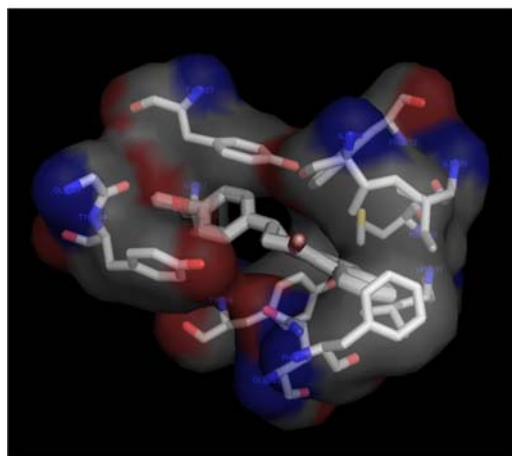
(b)

Figure 2. Three-dimensional structural models of the complex of (a) harmine and (b) DK382 docked into monoamine oxidase A (2Z5X.pdb), viewed in the PyMOL program.

Figure 3. Three-dimensional structural model of DK382 docked to the binding site of monoamine oxidase A, viewed in the PyMOL program.

porting information Figs. S5a and S5b), and the buffer used as solvent appeared at 2.8 min (Supporting information Fig. S5c). After the reaction of tyramine with MAOA, HPA was observed at 17.6 min retention time (Supporting information Fig. S6). The inhibitory effect of the test compound was determined based on the ratio of the peak area of HPA as a reactant for the enzyme MAOA over that of tyramine as a product (Supporting information data 2). In the reaction of tyramine with MAOA, the ratio of two peak areas in the chromatogram was 2.29.

Clorgyline is known as an MAOA inhibitor.[27] It was purchased from Sigma-Aldrich Co. LLC. Its reaction solution was prepared as for tyramine. When clorgyline and tyramine were added to MAOA, the HPA peak diminished in the chromatogram (Supporting information Fig. S7a). The peak area ratio was 0.44 because little HPA was produced by the inhibitor, clorgyline (Supporting information data 3). The ratio of the peak area of HPA over that of tyramine in the chromatogram was 2.28 (Supporting information Fig. S7b). Each experiment was performed in triplicate.

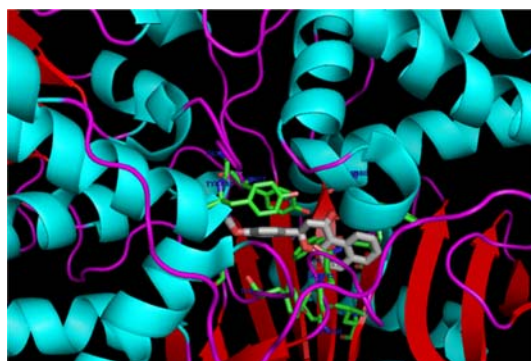The three compounds designed through *in silico* experi-

ments as candidates to inhibit MAOA were synthesized and they were evaluated in the same manner as clorgyline for their reaction with MAOA. The ratios observed in the chromatograms for DK382, DK264, and DK96 were 0.37, 2.23, and 1.82, respectively (Supporting information Figs. S8a, S8b, and S8c). In the reaction condition without any inhibitors where the enzyme and tyramine were provided at the concentrations of 300 μg/mL and 100 μg/mL, respectively, the ratio of the HPLC area of HPA over that of tyramine was 2.29 which can be expressed by 0% inhibitory effect. Then, the inhibitory effect by clorgyline is 80.8%, where it was provided at the concentration of 100 μg/mL. Likewise, the effects of DK382, DK264, and DK96 are 83.8%, 2.6%, and 20.5%, respectively (Supporting information Table 1). Therefore, the inhibitory effect of DK382 on MAOA (83.8%) is competitive to that of clorgyline (80.8%). Binding between DK382 and MAOA is stronger than between harmine and MAOA, as shown in Figure 3 and Supporting information Figure S9, respectively (Supporting information data 4).

In conclusion, even though the number of compounds tested here is not enough for evaluation, the current result demonstrates that phenylpyrazole moiety is not necessary for showing good inhibitory effects. Because benzoflavanones have not previously been reported to act on MAOA as inhibitors,[28-30] and the inhibitory effect of one of benzo-flavones, 3-(4-methoxyphenyl)-2,3-dihydro-1*H*-benzo[*f*]chromen-1-one used in this study is comparable to that of clorgyline which is known as MAOA inhibitor,[31] our findings are meaningful.

Experimental Section

To determine whether the compounds evaluated by the *in silico* experiments would truly inhibit MAOA, the MAOA enzyme was prepared (Supporting information data 5), and DK382, DK264, and DK96 were synthesized (Supporting information Figure S10). Chemical identification of DK382, DK264, and DK96 was made using one-dimensional and two-dimensional nuclear magnetic resonance (NMR) spectro-scopy (Bruker Avance 400, 9.4 Tesla, Karlsruhe, Germany).

All mass spectra were collected on a high-resolution electron impact ionization mass spectrometer (HREIMS, JMS700, Jeol Ltd., Tokyo, Japan). The synthesis of DK264, DK96, and DK382, and their assignments of spectral data are summarized in Supporting information data 6.

References

1. Youdim, M. B.; Bakhle, Y. S. *Br. J. Pharmacol.* **2006**, *147*, 287.
2. Bortolato, M.; Chen, K.; Shih, J. C. *Adv. Drug Deliv. Rev.* **2008**, *60*, 1527.
3. Lan, N. C.; Heinzmann, C.; Gal, A.; Klisak, I.; Orth, U.; Lai, E.; Grimsby, J.; Sparkes, R. S.; Mohandas, T.; Shih, J. C. *Genomics* **1989**, *4*, 552.
4. Grimsby, J.; Lan, N. C.; Neve, R.; Chen, K.; Shih, J. C. *J. Neurochem.* **1990**, *55*, 1166.
5. Westlund, K. N.; Denney, R. M.; Rose, R. M.; Abell, C. W. *Neuroscience* **1988**, *25*, 439.
6. Patkar, A. A.; Pae, C. U.; Masand, P. S. *CNS. Spectr.* **2006**, *11*, 363.
7. Da Prada, M.; Kettler, R.; Keller, H. H.; Burkard, W. P.; Muggli-Maniglio, D.; Haefely, W. E. *J. Pharmacol. Exp. Ther.* **1989**, *248*, 400.
8. Ma, J.; Yoshimura, M.; Yamashita, E.; Nakagawa, A.; Ito, A.; Tsukihara, T. *J. Mol. Biol.* **2004**, *338*, 103.
9. Dreiseitel, A.; Korte, G.; Schreier, P.; Oehme, A.; Locher, S.; Domani, M.; Hajak, G.; Sand, P. G. *Pharmacol. Res.* **2009**, *59*, 306.
10. Johnson, S.; Stockmeier, C. A.; Meyer, J. H.; Austin, M. C.; Albert, P. R.; Wang, J.; May, W. L.; Rajkowska, G.; Overholser, J. C.; Jurjus, G.; Dieter, L.; Johnson, C.; Sittman, D. B.; Ou, X. M. *Neuropsychopharmacology* **2011**, *36*, 2139.
11. Cryan, J. F.; Dalvi, A.; Jin, S. H.; Hirsch, B. R.; Lucki, I.; Thomas, S. A. *J. Pharmacol. Exp. Ther.* **2001**, *298*, 651.
12. Cases, O.; Seif, I.; Grimsby, J.; Gaspar, P.; Chen, K.; Pournin, S.; Müller, U.; Aguet, M.; Babinet, C.; Shih, J.; De Maeyer, E. *Science* **1995**, *268*, 1763.
13. Gingrich, J. A.; Ansorge, M. S.; Merker, R.; Weisstaub, N.; Zhou, M. *CNS Spectr.* **2003**, *8*, 572.
14. Amsterdam, J. D.; Chopra, M. *Psychiatr. Ann.* **2001**, *31*, 361.
15. Ban, T. A. *J. Neural. Transm.* **2001**, *108*, 707.
16. López-Muñoz, F.; Alamo, C. *Curr. Pharm. Des.* **2009**, *15*, 1563.
17. Curet, O.; Damoiseau, G.; Aubin, N.; Sontag, N.; Rovei, V.; Jarreau, F. X. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 253.
18. Medvedev, A. E.; Kirkel, A. A.; Kamyshanskaya, N. S.; Moskvitina, T. A.; Axenova, L. N.; Gorkin, V. Z.; Andreeva, N. I.; Golovina, S. M.; Mashkovsky, M. D. *Biochem. Pharmacol.* **1994**, *47*, 303.
19. Binda, C.; Newton-Vinson, P.; Hubálek, F.; Edmondson, D. E.; Mattevi, A. *Nat. Struct. Biol.* **2002**, *9*, 22.
20. De Colibus, L.; Li, M.; Binda, C.; Lustig, A.; Edmondson, D. E.; Mattevi, A. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 12684.
21. Son, S. Y.; Ma, J.; Kondou, Y.; Yoshimura, M.; Yamashita, E.; Tsukihara, T. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 5739.
22. Wallace, A. C.; Laskowski, R. A.; Thornton, J. M. *Protein Eng.* **1995**, *8*, 127.
23. Gao, G. Y.; Li, D. J.; Keung, W. M. *J. Med. Chem.* **2001**, *44*, 3320.
24. Han, X. H.; Hong, S. S.; Hwang, J. S.; Lee, M. K.; Hwang, B. Y.; Ro, J. S. *Arch. Pharm. Res.* **2007**, *30*, 13.
25. Pisani, L.; Muncipinto, G.; Miscioscia, T. F.; Nicolotti, O.; Leonetti, F.; Catto, M.; Caccia, C.; Salvati, P.; Soto-Otero, R.; Mendez-Alvarez, E.; Passeleu, C.; Carotti, A. *J. Med. Chem.* **2009**, *52*, 6685.
26. Stahl, S. M.; Felker, A. *CNS. Spectr.* **2008**, *13*, 855.
27. Murphy, D. L.; Karoum, F.; Pickar, D.; Cohen, R. M.; Lipper, S.; Mellow, A. M.; Tariot, P. N.; Sunderland, T. *J. Neural. Transm. Suppl.* **1998**, *52*, 39.
28. Xu, Y.; Li, S.; Chen, R.; Li, G.; Barish, P. A.; You, W.; Chen, L.; Lin, M.; Ku, B.; Pan, J.; Ogle, W. O. *Pharmacol. Biochem. Behav.* **2010**, *94*, 447.
29. Dixon Clarke, S. E.; Ramsay, R. R. *J. Neural. Transm.* **2011**, *118*, 1031.
30. Sloley, B. D.; Urichuk, L. J.; Morley, P.; Durkin, J.; Shan, J. J.; Pang, P. K.; Coutts, R. T. *J. Pharm. Pharmacol.* **2000**, *52*, 451.
31. Holmes, A. R.; Keniya, M. V.; Ivnitski-Steele, I.; Monk, B. C.; Lamping, E.; Sklar, L. A.; Cannon, R. D. *Antimicrob. Agents Chemother.* **2012**, *56*, 1508.