Liaoning Medical University, Jinzhou, China

Inhibition of catechol-o-methyltransferase (COMT) by myricetin, dihydromyricetin, and myricitrin

XU ZHU, YUN-HONG JIA

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Yun-Hong Jia, Liaoning Medical University, Jinzhou 121001, China
jiayunhongjinzhou@163.com

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Catechol O-methyltransferase (COMT) is an important enzyme involved in the metabolism of levodopa (L-dopa) which is clinically used to treat Parkinson's disease through boosting the concentration of dopamine in the brain. Development of COMT inhibitors can efficiently increase the bioavailability of L-dopa. The present study aims to evaluate the inhibition of COMT activity by three herbal components isolated from *Myrica rubra* Sieb. et Zucc.. The *in vitro* human liver cytosol-catalyzed L-dopa methylation reaction was utilized. The results showed that all these three compounds strongly inhibited COMT activity in a concentration-dependent manner. The inhibition was competitive for these three compounds, as demonstrated by Dixon and Lineweaver-Burk plots. The inhibition kinetic parameters (Ki) towards COMT activity were calculated to be 0.5, 0.2, and 0.9 μM for myricitrin, myricetin, and dihydromyricetin, respectively. From the view of structures, the deglycosylation biotransformation of myricitrin into myricetin can increase the inhibitory ability towards COMT. However, further structural alteration of myricetin towards dihydromyricetin weakens the inhibitory potential towards COMT.

1. Introduction

Catechol O-methyltransferase (COMT), discovered by Julius Axelrod in 1957, is an important enzyme catalyzing the transfer of a methyl group from S-adenosyl methionine (SAM) to a catechol substrate (Axelrod 1957). Among the variety of biology processes controlled by COMT, the best-studied role of COMT is its capability to metabolize L-DOPA which is a widely used drug to treat Parkinson's disease through boosting the concentration of dopamine in the brain (Bonifacio et al. 2007). The percentage of exogenous L-DOPA reaching the brain is approximately 1%, which needs high administered doses to achieve efficient concentrations in the brain. This method might induce peripheral side effects. Due to the extensive metabolism of L-DOPA mediated by COMT, development of COMT inhibitors has become an efficient strategy to increase the bioavailability of L-DOPA. The COMT inhibitors entacapone and tolcapone have been approved for clinical use (Nyholm et al. 2012). Due to their low bioavailability and short duration of action, more and more efforts have been made to find new COMT inhibitors with improved pharmacokinetic behaviour.

Herbs and many purified compounds isolated from herbs are able to efficiently treat diseases (Matkowski et al. 2013). Myricetin, dihydromyricetin, and myricitrin are important flavonoids components isolated from *Myrica rubra* Sieb. et Zucc., and have been clinically used for various purposes. For example, myricetin has been demonstrated to exhibit anti-tumor and anti-inflammatory effects (Sun et al. 2012; Wang et al. 2010). Dihydromyricetin can be successfully applied to treat alcohol use disorders (Shen et al. 2012). Purpurin and alizarin are two anthraquinone compounds isolated madder root, and have been reported to prevent genotoxicity or cytotoxicity (Takahashi et al. 2007).

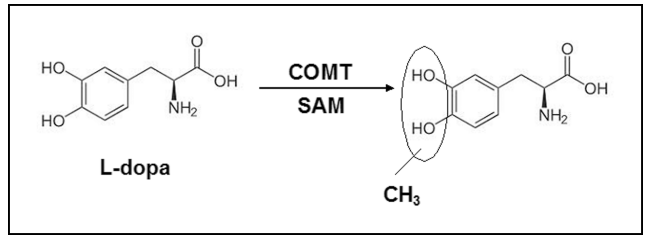

Fig. 1: Reaction equation for COMT-catalyzed L-dopa methylation.

The present study aimed to evaluate the inhibitory potential of these three herbal components towards the activity of COMT, trying to broaden their clinical utilization. *In vitro* human liver cytosol-catalyzed L-dopa methylation reaction was used as probe reaction, and the inhibition kinetic type and parameters (Ki) were determined.

2. Investigations and results

The representative reaction equation is shown in Fig. 1. Dose-dependent inhibition of myricitrin, myricetin, and dihydromyricetin towards COMT activity is shown in Fig. 2A, Fig. 3A, and Fig. 4A. Furthermore, the Dixon plot (1/(reaction velocity) versus the concentrations of inhibitors) and Lineweaver-Burk plot (1/(reaction velocity) versus 1/(L-dopa)) were employed to determine the inhibition kinetic type. The intersection point was located in the verticle axis in both Dixon plot (Fig. 2B, 3B and 4B) and Lineweaver-Burk plot (Fig. 2C, 3C and 4C), indicating a noncompetitive inhibition of these three compounds towards COMT activity. The inhibition kinetic

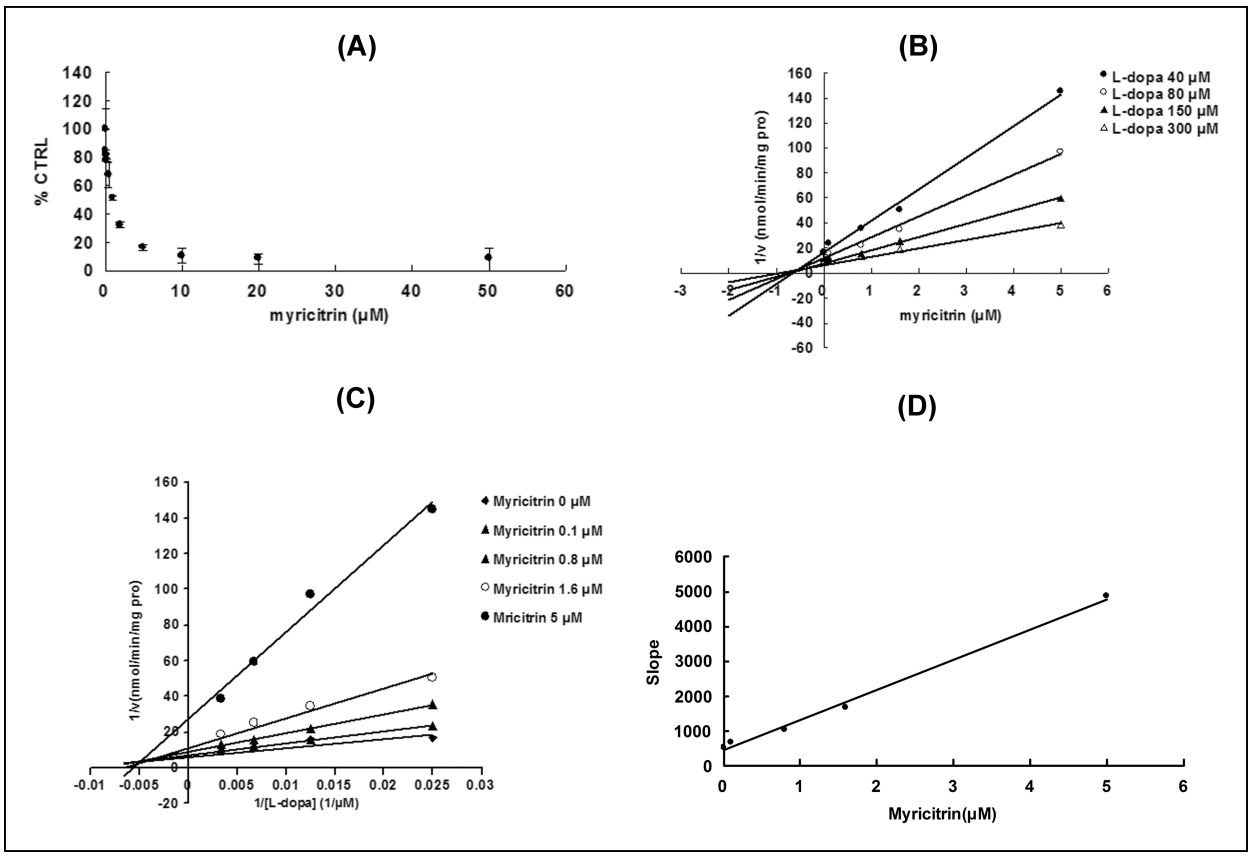

Fig. 2: The inhibition of myricitrin towards COMT-catalyzed L-dopa methylation. (A) Dose-dependent inhibition of myricitrin towards COMT-catalyzed L-dopa methylation. (B) Dixon plot of myricitrin's inhibition towards COMT-catalyzed L-dopa methylation. (C) Lineweaver-Burk plot of myricitrin's inhibition towards COMT-catalyzed L-dopa methylation. (D) Second plot of myricitrin's inhibition towards COMT-catalyzed L-dopa methylation. Each data point represents the mean value of two replicates.

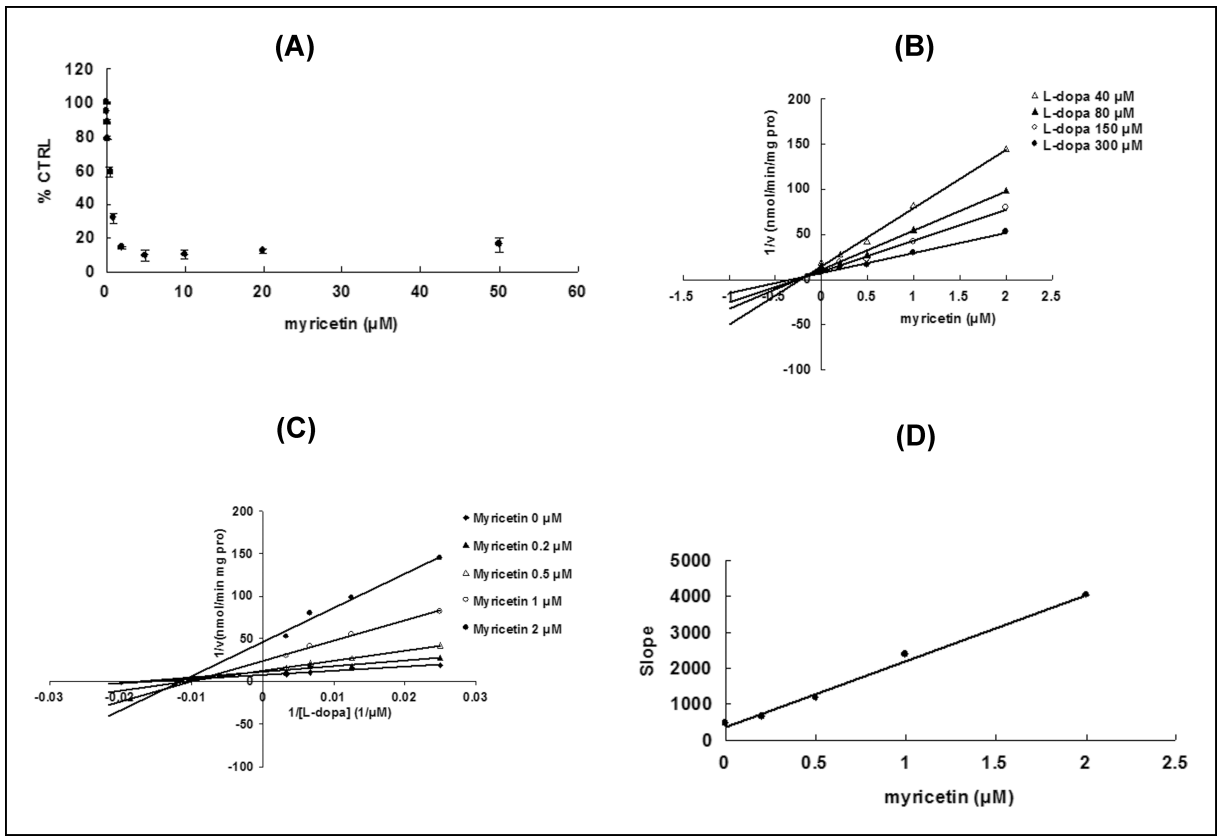

Fig. 3: The inhibition of myricetin towards COMT-catalyzed L-dopa methylation. (A) Dose-dependent inhibition of myricetin towards COMT-catalyzed L-dopa methylation. (B) Dixon plot of myricetin's inhibition towards COMT-catalyzed L-dopa methylation. (C) Lineweaver-Burk plot of myricetin's inhibition towards COMT-catalyzed L-dopa methylation. (D) Second plot of myricetin's inhibition towards COMT-catalyzed L-dopa methylation. Each data point represents the mean value of two replicates.

parameters (K_i) were calculated to be 0.5, 0.2, and 0.9 μM for the inhibition of myricitrin, myricetin, and dihydromyricetin towards COMT activity (Fig. 2D, 3D and 4D).

3. Discussion

Some Compounds with simple catechol and pyrogallol structures have been demonstrated to be good competitive inhibitors of COMT (Ross and Haljasmaa 1964). Additionally, some compounds with non-catechol groups can also inhibit COMT activity, including tropolone (Borchardt 1973). Reports have also indicated that the nitro substituent on catechol-derived inhibitors can remarkably increase the inhibitory potency (Borchardt et al. 1982).

The structures of myricetin, dihydromyricetin, and myricitrin (Fig. 5), are all containins the typical pyrogallol group. Therefore, it is not surprising to find that these three compounds exert strong inhibition towards COMT compartable to other reported COMT inhibitors. A previous study (Zhu et al. 2009) evaluated the inhibition ability of chlorogenic acid and caffeic acid towards COMT activity using O-methylation of 2- and 4-hydroxyestradiol as probe reaction. The IC_{50} values were calculated to be 1.3-1.4 and 6.3-12.5 μM for the inhibition of chlorogenic acid and caffeic acid towards towards O-methylation of 2-hydroxyestradiol. For the O-methylation of 4-hydroxyestradiol, the IC_{50} values were 0.7-0.8 and 1.3-3.1 μM for chlorogenic acid and caffeic acid, respectively. Another COMT inhibitor, 3',4'-dihydroxy-2-methyl-propriophenone (U-0521) can inhibit the COMT activity in red blood cells in a dose-dependent manner with an IC_{50} value of 6 μM (Reches et al. 1982). Compared with the inhibition potential of these compounds, myricetin, dihydromyricetin, and myricitrin exhibited relatively strong inhibition capability towards COMT.

Structure-inhibition relationship studies can elucidate what structure is important for entyme inhibition capability. Some research has been performed for drug-metabolizing enzymes (DMEs). For example, the structure-inhibition relationship was determined for the inhibition of ginsenosides towards UDP-glucuronosyltransferases (UGTs) isoforms (Fang et al. 2013). DeRuiter et al. (1991) already did structure-inhibition analyses of the N-benzoyl and N-(phenylsulfonyl) amino acid aldose reductase inhibitors (DeRuiter et al. 1991). Relatively few efforts have been made to study structure-inhibition relationships of COMT inhibitors. From the present study, we know that the deglycosylation biotransformation of myricitrin into myricetin can increase the inhibitory ability towards COMT activity. Further structural alteration of myricetin towards dihydromyricetin weakens the inhibitory potential towards COMT. However, a small number of compounds was used in the present study, and more compounds with similar structures are needed to establish more detailed structure-inhibition relationships in the future.

When these three compounds will be developed as things to increase the bioavailability of L-dopa, the *in vivo* concentration is a key determinant, and many factors might affect the *in vivo* exposure. For example, compounds with phenol hydroxyl groups are preferred substrates of UGT isoforms (Dong et al. 2013; Song et al. 2013). Therefore, the individual difference of UGT enzymes might affect the exposure of these three compounds.

In conclusion, the inhibition of COMT activity by three herbal components myricetin, dihydromyricetin, and myricitrin was demonstrated in the present study. The inhibition kinetic type and parameters (K_i) were determined. Additionally, potential structure-inhibition relationships towards COMT enzymes were initially discussed in the present study. Myricetin, dihy-

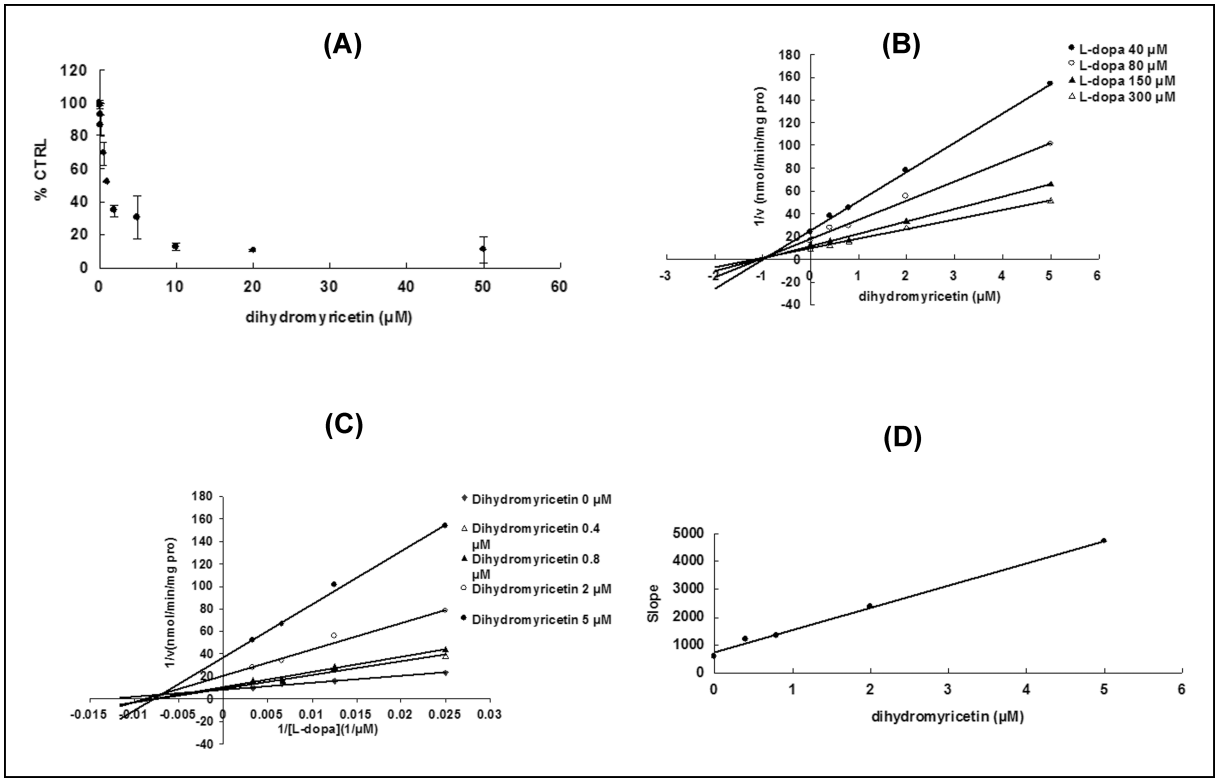

Fig. 4: The inhibition of dihydromyricetin towards COMT-catalyzed L-dopa methylation. (A) Dose-dependent inhibition of dihydromyricetin towards COMT-catalyzed L-dopa methylation. (B) Dixon plot of dihydromyricetin's inhibition towards COMT-catalyzed L-dopa methylation. (C) Lineweaver-Burk plot of dihydromyricetin's inhibition towards COMT-catalyzed L-dopa methylation. (D) Second plot of dihydromyricetin's inhibition towards COMT-catalyzed L-dopa methylation. Each data point represents the mean value of two replicates.

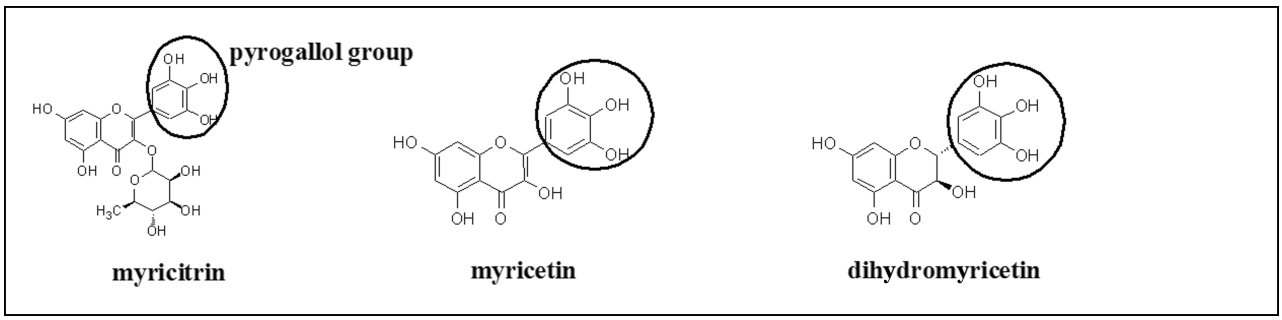

Fig. 5: Pyrogallol in the structures of myricitrin, myricetin and dihydromyricetin.

dromyricetin, and myricitrin might be clinically used as COMT inhibitors in the future.

4. Experimental

4.1. Chemicals and reagents

Levodopa (L-Dopa, purity ≥ 98%), $MgCl_2$, dithiothreitol (DTT, purity ≥ 98%), S-adenosyl methionine (SAM), Tris-HCl, and L-adrenaline was obtained from Sigma-Aldrich (St. Louis, MO). Myricetin, dihydromyricetin, and myricitrin were purchased from from Sichuan Weikeqi Bio-technology Co. Ltd (Sichuan, China). The human liver cytosol was purchased from BD Gentest (Woburn, MA). All other reagents were of HPLC grade or the highest purity commercially available.

4.2. In vitro evaluation of the inhibition of L-Dopa methylation

The inhibition of COMT activity by compounds was evaluated using the methylation reaction of L-Dopa as previously described with a slight modification (Kang et al. 2010). The incubation mixture (total volume = 200 µl) contained 5 mM $MgCl_2$, 50 mM Tris-HCl, 2 mM DTT, 150 µM L-Dopa, 1 mg/ml human liver cytosol, and various concentrations of herbal compounds (0.5–50 µM). After 3 min pre-incubation at 37 °C, the reaction was initiated with 0.2 mM SAM. After 30 min, the reaction was terminated by addition of 100 µl acetonitrile (50 µM L-adrenaline as internal standard). After centrifugation at 14,000 × g, the aliquot of supernatant was transferred into vials to perform analysis. The chromatography analysis was carried out with a Shimadzu (Kyoto, Japan) prominence ultra-fast liquid chromatography (UFLC) system, which was equipped with a CBM-20A communications bus module, a SIL-20ACHT autosampler, two LC-20AD pumps, a DGU-20A3 vacuum degasser and a CTO-20AC column oven. A Shim-pack XR-ODS column (75 mm × 2.0 mm, 2.2 mm, Shimadzu) was kept at 40 °C. The mobile phase consisted of methanol (A) and H_2O containing 0.2% (v/v) formic acid (B). The elution was performed using 4% B. The flow rate of the mobile phase was set at 0.3 ml/min. The column outlet was connected to ABI QTrap 4000. The relative MS parameters were as follows: GS1, 50 psi; GS2, 50 psi; Curtain gas, 20 psi; CAD, medium; IS, 5500 V; TEM, 500 °C. Multiple reaction monitoring (MRM) was utilized to determine L-dopa (198.1→107.1), the methylated product of L-dopa (212.2→195.1), and L-adrenaline (184.1→107.0).

4.3. Determination of inhibition kinetic type and parameters (K_i)

Several concentrations of L-dopa (40, 80, 150, and 300 µM) and inhibitors (0, 0.1, 0.8, 1.6 and 5 µM for myricitrin, 0, 0.2, 0.5, 1, 2 µM for myricitrin, and 0, 0.4, 0.8, 2, 5 µM for dihydromyricetin) were used to determine the reaction velocity. Dixon and Lineweaver-Burk plots were used to fit the data to determine the inhibition kinetic type as previously described (Fang et al. 2011; Huang et al. 2010). The second plot with the slopes from Lineweaver-Burk plot *versus* the concentrations of compounds was employed to calculate the inhibition kinetic parameters (K_i).

References

Axelrod J (1957) O-Methylation of epinephrine and other catechols *in vitro* and *in vivo*. Science 126: 400–401.

Bonifacio MJ, Palma PN, Almeida L, Soares-da-Silva P (2007) Catechol-O-methyltransferase and Its Inhibitors in Parkinson's Disease. CNS Drug Rev 13: 352–379.

Borchardt RT (1973) Catechol O-methyltransferase. 1. Kinetics of tropolone inhibition. J Med Chem 16: 377–382.

Borchardt RT, Huber JH, Houston M (1982) Catechol O-methyltransferase. 10. 5-Substituted, 3-hydroxy-4-methoxybenzoic acids (isovanillic acids) and 5-substituted, 3-hydroxy-4-methoxybenzaldehydes (isovanillins) as potential inhibitors. J Med Chem 25: 258–263.

DeRuiter J, Davis RA, Wandrekar VG, Mayfield CA (1991) Relative structure-inhibition analyses of the N-benzoyl and N-(phenylsulfonyl) amino acid aldose reductase inhibitors. J Med Chem 34: 2120–2126.

Dong RH, Fang ZZ, Zhu LL, Ge GB, Li XB, Hu CM, Cao YF, Xia YL, Yang L, Liu ZY (2013) Deep understanding of the interaction between thienorphine and UDP-glucuronosyltransferase (UGT) isoforms. Xenobiotica 43: 133–139.

Fang ZZ, Cao YF, Hu CM, Hong M, Sun XY, Ge GB, Liu Y, Zhang YY, Yang L, Sun HZ (2013) Structure-inhibition relationship of ginsenosides towards UDP-glucuronosyltransferases (UGTs). Toxicol Appl Pharmacol 267: 149–154.

Fang ZZ, Zhang YY, Ge GB, Liang SC, Sun DX, Zhu LL, Dong PP, Cao YF, Yang L (2011) Identification of cytochrome P450 (CYP) isoforms involved in the metabolism of corynoline, and assessment of its herb-drug interactions. Phytother Res 25: 256–263.

Huang T, Fang Z, Yang L (2010) Strong inhibitory effect of medroxyprogesterone acetate (MPA) on UDP-glucuronosyltransferase (UGT) 2B7 might induce drugdrug interactions. Pharmazie 65: 919–921.

Kang KS, Wen Y, Yamabe N, Fukui M, Bishop SC, Zhu BT (2010) Dual beneficial effects of (-)-epigallocatechin-3-gallate on levodopa methylation and hippocampal neurodegeneration: *in vitro* and *in vivo* studies. PLoS One 5: e11951.

Matkowski A, Jamiolkowska-Kozlowska W, Nawrot I (2013) Chinese medicinal herbs as source of antioxidant compounds-where tradition meets the future. Curr Med Chem 20: 984–1004.

Nyholm D, Johansson A, Lennernas H, Askmark H (2012) Levodopa infusion combined with entacapone or tolcapone in Parkinson disease: a pilot trial. Eur J Neurol 19: 820–826.

Reches A, Jiang D-H, Fahn S (1982) Effect of 3′, 4′-dihydroxy-2-methylpropriophenone (U-0521) on catechol-O-methyltransferase activity and on DOPA accumulation in rat red blood cells and corpus striatum. Biochem Pharmacol 31: 3415–3418.

Ross S, Haljasmaa O (1964) Catechol-O-Methyl transferase inhibitors. *In vitro* inhibition of the enzyme in mouse-brain extract. Acta Pharmacol Toxicol 21: 205–214.

Shen Y, Lindemeyer AK, Gonzalez C, Shao XM, Spigelman I, Olsen RW, Liang J (2012) Dihydromyricetin as a novel anti-alcohol intoxication medication. J Neurosci 32: 390–401.

Song JH, Fang ZZ, Zhu LL, Cao YF, Hu CM, Ge GB, Zhao DW (2013) Glucuronidation of the broad-spectrum antiviral drug arbidol by UGT isoforms. J Pharm Pharmacol 65: 521–527.

Sun F, Zheng XY, Ye J, Wu TT, Wang Jl, Chen W (2012) Potential anticancer activity of myricetin in human t24 bladder cancer cells both *in vitro* and *in vivo*. Nutrition Cancer 64: 599–606.

Takahashi E, Arimoto S, Okamoto K, Negishi T (2007) Enhancement of phase II enzyme activity by purpurin resulting in the suppression of MeIQx-DNA-adduct formation in mice. Mutat Res 626: 128–134.

Wang S-J, Tong Y, Lu S, Yang R, Liao X, Xu YF, Li X (2010) Anti-inflammatory activity of myricetin isolated from Myrica rubra Sieb. et Zucc. leaves. Planta Med 76: 1492–1496.

Zhu BT, Wang P, Nagai M, Wen Y, Bai HW (2009) Inhibition of human catechol-O-methyltransferase (COMT)-mediated O-methylation of catechol estrogens by major polyphenolic components present in coffee. J Steroid Biochem Mol Biol 113: 65–74.