

# Inhibitory Potential of Bilobetin Against CYP2J2 Activities in Human Liver Microsomes

Zhexue Wu<sup>1#</sup>, Su-Nyeong Jang<sup>2#</sup>, So-Young Park<sup>2#</sup>, Nguyen Minh Phuc<sup>2,3</sup>, and Kwang-Hyeon Liu<sup>2\*</sup>

<sup>1</sup>Mass spectrometry convergence research institute, Kyungpook National University, Daegu 41566, Korea

<sup>2</sup>BK21 FOUR Community-Based Intelligent Novel Drug Discovery Education Unit, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, South Korea

<sup>3</sup>Vietnam Hightech of Medicinal and Pharmaceutical JSC, Group 11 Quang Minh town, Hanoi 100000, Vietnam

Received December 13, 2020; Revised December 29, 2020; Accepted December 29, 2020

First published on the web December 31, 2020; DOI: 10.5478/MSL.2020.11.4.113

**Abstract :** Cytochrome P450 2J2 (CYP2J2) is a member of the cytochrome P450 superfamily, and is known to be arachidonic acid epoxygenase that mediates the formation of four bioactive regioisomers of epoxyeicosatrienoic acids (EETs). CYP2J2 is also involved in the metabolism of drugs such as albendazole, astemizole, danazol, ebastine, and terfenadine. CYP2J2 is highly expressed in the heart and cancer tissues. In this study, the inhibitory potential of ten natural products against CYP2J2 activity was evaluated using human liver microsomes and tandem mass spectrometry. Among them, bilobetin, which is a kind of biflavonoid, exhibits a strong inhibitory effect against the CYP2J2-mediated astemizole *O*-demethylation ( $IC_{50} = 0.73 \mu\text{M}$ ) and terfenadine hydroxylation ( $IC_{50} = 0.89 \mu\text{M}$ ). This result suggests that bilobetin can be used as strong CYP2J2 inhibitor in drug metabolism study.

**Keywords :** bilobetin, CYP2J2, human liver microsomes, liquid chromatography-tandem mass spectrometry, natural products

## Introduction

Cytochrome P450 2J2 (CYP2J2) is an arachidonic acid-metabolizing enzyme highly expressed in the heart, kidneys, lungs, small intestine and gastrointestinal tract.<sup>1,4</sup> CYP2J2 plays an important role in the metabolism of human endogenous substances, such as arachidonic acid,<sup>1</sup> linoleic acid,<sup>5</sup> docosahexaenoic acid,<sup>6,7</sup> eicosapentaenoic acid<sup>7</sup> and vitamin D3.<sup>8</sup> CYP2J2 also involved in the metabolism of xenobiotics such as albendazole,<sup>9</sup> apixaban,<sup>10</sup> danazol,<sup>11</sup> ebastine,<sup>12</sup> eperisone,<sup>13</sup> thioridazine,<sup>11</sup> and vorapaxar.<sup>14</sup>

Meanwhile, it has been reported that CYP2J2 is overexpressed in human tumor tissues and tumor cells.<sup>15-17</sup> In various tumor types, overexpression of CYP2J2 and elevated epoxyeicosatrienoic acids (EETs) promote cancer

cell proliferation, migration and adhesion.<sup>18,19</sup> In addition, accumulating evidence on the relationship between CYP2J2 and anti-cancer activity in hepatocellular carcinoma suggest that inhibition of CYP2J2 enzyme activity by chemicals might introduce novel therapeutics for the treatment of cancer via down-regulation of EETs biosynthesis.<sup>15</sup> Several CYP2J2 inhibitors such as decursin,<sup>20</sup> tanshinone IIA,<sup>17</sup> acetylshikonin,<sup>16</sup> and broussonchalcone A<sup>15</sup> showed anti-cancer effects *in vitro* and *in vivo* by reducing EET biosynthesis. However, little data are available on the CYP2J2 inhibitors to date.

To identify a new CYP2J2 inhibitor, 10 natural products obtained from medicinal plants were screened for their CYP2J2 inhibitory potential in human liver microsomes (HLMs) using astemizole as CYP2J2 substrate. The ten classes of natural products include 6,8-diprenylorobol (flavonoid), bilobetin (biflavonoid), geniposide (iridoid glycoside), gomisin A (lignan), physcion (anthraquinone), patchouli alcohol (sesquiterpenoid), pellitorine (amide alkaloid), resveratrol (stilbenoid), syringin (phenylpropanoid), and tomentosin (sesquiterpene lactone) (Figure 1).

## Experimental

### Materials

Astemizole, *O*-desmethyl astemizole, terfenadine, and terfenadine alcohol were purchased from Toronto Research chemicals (North York, Canada). Glucose-6-phosphate

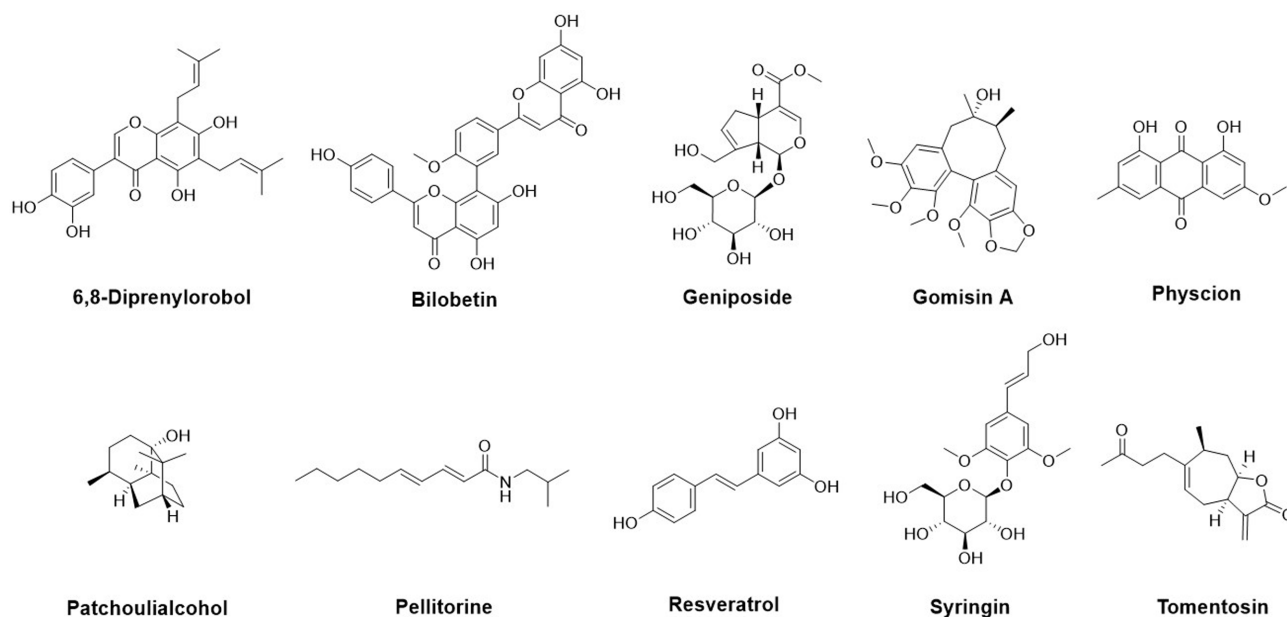
### Open Access

<sup>#</sup>These authors are equally contributed.

\*Reprint requests to Kwang-Hyeon Liu

E-mail: dstlkh@knu.ac.kr

All MS Letters content is Open Access, meaning it is accessible online to everyone, without fee and authors' permission. All MS Letters content is published and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0/>). Under this license, authors reserve the copyright for their content; however, they permit anyone to unrestrictedly use, distribute, and reproduce the content in any medium as far as the original authors and source are cited. For any reuse, redistribution, or reproduction of a work, users must clarify the license terms under which the work was produced.



**Figure 1.** Chemical structure of ten natural products used in this study.

(G6P), glucose-6-phosphate dehydrogenase (G6PDH), and b-nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>) were obtained from Sigma–Aldrich (St. Louis, MO). Ten natural products were purchased from BioCrick Biotech (Chengdu, China), Sigma-Aldrich (St. Louis, MO, USA), and Ensol Biosciences Inc (Daejeon, Korea). Pooled human liver microsomes (HLMs; H0630) were purchased from Xenotech (Lenexa, KS, USA). All the other solvents were LC-MS grade (Fisher Scientific Co., Pittsburgh, PA, USA).

#### CYP2J2 inhibitor screening

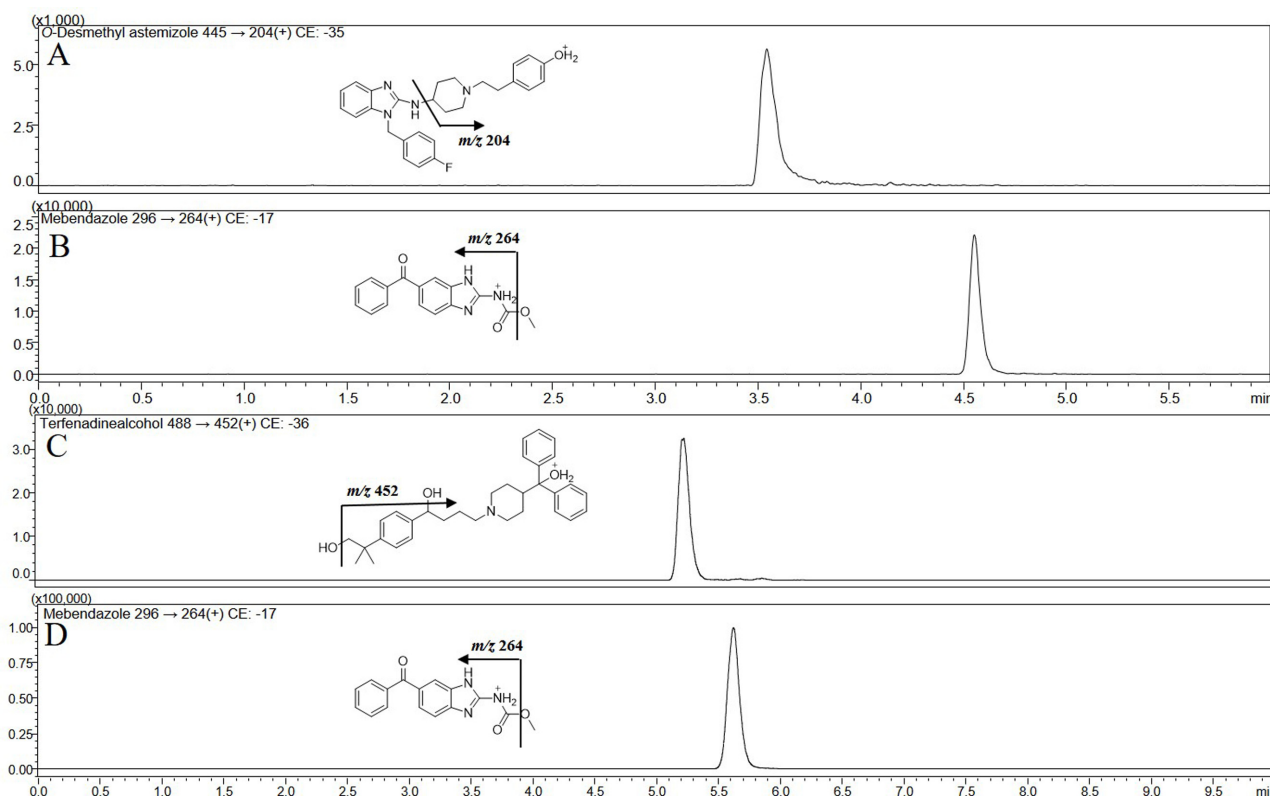
All incubations were performed in triplicate and the data are presented as average values. The inhibitory effect of ten natural products against CYP2J2-mediated astemizole *O*-demethylase activity was determined using pooled HLMs in both the presence and absence of test chemicals.<sup>21</sup> In brief, incubation mixtures (final volume, 100  $\mu$ L) were composed of HLMs (0.25 mg/mL), astemizole (1  $\mu$ M), and inhibitor (5  $\mu$ M). The reaction was initiated by the addition of NADPH-generating system (containing 1.3 mM NADP<sup>+</sup>, 3.3 mM G6P, 3.3 mM MgCl<sub>2</sub>, and 500 unit/mL G6PDH) after pre-incubation for 5 minutes at 37°C. To determine the inhibitory potentials (IC<sub>50</sub> values) of bilobetin for CYP2J2-catalyzed astemizole *O*-demethylation<sup>22</sup> and terfenadine hydroxylation<sup>23</sup> in HLMs, bilobetin (0, 0.2, 0.5, 2, 5, 20  $\mu$ M) was added to reaction mixtures containing astemizole (1  $\mu$ M) or terfenadine (0.2  $\mu$ M). After pre-incubation at 37°C, the reaction was initiated by the addition of NADPH generating system. The reaction was terminated by the addition of 100  $\mu$ L ice-cold

acetonitrile containing 100 nM of mebendazole (internal standard, IS) after 20 minutes incubation. After mixing and centrifugation at 14,000g for 5 minutes, supernatants (1  $\mu$ L) were analyzed by liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS). The reaction rates were linear with incubation time and microsomal protein amount.<sup>20</sup>

#### Analysis of desmethylastemizole and terfenadine alcohol

*O*-Desmethylastemizole and terfenadine alcohol were analyzed by LC-MS/MS as described previously,<sup>20</sup> using a Shimadzu LCMS-8040 triple quadrupole mass spectrometer (Shimadzu, Tokyo, Japan). The analytes were separated on a reversed-phase column (Luna C18, 50  $\times$  2 mm i.d., 3  $\mu$ m particle size; Phenomenex, Torrance, CA). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The flow rate was 0.2 mL/min. For the analysis of *O*-desmethylastemizole, the mobile phase B was linearly increased from 10 to 50% over 3 min, held at 50% for 0.1 min, and then immediately stepped back down to 10% for re-equilibration for 6 min. The retention time of *O*-desmethylastemizole and mebendazole were 3.58 min and 4.59 min (Figure 2A and 2B). For the analysis of terfenadine alcohol, the mobile phase B was linearly increased from 10 to 50% over 6 min, held at 50% for 0.1 min, and then immediately stepped back down to 10% for re-equilibration. The retention time of terfenadine alcohol and mebendazole were 5.23 min and 5.61 min (Figure 2C and 2D). Quantitation was carried out in the selected reaction monitoring (SRM) of the [M+H]<sup>+</sup> ion and the related product ion for each metabolite and IS. The

## Inhibitory Potential of Bilobetin Against CYP2J2 Activities in Human Liver Microsomes



**Figure 2.** LC-MS/MS selected ion chromatograms of O-desmethyl astemizole (A), mebendazole (B and D), and terfenadine alcohol (C).

following SRM transition ions were monitored: O-desmethylastemizole ( $m/z$  445  $\rightarrow$  204), terfenadine alcohol ( $m/z$  488  $\rightarrow$  452), and mebendazole ( $m/z$  296  $\rightarrow$  264) (Figure 2).

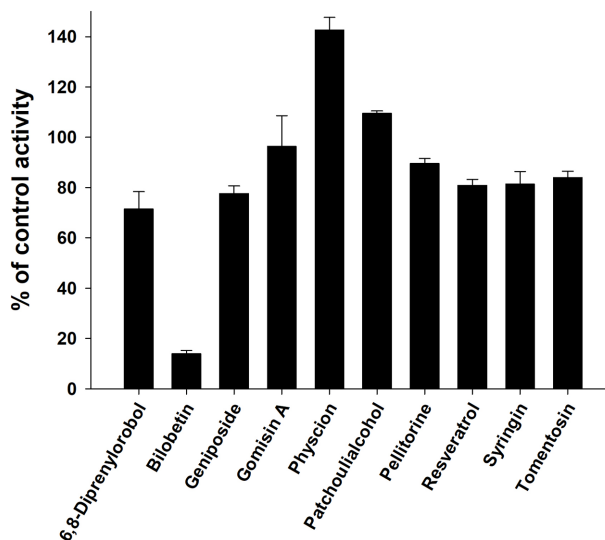
### Data analysis

Results are expressed as the mean  $\pm$  S.D. of estimates obtained from pooled HLMS triplicate experiments. Percentage inhibition was calculated as the ratio of metabolite formation in the presence and absence of the specific inhibitor. Calculations were performed using WinNonlin software (Pharsight, Mountain View, CA).

### Results and Discussion

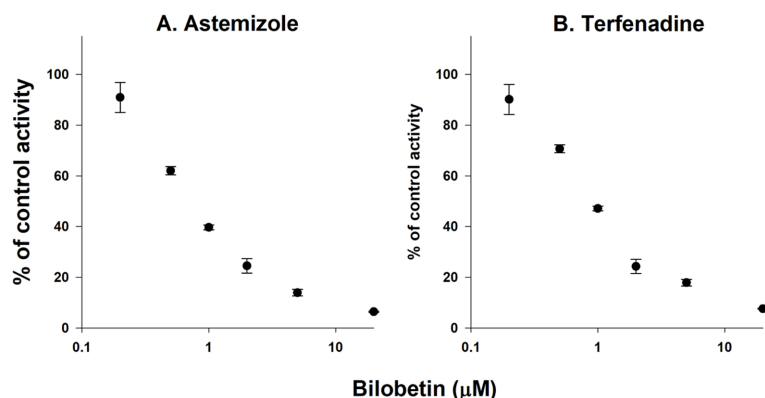
CYP2J2 is a metabolic enzyme mainly expressed in extrahepatic tissues.<sup>24</sup> In addition, CYP2J2 is highly expressed in a variety of cancer cell lines and tissues.<sup>18</sup> CYP2J2 plays an important role in the cancer cell proliferation, migration and adhesion.<sup>25</sup>

In this study, ten natural products having different structural scaffolds such as flavonoid, biflavonoid, iridoid glycoside, lignan, anthraquinone, phenylpropanoid, sesquiterpenoid, amide alkaloid, stilbenoid, and sesquiterpene lactone (Figure 1) were screened as potential



**Figure 3.** Inhibitory effects of ten natural products (5  $\mu$ M) against CYP2J2-mediated astemizole O-demethylation activity.

CYP2J2 inhibitors. Among ten natural products tested, bilobetin showed strong inhibition of astemizole O-demethylation activity. Bilobetin (5  $\mu$ M) strongly inhibited



**Figure 4.** Inhibitory effects of bilobetin on CYP2J2-mediated astemizole *O*-demethylation (A) and terfenadine hydroxylation (B) activities.

**Table 1.** Inhibitory potential of bilobetin against the activities of CYP2J2 in human liver microsomes

Substrate	Enzyme activity	IC <sub>50</sub> (µM)
Astemizole	Astemizole <i>O</i> -demethylation	0.73
Terfenadine	Terfenadine hydroxylation	0.89

CYP2J2-mediated astemizole *O*-demethylation activity (> 80%), while the inhibitory effects of other nine natural products were negligible or weak (< 30%) (Figure 3).

We performed further experiment for the estimation of IC<sub>50</sub> value of bilobetin because bilobetin showed stronger inhibitory potential against CYP2J2 activity than other nine compounds. Bilobetin strongly inhibited CYP2J2-mediated astemizole *O*-demethylation activity in a concentration-dependent manner, with an IC<sub>50</sub> value of 0.73 µM (Figure 4 and Table 1). Bilobetin also inhibited CYP2J2-catalyzed terfenadine hydroxylation activity with in a substrate-independent manner with an IC<sub>50</sub> value of 0.89 µM in HLMs (Figure 4 and Table 1). Its CYP2J2 inhibitory potential was more potent than that of acetylshikonin<sup>16</sup> (IC<sub>50</sub> = 4.3 µM), brousochalcone A<sup>15</sup> (IC<sub>50</sub> = 5.57 µM), decursin<sup>20</sup> (IC<sub>50</sub> = 6.95 µM), thelephoric acid<sup>21</sup> (IC<sub>50</sub> = 3.23 µM), and tanshinone IIA<sup>17</sup> (IC<sub>50</sub> = 2.5 µM). Its inhibitory potential was also stronger than that of LKY-047<sup>23</sup> (IC<sub>50</sub> = 1.7 µM) known as strong CYP2J2 inhibitor. Therefore, bilobetin, along with LKY-047, might be used as strong CYP2J2 inhibitor in drug metabolism study.

Bilobetin is a biflavonoid isolated from the leaves of *Ginkgo biloba*.<sup>26</sup> Selaginellin, a biflavonoid isolated from *Selaginella tamariscina*, also showed similar CYP2J2 inhibitory potential<sup>27</sup> (IC<sub>50</sub> = 0.8 µM). Bilobetin exhibited anti-proliferative activities by inducing apoptosis of cancer cells.<sup>28</sup> Finally, given that CYP2J2 may represent a potential target for cancer therapy,<sup>19</sup> studies are currently underway to evaluate bilobetin as a potential anti-cancer agent.

## Conclusions

In conclusion, we identified bilobetin as a strong CYP2J2 inhibitor through the screening of ten natural products. Bilobetin inhibited CYP2J2 activity with IC<sub>50</sub> values of 0.73–0.89 mM in a CYP2J2 substrate-independent manner. Our data suggest that bilobetin could be used as a strong CYP2J2 inhibitor in drug metabolism study.

## Acknowledgements

This research was supported by the Kyungpook National University Development Project Research Fund, 2018 (S.N.J).

## References

- Wu, S.; Moomaw, C. R.; Tomer, K. B.; Falck, J. R.; Zeldin, D. C. *J. Biol. Chem.* **1996**, *271*, 3460, DOI: 10.1074/jbc.271.7.3460.
- Enayetallah, A. E.; French, R. A.; Thibodeau, M. S.; Grant, D. F. *J. Histochem. Cytochem.* **2004**, *52*, 447, DOI: 10.1177/002215540405200403.
- Paine, M. F.; Hart, H. L.; Ludington, S. S.; Haining, R. L.; Rettie, A. E.; Zeldin, D. C. *Drug Metab. Dispos.* **2006**, *34*, 880, DOI: 10.1124/dmd.105.008672.
- Karkhanis, A.; Hong, Y.; Chan, E. C. Y. *Biochem. Pharmacol.* **2017**, *135*, 12, DOI: 10.1016/j.bcp.2017.02.017.
- King, L. M.; Ma, J.; Srettapunjong, S.; Graves, J.; Bradbury, J. A.; Li, L.; Spiecker, M.; Liao, J. K.; Mohrenweiser, H.; Zeldin, D. C. *Mol. Pharmacol.* **2002**, *61*, 840, DOI: 10.1124/mol.61.4.840.
- Arnold, C.; Markovic, M.; Blossey, K.; Wallukat, G.; Fischer, R.; Dechend, R.; Konkel, A.; von Schacky, C.; Luft, F. C.; Muller, D. N.; Rothe, M.; Schunck, W. H. *J. Biol. Chem.* **2010**, *285*, 32720, DOI: 10.1074/jbc.M110.118406.

7. Fer, M.; Dreano, Y.; Lucas, D.; Corcos, L.; Salaun, J. P.; Berthou, F.; Amet, Y. *Arch. Biochem. Biophys.* **2008**, *471*, 116, DOI: 10.1016/j.abb.2008.01.002.
8. Aiba, I.; Yamasaki, T.; Shinki, T.; Izumi, S.; Yamamoto, K.; Yamada, S.; Terato, H.; Ide, H.; Ohyama, Y. *Steroids* **2006**, *71*, 849, DOI: 10.1016/j.steroids.2006.04.009.
9. Wu, Z.; Lee, D.; Joo, J.; Shin, J. H.; Kang, W.; Oh, S.; Lee, D. Y.; Lee, S. J.; Yea, S. S.; Lee, H. S.; Lee, T.; Liu, K. H. *Antimicrob. Agents Chemother.* **2013**, *57*, 5448, DOI: 10.1128/AAC.00843-13.
10. Wang, L.; Zhang, D.; Raghavan, N.; Yao, M.; Ma, L.; Frost, C. E.; Maxwell, B. D.; Chen, S. Y.; He, K.; Goosen, T. C.; Humphreys, W. G.; Grossman, S. J. *Drug Metab. Dispos.* **2010**, *38*, 448, DOI: 10.1124/dmd.109.029694.
11. Lee, C. A.; Neul, D.; Clouser-Roche, A.; Dalvie, D.; Wester, M. R.; Jiang, Y.; Jones, J. P., 3rd; Freiwald, S.; Zientek, M.; Totah, R. A. *Drug Metab. Dispos.* **2010**, *38*, 347, DOI: 10.1124/dmd.109.030270.
12. Liu, K. H.; Kim, M. G.; Lee, D. J.; Yoon, Y. J.; Kim, M. J.; Shon, J. H.; Choi, C. S.; Choi, Y. K.; Desta, Z.; Shin, J. G. *Drug Metab. Dispos.* **2006**, *34*, 1793, DOI: 10.1124/dmd.106.010488.
13. Yoo, H. H.; Kim, N. S.; Lee, J.; Sohn, D. R.; Jin, C.; Kim, D. H. *Xenobiotica* **2009**, *39*, 1, DOI: 10.1080/00498250802509448.
14. Nair, A. S. *J. Anaesthesiol. Clin. Pharmacol.* **2017**, *33*, 269, DOI: 10.4103/joacp.JOACP\_363\_16.
15. Park, S. H.; Lee, J.; Shon, J. C.; Phuc, N. M.; Jee, J. G.; Liu, K. H. *Phytomedicine* **2018**, *42*, 199, DOI: 10.1016/j.phymed.2018.03.032.
16. Park, S. H.; Phuc, N. M.; Lee, J.; Wu, Z.; Kim, J.; Kim, H.; Kim, N. D.; Lee, T.; Song, K. S.; Liu, K. H. *Phytomedicine* **2017**, *24*, 134, DOI: 10.1016/j.phymed.2016.12.001.
17. Jeon, Y. J.; Kim, J. S.; Hwang, G. H.; Wu, Z.; Han, H. J.; Park, S. H.; Chang, W.; Kim, L. K.; Lee, Y. M.; Liu, K. H.; Lee, M. Y. *Eur. J. Pharmacol.* **2015**, *764*, 480, DOI: 10.1016/j.ejphar.2015.07.047.
18. Jiang, J. G.; Chen, C. L.; Card, J. W.; Yang, S.; Chen, J. X.; Fu, X. N.; Ning, Y. G.; Xiao, X.; Zeldin, D. C.; Wang, D. W. *Cancer Res.* **2005**, *65*, 4707, DOI: 10.1158/0008-5472.CAN-04-4173.
19. Jiang, J. G.; Ning, Y. G.; Chen, C.; Ma, D.; Liu, Z. J.; Yang, S.; Zhou, J.; Xiao, X.; Zhang, X. A.; Edin, M. L.; Card, J. W.; Wang, J.; Zeldin, D. C.; Wang, D. W. *Cancer Res.* **2007**, *67*, 6665, DOI: 10.1158/0008-5472.CAN-06-3643.
20. Lee, B.; Wu, Z.; Sung, S. H.; Lee, T.; Song, K. S.; Lee, M. Y.; Liu, K. H. *Food Chem. Toxicol.* **2014**, *70*, 94, DOI: 10.1016/j.fct.2014.04.020.
21. Wu, Z.; Lee, B.; Song, K.-S.; Liu, K.-H. *J. Life Sci.* **2013**, *23*, 1126, DOI: 10.5352/JLS.2013.23.9.1126.
22. Lee, E.; Wu, Z.; Shon, J. C.; Liu, K. H. *Drug Metab. Dispos.* **2015**, *43*, 1250, DOI: 10.1124/dmd.115.064345.
23. Phuc, N. M.; Wu, Z.; O, Y.; Lee, J. H.; Oh, S.; Song, G. Y.; Liu, K. H. *Drug Metab. Dispos.* **2017**, *45*, 765, DOI: 10.1124/dmd.117.075036.
24. Evangelista, E. A.; Kaspera, R.; Mokadam, N. A.; Jones, J. P., 3rd; Totah, R. A. *Drug Metab. Dispos.* **2013**, *41*, 2087, DOI: 10.1124/dmd.113.053389.
25. Hwang, G. H.; Park, S. M.; Han, H. J.; Baek, K. M.; Kim, J. S.; Chang, W.; Lee, H. J.; Yun, S. P.; Ryu, J. M.; Lee, M. Y. *Oncol. Lett.* **2017**, *14*, 5484, DOI: 10.3892/ol.2017.6846.
26. Briancon-Scheid, F.; Lobstein-Guth, A.; Anton, R. *Planta Med.* **1983**, *49*, 204, DOI: 10.1055/s-2007-969851.
27. Heo, J. K.; Nguyen, P. H.; Kim, W. C.; Phuc, N. M.; Liu, K. H. *Molecules.* **2017**, *22*, 1590, DOI: 10.3390/molecules22101590.
28. Li, M.; Li, B.; Xia, Z. M.; Tian, Y.; Zhang, D.; Rui, W. J.; Dong, J. X.; Xiao, F. J. *Molecules.* **2019**, *24*, 1496, DOI: 10.3390/molecules24081496.