

Inhibitory Effects of Phenylpropanoids Isolated from the Bark of *Ailanthus altissima* on COX-2 Activity

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Prostaglandin (PG) and thromboxane biosynthesis involves the catalyzed conversion of arachidonic acid, by the sequential actions of cyclooxygenases (COXs) and prostaglandin endoperoxide synthase (PGHS), to prostaglandin H₂ (PGH₂).¹ Three isozymes of COX (COX-1, COX-2, and COX-3) are known to date, and COX-1 and COX-2 have been well defined. COX-1 is constitutively expressed in many organs or tissues, while COX-2 is induced by various stimuli.² However, recent molecular-biological studies show that this simple paradigm has many exceptions. For example, COX-1 can be regulated during development,³ whereas COX-2 is constitutively expressed in the brain⁴ and in reproductive tissues.⁵ Often, both isozymes are involved under physiological and pathophysiological conditions, whereas under other conditions they play distinctly different roles. These COX-1 and COX-2 are the key enzymes during prostaglandin (PG) biosynthesis, and the inhibition of PG synthesis is the strategy underlying current anti-inflammatory therapies. Furthermore, accumulating evidence indicates that COX-2 is involved in many inflammatory processes and in the pathogenesis of various cancers, which suggests that COX-2 plays a key role in inflammation and tumorigenesis.^{6,7} Thus, the direct inhibition of COX-2 has been actively pursued as

another potential pharmacological approach, as is exemplified by the development of COX-2 inhibitors like celecoxib.⁸

The bark of *Ailanthus altissima* Swingle (Simaroubaceae) is used in Chinese folk medicine as an astringent, antispasmodic, anthelmintic, antiparasitic, and as a narcotic.^{9,10} In the previous work, three coumarins, namely, artelin (**7**), scopoletin (**8**), and isofraxidin (**9**) were obtained from this plant.¹¹ In the present study, repeated chromatography of the MeOH extract of the bark of *A. altissima* led to the isolation of six phenylpropanoids (**1-6**) (Fig. 1). These compounds were identified as 3-hydroxy-1-(4-hydroxyphenyl)-propan-1-one (**1**),¹² *p*-coumaric acid (**2**),¹³ 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-propan-1-one (**3**),¹⁴ 2,3-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-propan-1-one (**4**),¹⁴ coniferyl alcohol (**5**),¹⁵ and coniferyl aldehyde (**6**)¹⁶ using physical and spectroscopic data (mp, ¹H-, ¹³C-NMR, and MS) and by comparison with published data.¹²⁻¹⁶ To our knowledge, this is the first report to be issued on the isolation of phenylpropanoids **1**, **3**, and **4** from the genus *Ailanthus*. Plant-derived phenylpropanoids compose the largest group of secondary metabolites produced by higher plants, and are mainly used for protection against biotic or abiotic stresses, such as, infections, wounding, UV radiation, exposure to

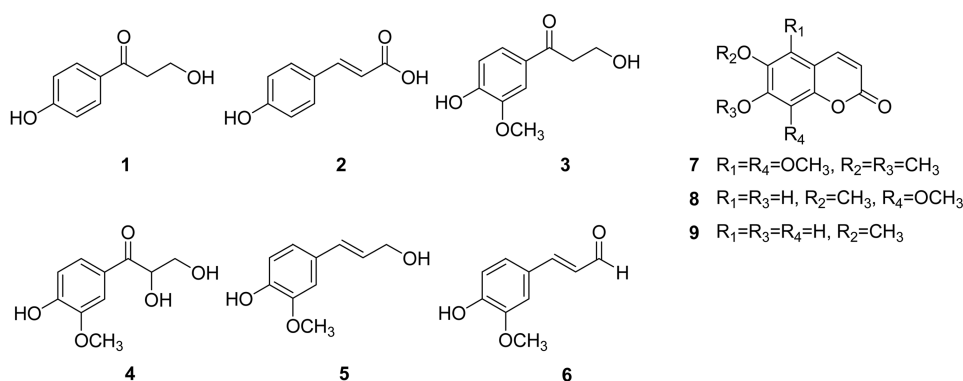


Figure 1. Structures of **1-9** isolated from the bark of *A. altissima*.

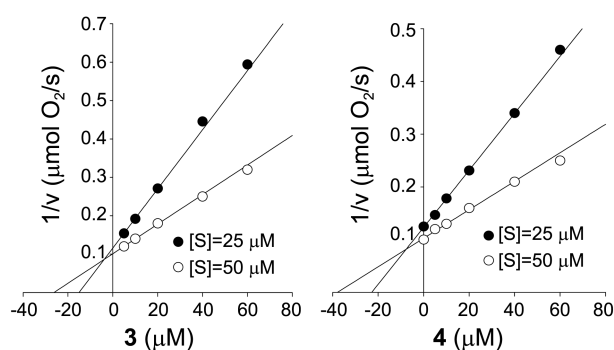


Figure 2. Dixon plots of oxygen consumption by COX-2 in the presence of **3** and **4** in Tris buffer (pH 7.0) at 37 °C.

ozone, pollutants, and herbivores.¹⁷ Some of these phenylpropanoids are considered to be biologically active and to have antibacterial, antiviral, analgesic, antispasmodic, neuroprotective, cytostatic, anti-inflammatory, and radical scavenging activities.¹⁸ Thus, phenylpropanoids are viewed as new source of natural antioxidants that are functionally related to the inhibition of cyclooxygenases activity.

During our continued efforts to identify novel COX-2 inhibitors in natural products, six phenylpropanoids (**1-6**) and three coumarins (**7-9**) from the bark of *A. altissima* were examined for COX-1 and COX-2 inhibitory activities. It was found that phenylpropanoids **1-5** have significant COX-2 inhibitory activity (Table 1), but that none of these compounds potentially inhibited COX-1 activity. In particular, **3** and **4** were found to significantly inhibit COX-2 activity and to have little effect on COX-1 activity at concentrations up to 60 μM. To explore the mechanism responsible for the inhibition of COX-2 activity, we investigated the inhibitory effects of **3** and **4** in detail. As shown in Figure 2, kinetic analysis was performed at different COX-2 (25 and 50 μM) and compound concentrations (5, 10, 20, 40, and 60 μM). Inhibition constants (K_i) were determined using Dixon plots. The inhibition mode of **3** or **4** was found to be competitive inhibition against COX-2 with a substrate in the Dixon plot (Fig. 2), which indicates that **3** and **4** bind to the active site in COX-2. The inhibition constants (K_i) of **3** and **4** were found to be 3.8 and 7.7 μM, respectively.

The inhibitory activities of **3** and **4** prompted us to perform molecular docking studies to understand the ligand-protein interactions concerned, and their COX-1 and COX-2 selectivities.¹⁹⁻²² All calculations were performed using Molegro Virtual Docker (MVD) 2010.4.2 for Windows.²³ The docking studies were carried out using the crystal structures of COX-1 (PDB code: 1EQH)²⁴ and COX-2 (code: 3LN1)²⁵ complexed with flurbiprofen and celecoxib, respectively. The active site of the enzyme was defined to include residues within a 10.0 Å radius to each inhibitor atoms. The docking wizard in MVD 2010.4.2 was used to dock the isolates **1-6** to the active sites of COX-1 and COX-2. Most stable docking models were selected according to the best Rerank score conformation predicted by the MVD scoring function (Table 1) for each crystal structure. Compound **3** was found to dock into the active site of COX-2 with a

Table 1. Enzymatic inhibitory activities and docking results for **1-9** from the bark of *A. altissima* against COX-1 and COX-2

Compound	IC ₅₀ (μM) ^a		Rerank Score (kcal/mol) ^b	
	COX-1	COX-2	COX-1 (PDB: 1EQH)	COX-2 (PDB: 3LN1)
1	> 60	58.4 ± 2.6	-65.82	-68.34
2	> 60	56.7 ± 1.8	-66.78	-65.25
3	> 60	17.3 ± 0.4	-76.44	-79.75
4	> 60	23.0 ± 0.5	-80.89	-82.03
5	> 60	57.2 ± 1.7	-74.43	-74.38
6	> 60	> 60	-72.73	-74.41
7	> 60	> 60	ND	ND
8	> 60	> 60	ND	ND
9	> 60	> 60	ND	ND
Naproxen ^c	34.0 ± 1.0	> 60	-92.52	-84.37
Celecoxib ^d	12.0 ± 0.4	0.06 ± 0.01	-7.56	-128.81

^aIC₅₀ value is the compound concentration required to produce 50% inhibition of COX-1 and COX-2 activity. ^bRerank Score in MVD2010.4.2. ^{c,d}Naproxen and celecoxib as positive controls were docked for comparison.

Rerank score of -79.75 and a score of -76.44 for COX-1. Compound **4** was found to dock into the active sites of COX-2 and COX-1 with Rerank scores of -82.03 and -80.89, respectively. Both **3** and **4** had higher Rerank scores for COX-2 and COX-1 than **1**, **2**, **5**, or **6**, but like naproxen, exhibited low COX-2 selectivity as compared with celecoxib. These docking results are consistent with those of the enzymatic experiment shown in Table 1.

In conclusion, the phenylpropanoids (**1-6**) and coumarins (**7-9**) isolated from *A. altissima* were screened for COX-1/COX-2 inhibition. Biological results showed that **3** and **4** exhibited moderate inhibitory and selective profiles against COX-2, and this was consistent with molecular docking results for **3** and **4** at the COX-2 active site. These findings suggest that new synthetic phenylpropanoid derivatives with appropriate substitutions that fill the adjunct pocket and interact with the other residues in COX-2 would be potential selective COX-2 inhibitors.

Experimental Section

Plant Material. The barks of *Ailanthus altissima* Swingle (Simaroubaceae) were collected in Jinju, South Korea. A voucher specimen (*S. W. Hwang & M. S. Yang 022*) of this raw material has been deposited at Herbarium of the Gyeongsang National University (GSNU).

Extraction and Isolation. The air-dried barks (2 kg) of *A. altissima* were extracted with MeOH (10 L × 3) at room temperature for 72 h. The combined extract was concentrated *in vacuo* to afford a brown gum (120 g), which was partitioned with CHCl₃ and water. The CHCl₃ layer was washed with brine, dried over anhydrous Na₂SO₄, and then concentrated to give a thickish residue (36 g). The residue was chromatographed on a silica gel (500 g) column eluted with a gradient of 100% CHCl₃ to 100% MeOH. Fifteen

pooled fractions (F1-F15) were obtained after combining fractions with similar TLC profiles from this initial column chromatography. Among the fractions, the fraction F8 (0.94 g) was chromatographed over silica gel as stationary phase using a *n*-hexane-EtOAc gradient (from 4:1 to 1:1 v/v) as mobile phase to afford 14 fractions (F8-1-F8-14). Compounds **6** (11 mg, $R_f=0.55$, CHCl_3 -acetone = 4:1) and **5** (30 mg, $R_f=0.35$, CHCl_3 -acetone = 9:1) were isolated from the fractions F8-7 (0.7 g) and F8-12 (0.8 g), respectively. The fraction F9 (3.2 g) was chromatographed over silica gel as stationary phase using a CHCl_3 -acetone gradient (from 99:1 to 1:1 v/v) as mobile phase to afford 12 fractions (F9-1-F9-12). Of these, the fraction F9-2 (210 mg) was chromatographed over silica gel, with CHCl_3 -acetone gradient (from 99:1 to 1:1 v/v) to isolate **3** (16 mg, $R_f=0.61$, CHCl_3 -MeOH = 9:1). The fraction F11 (1.3 g) was chromatographed over silica gel as stationary phase using a *n*-hexane-EtOAc gradient (from 3:2 to 2:3 v/v) as mobile phase to afford 8 fractions (F11-1-F11-8). Of these, fraction F11-2 (330 mg) was chromatographed over silica gel, with CHCl_3 -acetone gradient (from 99:1 to 1:1 v/v) to give **2** (18 mg, $R_f=0.53$, CHCl_3 -MeOH = 9:1). Compounds **1** (54 mg, $R_f=0.71$, CHCl_3 -MeOH = 9:1) and **4** (13 mg, $R_f=0.70$, CHCl_3 -MeOH = 9:1) were isolated from the fraction F11-3 (420 mg) and F11-6 (140 mg), respectively.

In vitro COX Inhibition Assay. Cyclooxygenase enzyme inhibitory activities of the different concentrations of each isolated compound were evaluated by using PGHS-1 enzyme vesicles (*ca.* 5 mg protein/mL in 0.1 M Tris/HCl, pH 7.8), a homogeneous protein purified from ram seminal according to the previously published procedures.²⁶ The rate of oxygen consumption during the initial phase of the enzyme-mediated reaction, with arachidonic acid as substrate was measured using a Model 5300 biological oxygen monitor (Yellow Spring Instruments, Inc., Yellow Springs, OH). Each assay mixture consisted of 0.1 M Tris/HCl, pH 7.8, 1.0 mM phenol, 17 μg hemoglobin, and 10 μM arachidonic acid. Reactions were initiated by the addition of 5-25 μg of microsomal protein in a volume of 15-50 μL . Instantaneous inhibition (in a 600 μL micro chamber; Instech Laboratory, Plymouth Meeting, PA) of enzyme activity was determined by measuring the cyclooxygenase activity initiated by adding aliquots of microsomal suspensions of PGHS-1 or PGHS-2 (10 μM O_2 /min cyclooxygenase activity/aliquot) to assay mixtures containing 10 μM arachidonic acid and various concentrations (0.1-60 μM) of the tested compounds. Data were recorded using Quicklog for Windows (Strawberry Tree Inc., Sunnyvale, CA). The percentage of inhibition was calculated with respect to the DMSO blank. Celecoxib and naproxen were used as positive controls for COX-2/COX-1 inhibitors. The efficacy of compound was determined as the concentration causing

50% enzyme inhibition (IC_{50}).

Docking Methodology. Docking studies have been performed using MVD 2010.4.2. With this purpose, crystal structures of COX-1/flurbiprofen and COX-2/celecoxib complex (PDB codes: 1EQH and 3LN1) were obtained from the Protein Data Bank in order to prepare the protein for docking studies. Docking procedure was followed using the standard protocol implemented in MVD 2010.4.2 and the geometry of resulting complexes was studied using the MVD's Pose Viewer utility.

References

1. Hawkey, C. J. *Lancet*. **1999**, 353, 307.
2. Smith, W. L.; Song, I. *Prostaglandins Other Lipid Mediat.* **2002**, 69, 115.
3. Smith, W. L.; Langenbach, R. *J. Clin. Invest.* **2001**, 12, 1491.
4. Yamagata, K.; Andreasson, K. I.; Kaufmann, W. E.; Barnes, C. A.; Worley, P. F. *Neuron*. **1993**, 11, 371.
5. Kniss, D. A. *J. Soc. Gynecol. Investig.* **1999**, 6, 285.
6. Fujimura, T.; Ohta, T.; Oyama, K.; Miyashita, T.; Miwa, K. *J. Gastrointest. Cancer* **2007**, 38, 78.
7. Mutoh, M.; Takahashi, M.; Wakabayashi, K. *Curr. Pharm. Des.* **2006**, 12, 2375.
8. Deeks, J. J.; Smith, L. A.; Bradley, M. D. *BMJ*. **2002**, 325, 619.
9. Kowarik, I.; Säumel, I. *Perspect. Plant Ecol.* **2007**, 8, 207.
10. Vincenzo, D. M.; Laura, D. M.; Emilia, Q.; Cosimo, P. *J. Agric. Food Chem.* **2003**, 51, 1177.
11. Hwang, S. W.; Lee, J. R.; Lee, J.; Kwon, H. S.; Yang, M. S.; Park, J. H. *Heterocycles* **2005**, 65, 1963.
12. Le, H. T.; Ha, D. T.; Minh, C. T. A.; Kim, T. H.; Kiem, P. V.; Nguyen, D. T.; Na, M. K. *Arch. Pharm. Res.* **2012**, 35, 87.
13. Korp, R.; Vonk, H.; Xu, X.; Hoff, W. D.; Crielard, W.; Hellingwerf, K. *J. FEBS Lett.* **1996**, 382, 73.
14. Jones, L.; Bartholomew, B.; Latif, Z.; Sarker, S. D.; Nash, R. *J. Fitoterapia* **2000**, 71, 580.
15. Quideau, S.; Ralph, J. *J. Agric. Food Chem.* **1992**, 40, 1108.
16. Lim, E. K.; Jackson, R. G.; Bowles, D. J. *FEBS Lett.* **2005**, 579, 2802.
17. Korkina, L.; Kostyuk, V.; De Luca, C.; Pastore, S. *Mini. Rev. Med. Chem.* **2011**, 11, 823.
18. Diaz, A. M.; Abad, M. J.; Fernandez, L.; Silvan, A. M.; DeSantos, J.; Bermejo, P. *Life Sci.* **2004**, 74, 2515.
19. Manivannan, E.; Chaturvedi, S. C. *Bioorg. Med. Chem.* **2011**, 19, 4520.
20. Abdel-Aziz, A. A.; ElTahir, K. E.; Asiri, Y. A. *Eur. J. Med. Chem.* **2011**, 46, 1648.
21. Gautam, R.; Jachak, S. M.; Kumar, V.; Mohan, C. G. *Bioorg. Med. Chem. Lett.* **2011**, 21, 1612.
22. Singh, P.; Bhardwaj, A.; Kaur, S.; Kumar, S. *Eur. J. Med. Chem.* **2009**, 44, 1278.
23. MVD 2010.4.2 for Windows in MolegroApS.
24. Selinsky, B. S.; Gupta, K.; Sharkey, C. T.; Loll, P. J. *Biochemistry* **2001**, 40, 5172.
25. Wang, J. L.; Limburg, D.; Graneto, M. J.; Springer, J.; Hamper, J. R.; Liao, S.; Pawlitz, J. L.; Kurumbail, R. G.; Maziasz, T.; Talley, J. J.; Kiefer, J. R.; Carter, J. *Bioorg. Med. Chem. Lett.* **2010**, 20, 7159.
26. Wang, H.; Nair, M. G.; Strasburg, G. M.; Chang, Y. C.; Booren, A. M.; Gray, I. J.; DeWitt, D. L. *J. Nat. Prod.* **1999**, 62, 294.