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Two novel *CYP2D6*10* haplotypes as possible causes of a poor metabolic phenotype in Japanese

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Running title: Novel *CYP2D6*10* haplotype in Japanese

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Abbreviations: P450, cytochrome P450; PM, poor metabolizer; EM, extensive metabolizer; IM, intermediate metabolizer; CPR, NADPH-cytochrome P450 reductase; *E. coli*, *Escherichia coli*; LC/MS, liquid chromatography mass spectrometry.

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Abstract

During the course of sequencing for the *CYP2D6* gene, we found a novel SNP of g.3318G>A (E383K) associated with *CYP2D6*10*, termed as *CYP2D6*72*. We also found a g.1611T>A (F120I) in the *CYP2D6*49*, which was previously identified as a *CYP2D6*10*-associated allele in an independent Japanese population. To clarify the effects of these novel *CYP2D6*10* haplotypes on the functions of CYP2D6, kinetic analysis for dextromethorphan *O*-demethylation was performed using the *Escherichia coli* expression system and human liver microsomes. The V_{max}/K_m values for dextromethorphan *O*-demethylation catalyzed by recombinant CYP2D6 forms encoded by *CYP2D6*10*, *CYP2D6*49* and *CYP2D6*72* were 3.0%, 0.5% and 1.3%, respectively, as compared to that catalyzed by CYP2D6.1. Liver microsomes from a human subject genotyped as *CYP2D6*10/*49* also showed a reduced dextromethorphan *O*-demethylase activity. CYP2D6.49 formed a 7-hydroxydextromethorphan, with a roughly similar V_{max}/K_m value to that of *O*-demethylation. These results suggest that these two *CYP2D6*10* haplotypes are possible causes of inter-individual variation in the activities and the substrate specificity of CYP2D6. (160 words)

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Introduction

Cytochrome P450 (CYP) 2D6 is one of the most important isoforms due to its central role in the metabolism of a number of drugs such as opioids, antidepressants and neuroleptics. The genetic polymorphisms of CYP2D6 cause inter-individual difference in the efficacy and toxicity of drugs (Ingelman-Sundberg, 2005). An ethnic difference is found not only in the frequency of poor metabolizers (PMs) but also in the activity of debrisoquine hydroxylase within subjects categorized as apparent extensive metabolizers (EMs). The mean metabolic ratio for debrisoquine metabolism in an EM population has been reported to be slightly lower in Asians than in Caucasians. This seems to be caused by a higher frequency of individuals classified as intermediate metabolizers (IMs) showing reduced activities. In fact, the lower CYP2D6 activity in IMs is associated with the *CYP2D6*10* allele (Tateishi et al., 1999). The *CYP2D6*10* allele contains a g.100C>T (where the A of the ATG start codon is +1) that causes a Pro34Ser amino acid substitution, leading to the formation of an unstable enzyme with lower metabolic activity (Nakamura et al., 2002). The large variation of dextromethorphan *O*-demethylase activities has been observed even among subjects with *CYP2D6*10* genotype (Tateishi et al., 1999).

To clarify the causes of this variation, we previously investigated the *CYP2D6* gene of 254 Japanese subjects and identified 5 novel non-synonymous SNPs (Soyama et al., 2004). In the present study, we found one novel SNP associated with the *CYP2D6*10* allele (*CYP2D6*72*) in an independent population of 349 individuals. We also found one previously identified *CYP2D6*10* haplotype (*CYP2D6*49*). We clarified that these haplotypes were possible factors causing the impaired function of CYP2D6.

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Materials and Methods

Chemicals

Dextromethorphan and dextrorphan were purchased from Wako Pure Chemicals (Osaka, Japan). $NADP^+$, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were obtained from Oriental Yeast (Tokyo, Japan). All other chemicals were of the highest quality commercially available.

Subjects

A total of 349 unrelated healthy Japanese subjects, who received medical checkups in the Japanese Red Cross Society Kumamoto Health Care Center (Kumamoto, Japan), were recruited in this study. Liver samples were obtained as described previously (Yamaori et al., 2004). Written informed consent was obtained from each subject. This study was approved by the Ethics Committees of Hokkaido University and Kumamoto University.

Genotyping for *CYP2D6*

Genomic DNA was prepared from peripheral leukocytes or liver samples according to the method of phenol-chloroform extraction followed by ethanol precipitation. Genotyping for the *CYP2D6*5* and *CYP2D6*1×2* alleles was carried out according to the method described by Johansson *et al.* (Johansson et al., 1996). Detection of the other mutations was performed by the direct sequencing method described previously (Yamazaki et al., 2003).

Enzyme sources

Expression plasmids carrying cDNA coding for the CYP2D6.10 (from *CYP2D6*10)*, CYP2D6.72 (from *CYP2D6*72*) or CYP2D6.49 (from *CYP2D6*49*) together with the NADPH-cytochrome P450 reductase (CPR) cDNA were constructed by site-directed mutagenesis

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using wild-type CYP2D6.1 (from *CYP2D6*1)* constructed by Iwata *et al*. as a template (Iwata et al., 1998). Each CYP2D6 together with CPR was expressed in *Escherichia coli* (*E. coli*) cells as described by Iwata *et al*. (Iwata et al., 1998).

Microsomes from human livers were suspended in 10 mM Tris-HCl buffer (pH 7.4) containing 1.0 mM ethylenediaminetetraacetic acid and 20% (v/v) glycerol. Microsomal protein contents were determined using Pierce BCA Protein Assay Kit (Pierce Chemical, Rockford, IL, USA) with bovine serum albumin as a standard.

Measurement of dextromethorphan *O*-demethylase activity

Dextromethorphan *O*-demethylase activity was determined according to the method as described below. A typical incubation mixture consisted of the membrane fraction of *E. coli* expressing CYP2D6 (50 pmol/mL) or human liver microsomes (0.2 mg protein/mL), dextromethorphan (3.5-400 μM) and an NADPH-generating system (0.5 mM $NADP^+$, 5 mM glucose 6-phosphate and 1 unit/mL glucose phosphate dehydrogenase) and 50 mM potassium phosphate buffer (pH 7.4). After 15 min incubation at 37°C, the reaction was terminated by addition of 10 μL of 60% (w/v) $HClO_4$. Protein was removed from the reaction mixture by centrifugation. A supernatant (10 μL) was injected to a liquid chromatography (LC) system (L-7100 pump, L-7200 autosampler and L-7485 FL detector, Hitachi, Tokyo, Japan) equipped with a Mightysil RP-18 GP Aqua column (250 × 4.6 mm, 5 μm, Kanto Chemical, Tokyo, Japan). When incubated with *E. coli* membranes, dextromethorphan and its metabolites were separated following gradient program with mobile phase of 15% (v/v) CH_3CN containing 20 mM $NaClO_4$ (pH 2.5) for solvent A and 60% (v/v) CH_3CN containing 20 mM $NaClO_4$ (pH 2.5) for solvent B at 2.0 mL/min. Gradient program consisted of A at 100% for 20 min, and

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decrease of A from 100% to 0% from 20 to 35 min, followed by an isocratic segment marinating A at 0% to 45 min. Under this chromatographic condition, the analytes had the following retention times; dextrorphan, 15.1 min; new metabolite, 17.9 min and dextromethorphan, 41.3 min. When dextromethorphan *O*-demethylase activity in human liver microsomes was measured, elution was performed using a Mightysil RP-18 GP Aqua column (150 × 4.6 mm, 5 µm, Kanto Chemical) and mobile phase consisted of 25% (v/v) CH_3CN containing 20 mM $NaClO_4$ (pH 2.5) at a flow rate of 1.5 mL/min. Under this chromatographic condition, the analytes had the following retention times; dextrorphan, 3.0 min; new metabolite, 3.9 min; 3-methoxymorphinan, 4.7 min and dextromethorphan, 11.2 min. The elution of metabolites was monitored with excitation at 270 nm and emission at 312 nm.

Kinetic parameters for the dextromethorphan *O*-demethylation were estimated with a computer program (Microcal Origin, Microcal Software, Northampton, MA, USA) designed for a nonlinear regression analysis. Statistical comparisons were made with Dunnett's post hoc test using StatView v5.0 (SAS Institute, Cary, NC), and differences were considered to be statistically significant when the p value was < 0.05.

Results and Discussion

Novel SNP of g.3318G>A, causing amino acid substitution (E383K), was discovered by sequencing of the *CYP2D6* gene locus containing in the 5'-flanking region, all exons and introns, and 3'-untranslated region of 349 unrelated healthy Japanese. Haplotype analysis clarified that g.3318G>A was completely linked with *CYP2D6*10* allele. This *CYP2D6* haplotype was assigned as *CYP2D6*72* by the CYP Allele Nomenclature Committee (http://www.imm.ki.se/CYPalleles). The

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g.1611T>A, which was previously identified as *CYP2D6*49* (Soyama et al., 2004), was also found in this 349 Japanese population.

To examine functional alterations caused by F120I and E383K, we examined dextromethorphan *O*-demethylase activities of wild- and mutant-type CYP2D6 expressed in *E. coli* membrane (Table 1). The K_m values of recombinant CYP2D6.49 and CYP2D6.72 for dextromethorphan *O*-demethylation were significantly higher than that of wild-type CYP2D6.1. CYP2D6.49 also showed significantly higher K_m value compared to that of CYP2D6.10. The V_{max}/K_m of CYP2D6.10 was approximately 30-fold lower than that of CYP2D6.1, consistent with previous findings (Ramamoorthy et al., 2001). Thus, the V_{max}/K_m values of CYP2D6.49 and CYP2D6.72 were 16% and 42% of that of CYP2D6.10, and 0.5% and 1.3% of CYP2D6.1, respectively. CYP2D6.49 and CYP2D6.72 showed V_{max}/K_m values of 1.3% and 1.5% relative to CYP2D6.1 for bufuralol 1'-hydroxylation, and 9.9% and 11.6% for debrisoquine 4-hydroxylation (data not shown).

To investigate whether or not the novel genetic polymorphisms influenced the catalytic activities of CYP2D6 in hepatic microsomes, dextromethorphan *O*-demethylase activities of liver microsomes from subjects carrying different *CYP2D6* genotypes were measured (Table 2). The K_m values of dextromethorphan *O*-demethylation seen in liver microsomal samples genotyped as *CYP2D6*1/*1*, *CYP2D6*1/*10*, *CYP2D6*10/*10* and *CYP2D6*10/*49* were 10.2, 15.3, 23.9 and 39.7 μM, respectively. The V_{max} values for these liver microsomal samples were comparable, resulting in V_{max}/K_m values for microsomes from livers genotyped as *CYP2D6*1/*10*, *CYP2D6*10/*10* and *CYP2D6*10/*49* that were 51%, 40% and 23% of *CYP2D6*1/*1* (15.4 μL/min/mg protein), respectively. The impaired dextromethorphan *O*-demethylase activity of CYP2D6.49 was also seen in

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human liver microsomes from a subject heterozygous for the variant allele.

Interestingly, a new peak, suggesting the formation of a new metabolite eluted at approximately 18 min, was seen when dextromethorphan was incubated with *E. coli* membrane expressing CYP2D6.49 (data not shown). In liquid chromatography mass spectrometry (LC/ MS) analysis, an $[M+H]^+$ ion at 288 was observed for the new metabolite, demonstrating an addition of 16 units (a hydroxyl group) to dextromethorphan (MW = 271) (data not shown). Although information on the position of the hydroxyl group of dextromethorphan was not sufficient in the present study, the parent ion for the new metabolite appeared to correspond to 7-hydroxydextromethorphan, as recently described concerning CYP2D6.1 with substitution of F120A by site-directed mutagenesis (Flanagan et al., 2004). Since an authentic standard for 7-hydroxydextromethorphan was not available, we expressed the hydroxylation velocity as the peak area ratio of metabolite to the dextrorphan standard, because UV or fluorescence responses of dextromethorphan and dextrorphan were similar in our preliminary experiments. The K_m and V_{max} values for 7-hydroxylation were comparable to *O*-demethylation of dextromethorphan (Table 1). The 7-hydroxydextromethorphan was produced by liver microsomes from subjects carrying the *CYP2D6*10/*49* genotype as an approximately 5-min peak as well as in CYP2D6.49 expressed in the *E. coli* (Fig. 1). However, this peak was not detected in human liver microsomes prepared from subjects carrying other genotypes. Apparent K_m, V_{max} and V_{max}/K_m values for the formation of 7-hydroxydextromethorphan were calculated to be 107 μM, 139 pmol/min/mg protein and 1.3 μL/min/mg protein, respectively, with human liver microsomes (data not shown). To further confirm the contribution of CYP2D6.49 in the dextromethorphan hydroxylation, quinidine, a known typical and potent inhibitor of CYP2D6, was added to the reaction mixture.

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Quinidine inhibited the 7-hydroxylation in a concentration-dependent manner (data not shown). These results suggested that CYP2D6.49 was capable of catalyzing 7-hydroxylation of dextromethorphan in human liver microsomes. The toxicological or pharmacological significance of the formation of 7-hydroxydextromethorphan are unknown yet. This is the first report, to our knowledge, suggesting that genetic polymorphism of *CYP2D6* causes a different orientation of substrates leading to different oxidative metabolite formation. Unmetabolized compounds in the body or unusual metabolite(s) mediated by CYP2D6 variants might cause unexpected side effects. Further studies are necessary in this context.

In conclusion, two novel *CYP2D6*10* haplotypes (*CYP2D6*49* and *CYP2D6*72*) can be expected to demonstrate a reduced metabolic capacity toward drugs metabolized primarily by CYP2D6. We were able to explain one of the causes of a large inter-individual variation in CYP2D6 enzyme activity within *CYP2D6*10* genotype group.

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References

Flanagan JU, Marechal JD, Ward R, Kemp CA, McLaughlin LA, Sutcliffe MJ, Roberts GC, Paine MJ and Wolf CR (2004) Phe^{120} contributes to the regiospecificity of cytochrome P450 2D6: mutation leads to the formation of a novel dextromethorphan metabolite. *Biochem J* **380:**353-360.

Ingelman-Sundberg M (2005) Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J* **5:**6-13.

Iwata H, Fujita K, Kushida H, Suzuki A, Konno Y, Nakamura K, Fujino A and Kamataki T (1998) High catalytic activity of human cytochrome P450 co-expressed with human NADPH-cytochrome P450 reductase in *Escherichia coli*. *Biochem Pharmacol* **55:**1315-1325.

Johansson I, Lundqvist E, Dahl ML and Ingelman-Sundberg M (1996) PCR-based genotyping for duplicated and deleted *CYP2D6* genes. *Pharmacogenetics* **6:**351-355.

Nakamura K, Ariyoshi N, Yokoi T, Ohgiya S, Chida M, Nagashima K, Inoue K, Kodama T, Shimada N and Kamataki T (2002) CYP2D6.10 present in human liver microsomes shows low catalytic activity and thermal stability. *Biochem Biophys Res Commun* **293:**969-973.

Ramamoorthy Y, Tyndale RF and Sellers EM (2001) Cytochrome P450 2D6.1 and cytochrome P450 2D6.10 differ in catalytic activity for multiple substrates. *Pharmacogenetics* **11:**477-487.

Soyama A, Kubo T, Miyajima A, Saito Y, Shiseki K, Komamura K, Ueno K, Kamakura S, Kitakaze M, Tomoike H, Ozawa S and Sawada J (2004) Novel nonsynonymous single nucleotide polymorphisms in the *CYP2D6* gene. *Drug Metab Pharmacokinet* **19:**313-319.

Tateishi T, Chida M, Ariyoshi N, Mizorogi Y, Kamataki T and Kobayashi S (1999) Analysis of the *CYP2D6* gene in relation to dextromethorphan *O*-demethylation capacity in a Japanese population. *Clin Pharmacol Ther* **65:**570-575.

Yamaori S, Yamazaki H, Iwano S, Kiyotani K, Matsumura K, Honda G, Nakagawa K, Ishizaki T and Kamataki T (2004) CYP3A5 contributes significantly to CYP3A-mediated drug oxidations in

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DMD Fast Forward. Published on January 21, 2009 as DOI: 10.1124/dmd.108.026096
This article has not been copyedited and formatted. The final version may differ from this version.

liver microsomes from Japanese subjects. *Drug Metab Pharmacokinet* **19:**120-129.

Yamazaki H, Kiyotani K, Tsubuko S, Matsunaga M, Fujieda M, Saito T, Miura J, Kobayashi S and Kamataki T (2003) Two novel haplotypes of *CYP2D6* gene in a Japanese population. *Drug Metab Pharmacokinet* **18:**269-271.

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Footnotes

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Legends for Figures

Figure 1. Representative LC chromatograms for metabolite formation from dextromethorphan catalyzed by human liver microsomes. Dextromethorphan (400 μM) were incubated in the presence of human liver microsomes genotyped as *CYP2D6*10/*10* (a), *CYP2D6*10/*49* (b). The peak of dextromethorphan was noted at a retention time of 11 min. Abbreviations used in this figure: DOR, dextrorphan; 3MM, 3-methoxymorphinan; and NM, new metabolite.

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Table 1. Kinetic parameters for dextromethorphan *O*-demethylation catalyzed by wild and mutant types of CYP2D6 expressed in *E. coli* membranes.

CYP2D6 allele	CYP2D6 protein	Amino acid changes	*O*-demethylation			7-hydroxylation[a]		
			K_m (μM)	V_{max} (nmol/min/nmol CYP2D6)	V_{max}/K_m (mL/min/nmol CYP2D6)	K_m (μM)	V_{max} (nmol/min/nmol CYP2D6)	V_{max}/K_m (mL/min/nmol CYP2D6)
**1*	CYP2D6.1	None	10 ± 1	6.3 ± 0.9	0.63	N.D.	N.D.	N.D.
**10*	CYP2D6.10	P34S, S486T	63 ± 7	$1.2 \pm 0.2^{**}$	0.019	N.D.	N.D.	N.D.
**49*	CYP2D6.49	P34S, F120I, S486T	$323 \pm 44^{**, \ddagger}$	$0.9 \pm 0.1^{**}$	0.003	391 ± 23	0.8 ± 0.2	0.002
**72*	CYP2D6.72	P34S, E383K, S486T	$87 \pm 21^{*}$	$0.7 \pm 0.3^{**, \dagger}$	0.008	N.D.	N.D.	N.D.

Dextromethorphan (3.5-2000 μM) was incubated with membrane fractions prepared from genetically engineered *E. coli*. Kinetic parameters were calculated by nonlinear regression analysis. Each value represents the mean ± SD from three determinations. N.D., product not detectable.

Significantly different from wild-type *CYP2D6*1* by Dunnett test (* $p<0.05$, ** $p<0.01$).

Significantly different from *CYP2D6*10* by Dunnett test († $p<0.05$, ‡ $p<0.01$).

[a]Hydroxylation velocity was determined as the peak area ratio of metabolite to dextrorphan standard.

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Table 2. Kinetic parameters for dextromethorphan *O*-demethylation catalyzed by human liver microsomes with different *CYP2D6* genotypes

CYP2D6 genotype	K_m (μM)[a]	V_{max} (pmol/min/mg protein)[a]	V_{max}/K_m (μL/min/mg protein)
**1/*1* (*n* = 3)	10.2 ± 1.5	157 ± 77	15.4
**1/*10* (*n* = 3)	15.3 ± 9.1	120 ± 14	7.8
**10/*10* (*n* = 3)	23.9 ± 8.2	148 ± 103	6.2
**10/*49* (*n* = 1)	39.7	141	3.6

Dextromethorphan (10-400 μM) was incubated with human liver microsomes from subjects genotyped as shown in the table. Kinetic parameters were calculated by nonlinear regression analysis.

[a]Data are represented as mean ± SD of liver microsomes from different individuals.

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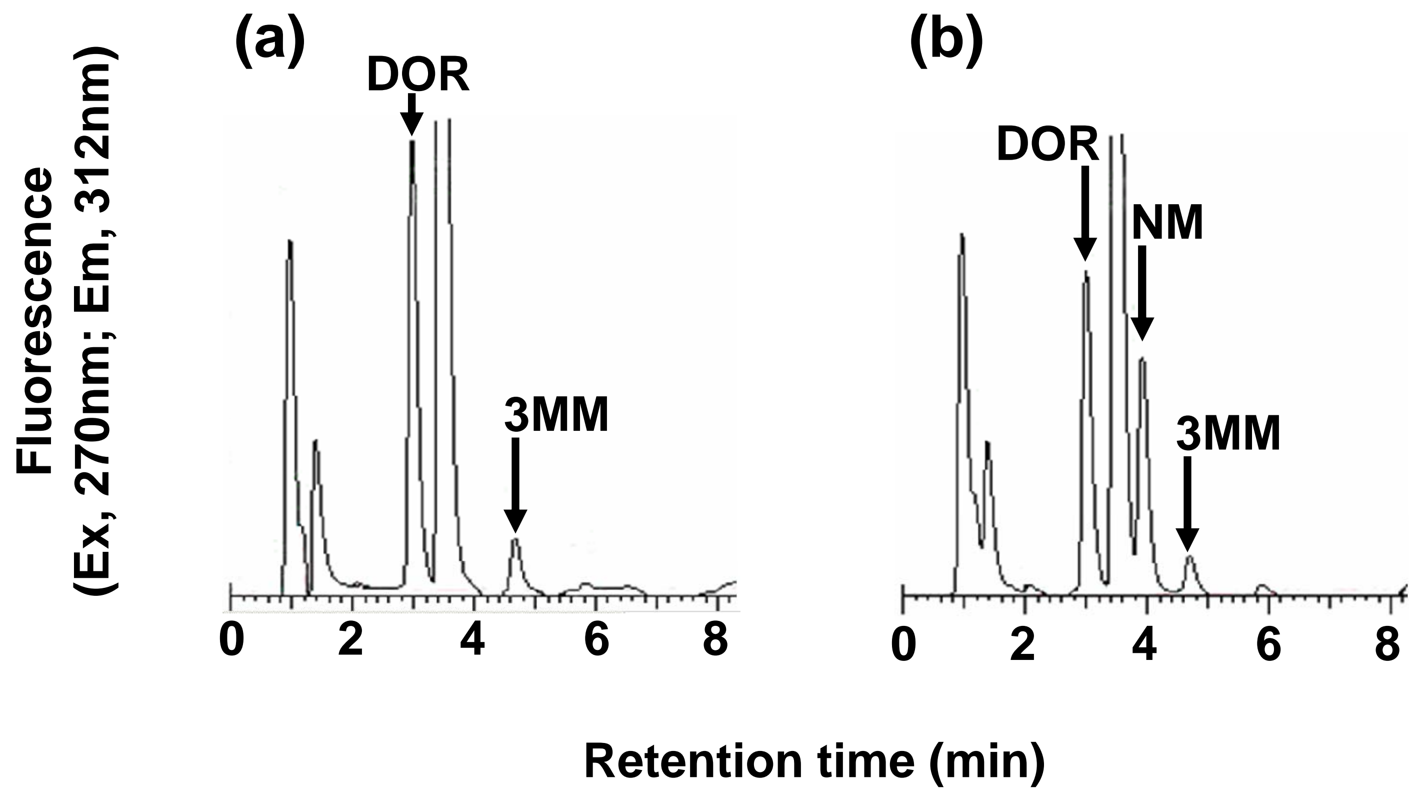
Fig.1



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