European Journal of Medicinal Chemistry 48 (2012) 321–329

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design, synthesis and evaluation of caffeic acid phenethyl ester-based inhibitors targeting a selectivity pocket in the active site of human aldo–keto reductase 1B10

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ARTICLE INFO

Article history:
Received 2 September 2011
Received in revised form
20 December 2011
Accepted 22 December 2011
Available online 29 December 2011

Keywords:
AKR1B10
Aldose reductase
Caffeic acid phenethyl ester
Inhibitor
Selectivity
Cancer

ABSTRACT

Inhibitors of a human aldo–keto reductase, AKR1B10, are regarded as promising therapeutics for the treatment of cancer, but those with both high potency and selectivity compared to the structurally similar aldose reductase (AKR1B1) have not been reported. In this study, we have found that, among honeybee propolis products, caffeic acid phenethyl ester (CAPE) inhibited AKR1B10 (IC_{50} = 80 nM) with 7-fold selectivity over AKR1B1. Based on a model of docked CAPE in AKR1B10, its derivatives were designed, synthesized and evaluated for inhibitory potency. Among them, 3-(4-hydroxy-2-methoxyphenyl)acrylic acid 3-(3-hydroxyphenyl)propyl ester (**10c**) was the most potent competitive inhibitor (K_i = 2.6 nM) with 790-fold selectivity for AKR1B10 over AKR1B1. Molecular docking of **10c** and site-directed mutagenesis of AKR1B10 residues suggested that the interactions between the 2-methoxy and 3-hydroxy groups of **10c** and the enzyme's Val301 and Gln114, respectively, are important for the inhibitor's selectivity. Additionally, the sub-μM concentration of **10c** significantly suppressed the farnesal metabolism and cellular proliferation in AKR1B10-overexpressing cells.

1. Introduction

A human member of the aldo–keto reductase (AKR) superfamily, AKR1B10, is a NADPH-dependent reductase, which was originally identified as a human aldose reductase (AKR1B1)-like protein that is up-regulated in hepatocellular carcinomas [1]. AKR1B10 is normally expressed in the intestine and colon with lower levels in the liver, but overexpressed in human tumors, such as smokers' non-small cell lung carcinomas [2], uterine carcinomas [3], cholangiocarcinomas [4], and early stage gastric cancer [5], as well as hepatocellular carcinomas. The silencing of the AKR1B10 gene results in growth inhibition of cancer cells [6–8] and hepatocellular carcinoma xenografts in mice [8]. Since AKR1B10 shows high catalytic efficiency towards aliphatic aldehydes, retinals and isoprenyl aldehydes [1,6,7,9–11], its roles in cell carcinogenesis and tumor development have been suggested to be detoxification of cytotoxic carbonyls derived from lipid peroxidation [6,7,9], decrease in retinoic acid synthesis [10], and regulation of cellular fatty acid synthesis and lipid metabolism [7,11], which lead to cell survival and proliferation. In addition, AKR1B10 is up-regulated in cancer cell lines resistant to anticancer drugs, pre-activated cyclophosphamide [12], and mitomycin C [13] of which the mitomycin C resistance is suggested to be mediated by the enzyme's ability to detoxify cytotoxic aldehydes. AKR1B10 catalyzes the reduction of anticancer drugs such as daunorubicin, oracin and idarubicin, which is also thought to cause the chemoresistance of cancer cells [14,15]. Thus, this enzyme has been recognized not only as a potential diagnostic and/or prognostic marker, but also as a target for the prevention and treatment of the above types of cancer [16].

Previously reported inhibitors of AKR1B10 included bile acids [11,14], synthetic aldose reductase inhibitors [10,11,17],

Abbreviations: AKR, Aldo–keto reductase; AKR1B1, Human aldose reductase; AKR1B10, A human member in the AKR1B subfamily of the AKR superfamily; CAPE, Caffeic acid phenethyl ester.

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doi:10.1016/j.ejmech.2011.12.034

anti-inflammatory agents, flavonoids [18], additional plant polyphenols [19], 9-methyl-2,3,7-trihydroxy-6-fluorone [20], chromene-3-carboxamide derivatives [21], triterpenoids [22], fibrates [23], and prostaglandin A_1 [24]. Among them, tolrestat, an aldose reductase inhibitor, and the chromene-3-carboxamide derivatives are potent inhibitors, but almost equally inhibit the structurally similar AKR1B1. While AKR1B1 is involved in diabetic complications under hyperglycaemia conditions, it also plays important roles in prostaglandin metabolism and detoxification of lipid peroxidation products and precursors of advanced glycation end products [25–27]. In this respect, selective inhibition of AKR1B10 over AKR1B1 is required for the development of anticancer agents mainly targeting AKR1B10. Although a triterpenoid, oleanolic acid, was reported to be the most selective inhibitor to AKR1B10 over AKR1B1, compared to other inhibitors its potency is not high [22]. Recent studies reported the crystal structure [28] and docked models of AKR1B10-coenzyme-inhibitor complexes [11,18–22]. The structural studies suggested several selectivity determinant residues (Gln114, Lys125, Val301, Gln303 and/or Ser304) that are not conserved in AKR1B1, forming an inhibitor selectivity pocket in the active site AKR1B10.

In order to synthesize more potent and selective inhibitors of ARK1B10, we have searched lead compounds that can be structurally modified to bind tightly and target the selectivity pocket of the enzyme. Since natural substances, cinnamic acid and its phenolic analogs, have been received attention as anticancer agents [29], we compared the inhibitory effects of cinnamic acid derivatives isolated from propolis on recombinant AKR1B10 and AKR1B1, and found that caffeic acid phenethyl ester (CAPE) is the most potent and selective inhibitor for AKR1B10. Based on the subsequent molecular docking of CAPE in AKR1B10, we designed and synthesized CAPE-based derivatives that are expected to interact more tightly with the residues in the selectivity pocket, and evaluated them for enzyme inhibitory activity. We show that a derivative, 3-(4-hydroxy-2-methoxyphenyl)acrylic acid 3-(3-hydroxyphenyl)propyl ester (**10c**) is a highly potent and selective inhibitor for AKR1B10. Additionally, we analysed **10c** for cellular efficacy and proposed the binding mode of the inhibitor in the active site of the enzyme.

2. Results and discussion

2.1. Inhibitory potency of constituents of propolis

The inhibitory effects of eight cinnamic acid derivatives isolated from Brazilian propolis on the pyridine-3-aldehyde reductase activities of AKR1B10 and AKR1B1 were compared (Table 1). The compounds, except for 3,5-dicaffeoylquinic acid AKR1B10 [30], were evaluated for the first time in this study. Among them, CAPE exhibited the lowest IC_{50} value and highest selectivity to AKR1B10 (7-fold versus AKR1B1), contrast to selectivity of the other derivatives to AKR1B1. In addition to its anticancer property [29], CAPE exhibits inhibitory effects on the activities of matrix metalloproteinase-2 and -9 [31], cyclooxygenase-1 and -2 [32], 5-lipoxygenase [33], xanthine oxidase [34], 5α-reductase type 1 and type 2 [35], catechol-O-methyltransferase [36], and HIV integrase [37]. The IC_{50} values for these enzymes are 2–82 μM, and are much higher than that for AKR1B10 (0.08 μM), suggesting that CAPE tightly binds to AKR1B10. CAPE exhibited a mixed-type inhibition with respect to pyridine-3-aldehyde in the reduction reaction by AKR1B10, and displayed a competitive inhibition with respect to the alcohol substrate, geraniol, in the reverse reaction (the K_i value was 46 ± 2 nM). The different inhibition patterns in the forward and reverse reactions were similar to other AKR1B10 inhibitors [11,18–22], including tolrestat that binds to the substrate-binding site in the crystal structure of the enzyme-$NADP^+$-inhibitor ternary complex [28]. Thus, CAPE binds to the substrate-binding site of AKR1B10, and its affinity for the enzyme-$NADP^+$ binary complex can be expressed only as the K_i value determined in the geraniol oxidation, but not as the two K_i values obtained from the mixed-type inhibition of the pyridine-3-aldehyde reductase activity. The affinity (K_i value) for CAPE is higher than those for the chromene-3-carboxamide derivatives (2.7–24 nM) [21], isolithocholic acid (15 nM), tolrestat (46 nM) [11] and bisdemethoxycurcumin (22 nM) [19], but is lower than those for other known AKR1B10 inhibitors [18–20,22].

Table 1
Inhibitory effects of cinnamic acid derivatives of propolis on activities of AKR1B10 and AKR1B1.

Compound	IC_{50} (μM)	
	AKR1B10	AKR1B1
CAPE	0.080 ± 0.010	0.57 ± 0.06
3,5-Dicaffeoylquinic acid	0.13 ± 0.01	0.088 ± 0.003
3,4-Dicaffeoylquinic acid	0.24 ± 0.01	0.078 ± 0.01
3-Caffeoylquinic acid	7.9 ± 0.3	0.30 ± 0.01
Drupanin	49 ± 6	38 ± 2
Artepillin C	53 ± 4	23 ± 1
p-Coumaric acid	63 ± 6	76 ± 3
Caffeic acid	90 ± 8	32 ± 1

2.2. Synthesis of CAPE derivatives based on its docked model

To design more potent and selective CAPE-based inhibitors, we constructed a model of docked CAPE in the AKR1B10-$NADP^+$ complex (Fig. 1). In this model, CAPE was surrounded by 16 amino acids, including the catalytic residues (Tyr49 and His111), which form hydrogen bond interactions with the 4-hydroxy group of the caffeic acid moiety. Van der Waals contacts were present between

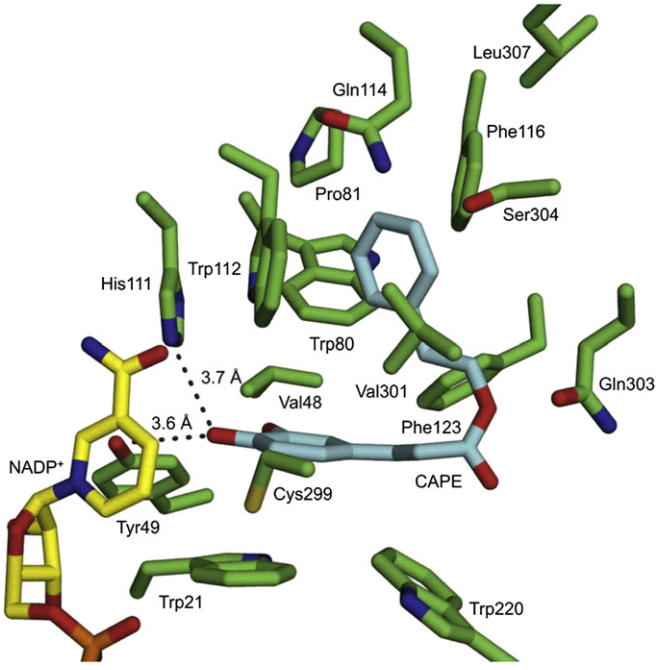


Fig. 1. Model of docked CAPE in AKR1B10-$NADP^+$ complex. The bound $NADP^+$ and the enzyme's residues within 4.0 Å from CAPE are depicted. The distances between the 4-hydroxy group of CAPE and catalytic residues (Tyr49 and His111) are given in angstroms.

Trp220 and the aromatic ring of the caffeic acid moiety (3.77 Å), which was also stacked against Trp21. The phenethyl ester moiety was positioned in the so-called selectivity pocket that is composed of non-conserved residues Gln114, Val301, Q303 and Ser304 in AKR1B10 (the respective residues in AKR1B1 are Thr, Leu, Ser and Ala, respectively).

To confirm the interaction of the 4-hydroxy group of the caffeic acid moiety suggested from the docked model, we synthesized CAPE derivatives without both (**1**) [38] and either of the 3,4-dihydroxy groups (**2** and **3**) [39]. Compound **3** with only the 4-hydroxy group retained high inhibitory potency towards AKR1B10 in contrast to marked declines in inhibition by **1** and **2**, and showed 4-fold higher selectivity (IC_{50} ratio of AKR1B1/AKR1B10) than CAPE (Table 2). To evaluate the effect of the structure of the ester moiety on the inhibitory potency, the derivatives with benzyl (**4** [40] and **5** [41]), isopropyl (**6**) [42] and phenpropyl esters (**7** and **8**) were synthesized. The derivatives (**7**–**10d**) were synthesized using the approach outlined in Scheme 1. Compounds **4**–**6** with short ester moieties were less potent inhibitors compared to CAPE and **3**, whereas **8** with the 4-hydroxy group and phenpropyl ester moiety showed greater inhibitory potency and selectivity than **3**.

The CAPE-docked model suggested that the introduction of a small hydrophobic substituent at the 2-position of CAPE is expected to enhance the inhibitor potency through the hydrophobic interaction with the side-chain of Phe123 or Val301 (Fig. 1). We synthesized 2-methoxy derivatives of **3** and **8** (**9a** and **10a**, respectively). The introduction of the methoxy group slightly improved the inhibitor potency towards AKR1B10, but significantly decreased inhibition towards AKR1B1, resulting in a great increase in selectivity.

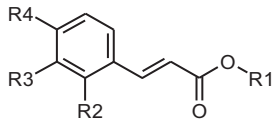
Derivatives of **9a** and **10a** with a hydroxyl group at 2-, 3- or 4-position on their ester phenyl rings were also synthesized (**9b**–**d** and **10b**–**d**), because an additional interaction between the hydroxyl group and the non-conserved residue(s) in the selectivity pocket of AKR1B10 was suggested by the CAPE-docked model. Although the introduction of the hydroxyl group into **9a** (**9b**–**d**) did not significantly improve the inhibitor potency, the 3-hydroxy derivative (**10c**) of **10a** showed the lowest IC_{50} value (6.2 nM) and highest selectivity (IC_{50} ratio of AKR1B1/AKR1B10 = 790) among the synthesized CAPE-based derivatives. In addition, 30 μM **10c** did not inhibit human aldehyde reductase (AKR1A1) that is functionally similar to AKR1B1 and AKR1B10. Compound **10c** inhibited competitively with respect to the alcohol substrate in the geraniol oxidation by AKR1B10, showing its K_i value of 2.6 nM. The K_i value is comparable to those for (Z)-2-(4-methoxyphenylimino)-7-hydroxy-N-(pyridin-2-yl)-2H-chromene-3-carboxamide [21], which had been known as the most potent AKR1B10 inhibitor. In addition, the inhibitory selectivity of **10c** is much superior to those of known potent inhibitors such as the chromene-3-carboxamide derivative, isolithocholic acid, bisdemethoxycurcumin and tolrestat, whose IC_{50} ratios of AKR1B1/AKR1B10 are 1.8, 255, 85 and 0.3, respectively [11,18,19,21].

2.3. Molecular docking and site-directed mutagenesis of inhibitor binding residues

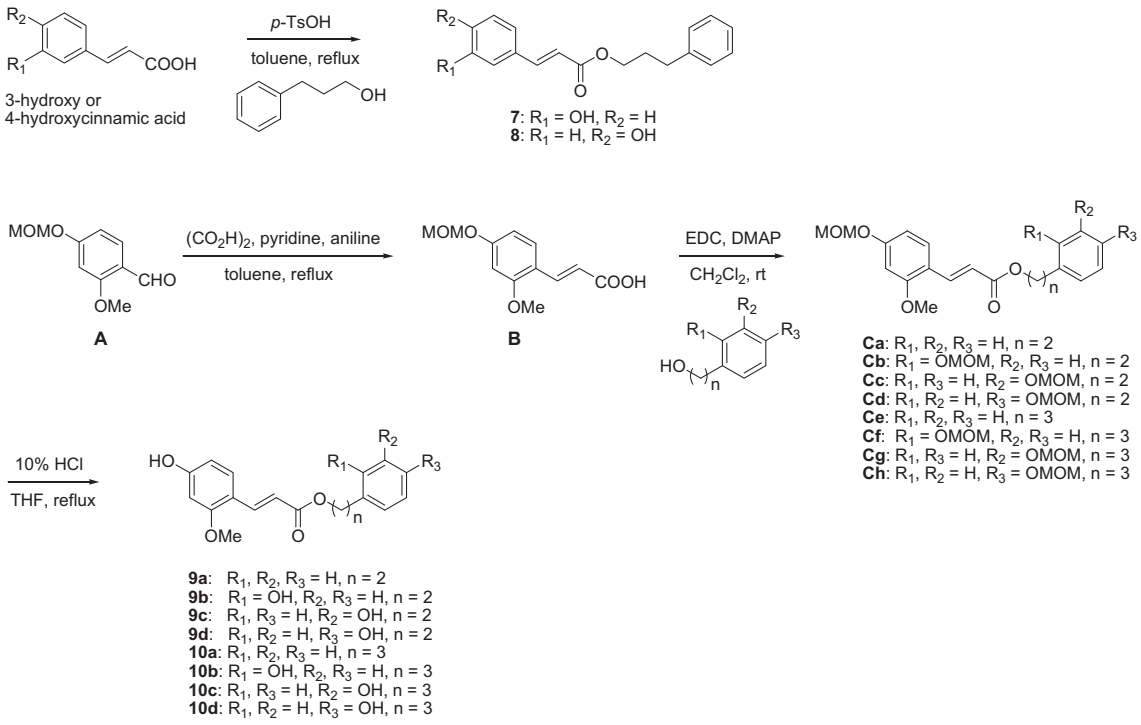
The underlying structural reason for the high inhibitory potency and selectivity of **10c** was examined by constructing a model of docked **10c** in the AKR1B10-$NADP^+$ complex (Fig. 2). In this model, the 4-hydroxy group of **10c** closely interacted with the catalytic residues, Tyr49 (3.40 Å) and His111 (2.94 Å), and thus the modeled **10c** was bound more deeply to the active site of the enzyme than the case of CAPE. The 2-methoxy group of **10c** was close (3.25 Å) to the side-chain of Val301, forming a tight hydrophobic interaction. The indole ring of Trp220 also formed hydrophobic/van der Waals interactions with the cinnamic acid moiety of **10c**. The 3-hydroxyphenpropyl moiety of **10c** occupied the selectivity pocket, and its 3-hydroxy group formed a hydrogen bond with the side-chain of Gln114 (3.19 Å). The other part of the 3-hydroxyphenpropyl moiety was surrounded by the side-chains of Gln303 (3.29 Å), Phe123 (3.42 Å) and Phe116 (3.66 Å), which interacted with this moiety through hydrophobic/van der Waals contacts.

Table 2
Inhibition of AKR1B10 and AKR1B1 by the CAPE-based derivatives.

Compound	R1[a]	R2[a]	R3[a]	R4[a]	IC_{50} (nM)		Ratio[b]
					AKR1B10	AKR1B1	
CAPE	$(CH_2)_2Ph$	H	OH	OH	80 ± 10	570 ± 60	7
1	$(CH_2)_2Ph$	H	H	H	18000 ± 2000	23000 ± 2000	1
2	$(CH_2)_2Ph$	H	OH	H	19000 ± 2000	73000 ± 4000	4
3	$(CH_2)_2Ph$	H	H	OH	69 ± 12	2200 ± 300	31
4	CH_2Ph	H	OH	OH	210 ± 10	400 ± 30	2
5	CH_2Ph	H	H	OH	130 ± 10	2200 ± 300	17
6	$CH(CH_3)_2$	H	H	OH	920 ± 10	6100 ± 70	7
7	$(CH_2)_3Ph$	H	OH	H	3100 ± 400	41000 ± 3000	13
8	$(CH_2)_3Ph$	H	H	OH	21 ± 3	1100 ± 70	52
9a	$(CH_2)_2Ph$	OMe	H	OH	13 ± 2	7200 ± 500	554
9b	$(CH_2)_2Ph(2–OH)$	OMe	H	OH	17 ± 3	9500 ± 600	558
9c	$(CH_2)_2Ph(3–OH)$	OMe	H	OH	13 ± 2	7300 ± 500	562
9d	$(CH_2)_2Ph(4–OH)$	OMe	H	OH	14 ± 1	3200 ± 400	229
10a	$(CH_2)_3Ph$	OMe	H	OH	16 ± 2	11000 ± 1000	688
10b	$(CH_2)_3Ph(2–OH)$	OMe	H	OH	9.0 ± 0.2	6900 ± 100	766
10c	$(CH_2)_3Ph(3–OH)$	OMe	H	OH	6.2 ± 0.1	4900 ± 400	790
10d	$(CH_2)_3Ph(4–OH)$	OMe	H	OH	23 ± 1	11000 ± 600	478

[a] 

[b] Selectivity, IC_{50} ratio of AKR1B1/AKR1B10.



Scheme 1. Synthesis of CAPE-based derivatives.

The above interactions suggested from the docked model were supported by the site-directed mutagenesis of the AKR1B10 residues (Gln114, Val301, and Gln303) into the corresponding residues in AKR1B1, as well as the mutations of Phe123Ala and Trp220Tyr (Table 3). All the mutations, except for Ser304Ala, resulted in 2–5 fold increases in the K_i value for **10c** compared to the wild-type enzyme. While the effects caused by these single mutations were low, that caused by the double Val301Leu and Glu303Ser mutation was large and synergistic, suggesting that at least Val301 and Gln303 have a combined role in inhibitor binding and selectivity.

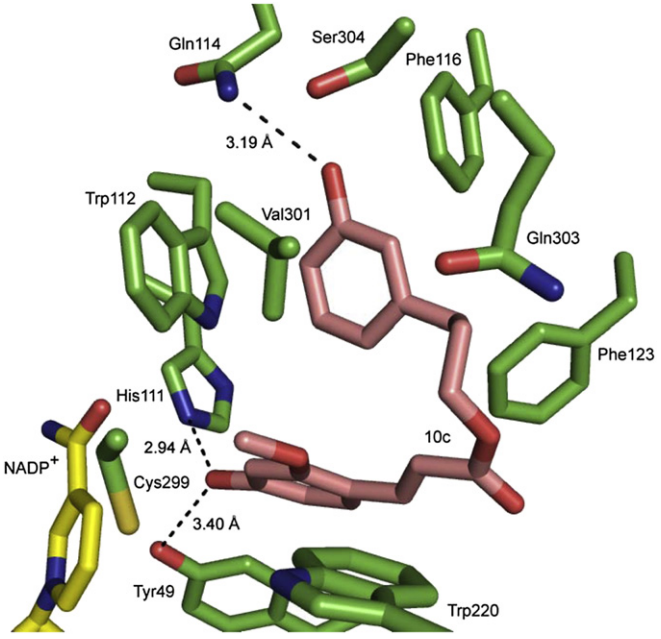


Fig. 2. Model of docked compound **10c** in the AKR1B10-NADP$^+$ complex. The bound NADP$^+$ and the enzyme's residues within 3.5 Å from **10c** are depicted. The possible hydrogen bond interactions were illustrated as dotted lines with their distance given in angstroms.

2.4. *Effect of* **10c** *on cellular metabolism and proliferation of AKR1B10-overexpressing cancer cells*

The inhibitory effects of **10c** and CAPE on AKR1B10 at a cellular level were compared in the metabolism of an isoprenyl aldehyde, farnesal, which is efficiently reduced into farnesol by AKR1B10 in vitro and in the enzyme-overexpressed HeLa cells [11]. Compound **10c** inhibited the farnesal metabolism more potently than CAPE, and was effective from 0.1 μM, showing an IC_{50} value of 0.3 μM (Fig. 3A). The IC_{50} value is lower than those of known AKR1B10 inhibitors determined under the same assay conditions (0.8–61 μM) [11,19,21,22].

As the silencing of AKR1B10 gene results in growth inhibition of cancer cells [6–8], we found that the growth of human lymphoma U937 cells was stimulated approximately 1.3-fold compared to the control cells, at 3 days after the transient transfection of the

Table 3
Effects of mutations of AKR1B10 on K_i values for compound **10c**.

Enzyme	K_i (nM)	Mu/Wt.[a]
Wild-type	2.6 ± 0.5	–
Gln114Thr	7.6 ± 0.2	3
Phe123Ala	5.1 ± 0.3	2
Trp220Tyr	15 ± 2	6
Val301Leu	5.8 ± 0.3	2
Gln303Ser	7.1 ± 0.2	3
Ser304Ala	3.5 ± 0.2	1
Val301Leu/Gln303Ser	23 ± 2	9

[a] K_i ratio of mutant enzyme/wild-type enzyme.

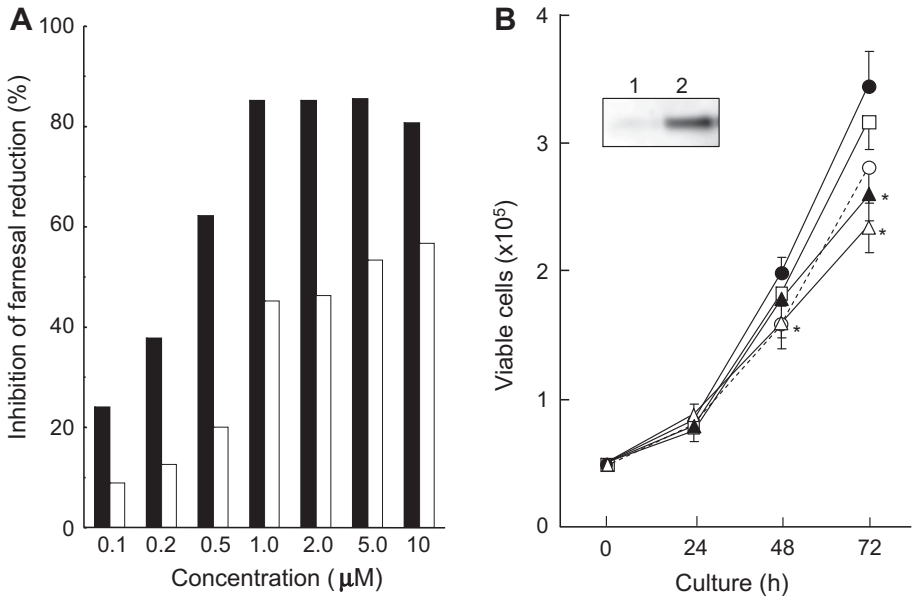


Fig. 3. Inhibitory effects of compound **10c** and CAPE on cellular AKR1B10. (A) effect on cellular farnesal reduction. The AKR1B10-expressed HeLa cells were pre-treated with the indicated concentrations of **10c** (closed bar) and CAPE (open bar) for 2 h, and then incubated with 20 μM [1-^{14}C] farnesol for 6 h. The inhibition percentages of the farnesal reduction by the inhibitors are expressed as the mean of duplicate experiments. (B) Effect on proliferation of AKR1B10-expressed U937 cells. Together with the control cells (○—○) that were transfected with the empty vector, the AKR1B10-expressed cells (5×10^4 cells/well) were incubated without (●) or with 1.0 μM CAPE (□), 0.2 μM **10c** (▲), and 1.0 μM **10c** (△), and then the viable cell numbers were estimated at the indicated times. *Significant difference between the group with 0.2 μM (▲) or 1.0 μM **10c** (△) and that without inhibitor (●), $p < 0.05$ (by statistical evaluation using the unpaired Student's *t*-test). The inset shows the Western blot analysis of AKR1B10 expression in the extracts (each 20 μg) of the control (lane 1) and AKR1B10-expressed cells (lane 2).

AKR1B10 cDNA (Fig. 3B). The transfected cells expressed AKR1B10, which was hardly detected in the control cells transfected with the vector alone. The addition of **10c** decreased the growth of the transfected cells, and its efficacy was evident at 0.2 μM and much superior to that of 1.0 μM CAPE. It should be noted that CAPE and **10c** were not cytotoxic at their low concentrations under the above assay conditions using HeLa and U937 cells. As CAPE was reported to be cytotoxic to several cancer cells [39], CAPE and **10c** showed high LD_{50} values of 56 ± 4 and 36 ± 3 μM, respectively, when HeLa cells were treated for 24 h. The results clearly indicate that the involvement of AKR1B10 in U937 cell proliferation, and suggests that usefulness of the selective inhibitor **10c** for validating the role of the up-regulated enzyme in other cancer cells.

3. Conclusion

The molecular docking of the new lead CAPE in the crystal structure of AKR1B10 has allowed the design of a novel CAPE-based inhibitor (**10c**) with improved potency ($K_i = 2.6$ nM) and selectivity (790-fold over AKR1B1). As the inhibitor was designed to target a selectivity pocket in the active site of AKR1B10, site-directed mutagenesis, together with the molecular docking of **10c** in AKR1B10, demonstrated that the high selectivity is attributed to the interactions of **10c** with Gln114, Val301 and Gln303 in the selectivity pocket. Especially, the interaction of the 2-methoxy group of **10c** with Val301 is a novel structural feature for selectivity, and may contribute to future synthesis of selective AKR1B10 inhibitors, in order to prevent possible side effects caused by inhibition of other AKR enzymes including AKR1B1. Compound **10c** significantly decreased the metabolism of farnesal in the cells with an IC_{50} value of 0.3 μM, which is superior to the IC_{50} values of the previously reported inhibitors of AKR1B10. Furthermore, the growth stimulation of U937 cells by the overexpression of AKR1B10 supports the involvement of the enzyme in proliferation of cancer cells [6–8], and the inhibition of the cell growth by 0.2 μM **10c** is indicative of its efficacy as a candidate of therapeutics for cancers, in which AKR1B10 is overexpressed.

4. Experimental

4.1. Compounds and materials

Brazilian propolis components listed in Table 1 were gifted by API Co. Ltd. (Gifu, Japan). [1-^{14}C] Farnesol was purchased from American Radiolabeled Chemicals (St. Louis, MO). Human HeLa, and U937 cells were obtained from American Type Culture Collection (Manassas, VA).

4.2. Preparation of recombinant enzymes

Recombinant AKR1B1, AKR1A1 [43] wild-type AKR1B10 with the N-terminal 6-His tag [11], and its mutant forms (Gln114Thr, Trp220Tyr, Val301Leu, Gln303Ser, Ser304Ala, and double mutant Val301Leu/Gln303Ser) [18,19] were expressed in *Escherichia coli* BL21 (DE3) pLysS cells transformed with expression plasmids harboring their cDNAs, and purified to homogeneity, as described previously. The Phe123Ala mutant enzyme of AKR1B10 was prepared by site-directed mutagenesis [19] using a sense primer (5′-AGTCTGGGGATGACCTTGCCCCCAAAGATGATAAAGG-3′) and the corresponding antisense primer, expressed in the *E. coli* cells, and purified to homogeneity as described above.

4.3. Assay of enzyme activity

The reductase and dehydrogenase activities of the enzymes were determined at 25 °C by measuring the rate of change in NADPH absorbance (at 340 nm) and fluorescence (at 455 nm with an excitation wavelength of 340 nm), respectively [11]. The IC_{50} values for inhibitors were determined in the reaction mixture that consisted of 0.1 M potassium phosphate, pH 7.4, 0.1 mM NADPH,

0.2 mM pyridine-3-aldehyde, and enzyme (AKR1B1 or AKR1B10), in a total volume of 2.0 mL. Kinetic studies in the presence of inhibitors were carried out in both pyridine-3-aldehyde reduction and $NADP^+$-linked geraniol oxidation over a range of five substrate concentrations ($0.5-5 \times K_m$) at a saturating concentration of coenzyme. The IC_{50} and K_i values are expressed as the means of at least three determinations.

4.4. Molecular docking

The coordinates for AKR1B10 (PDB code: 1ZUA) [28] were obtained from the RCSB Protein Data Bank. The structure was prepared using the Maestro (Schrödinger, LLC) software package Version 8.5, as described previously [11]. In order to eliminate any bond length and bond angle biases, the ligand (CAPE or **10c**) was subjected to a full minimization prior to the docking. The docking calculations were performed using the program Glide 5.0 [44] on a Linux workstation under the conditions described previously [11]. Figures were generated using *PyMOL* (DeLano Scientific).

4.5. Cell culture experiments

The human cancer cells (HeLa and U937) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified incubator containing 5% CO_2. The value of LD_{50} (50% lethal dose) was calculated from the viabilities of HeLa and A549 cells treated for 24 h with increasing concentrations of the for the tested compound. The cell viability was measured by a tetrazolium dye-based cytotoxicity assay using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt [45].

The transfection of the pGW1 plasmids harboring the cDNA for AKR1B10 into HeLa and U937 cells, Western blot analysis of the expressed enzyme and analysis of the metabolism of [1-^{14}C]farnesol in the HeLa cells were carried out as described previously [11]. In the experiments of growth of U937 cells, the overexpressing and control cells were suspended in fresh growth medium, seeded at a density of 5×10^4 cells/ml into a 24-well multiplate, and then treated for 0, 24, 48, 72 h with CAPE or **10c**. The number of the viable cells was estimated by the trypan blue dye-exclusion method.

4.6. Chemical synthesis

4.6.1. General

The known esters (**1**–**6**) were synthesized by literature procedure [38–42]. The esters (**7**, **8**, **9a**–**d**, and **10a**–**d**) were synthesized from corresponding carboxylic acids by Fischer esterification or condensation using EDC with appropriate alcohols, and then deprotection of the MOM group for **9a**–**d** and **10a**–**d**.

4.6.2. General procedure for Fischer esterification

To a stirred solution of carboxylic acid (1 mmol) in toluene (10 mL) were added alcohol (15 mmol), and *p*-TsOH•H_2O (0.04 mmol), and the resulting mixture was refluxed for 24 h. After cooling, the solvent was removed under reduced pressure, and the residue was chromatographed on silica gel (15 g, hexane:acetone = 20:1–15:1) to give corresponding ester.

*4.6.2.1. 3-(3-Hydroxyphenyl)acrylic acid phenpropyl ester (**7**).* Yield: 81%; mp: 77–78 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04 (2H, quint, J = 7.3 Hz), 2.75 (2H, t, J = 7.3 Hz), 4.23 (2H, t, J = 7.3 Hz), 4.19 (1H, br), 15.9 (1H, d, J = 15.9 Hz), 6.87 (1H, d-like, J = 7.8 Hz), 7.0 (1H, s), 7.11 (1H, d, J = 7.8 Hz), 7.20–7.32 (6H, m), 7.61 (1H, d, J = 15.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.17, 32.16, 64.20, 114.64, 117.68, 118.05, 120.58, 126.00, 128.37, 128.43, 130.06, 135.70, 141.09, 145.03, 156.35, 167.62; IR (KBr): 3398, 1686, 1636 cm^{-1}; MS (EI): m/z 282 (M^+); HRMS: Calcd for $C_{18}H_{18}O_3$ 282.1256, Found 282.1256.

*4.6.2.2. 3-(4-Hydroxyphenyl)acrylic acid phenpropyl ester (**8**).* Yield: 76%; mp: 82–84 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 (2H, quint, J = 7.8 Hz), 2.74 (2H, t, J = 7.8 Hz), 4.22 (2H, t, J = 7.8 Hz), 5.28 (1H, br), 6.31 (1H, d, J = 16.1 Hz), 6.85 (2H, d, J = 8.6 Hz), 7.20–7.32 (5H, m), 7.44 (2H, d, J = 8.6 Hz), 7.62 (1H, d, J = 16.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.25, 32.20, 64.07, 115.04, 115.93, 125.99, 128.40, 128.43, 130.02, 130.05, 141.16, 145.01, 158.19, 168.12; IR (KBr): 3395, 1690, 1601 cm^{-1}; MS (EI): m/z 282 (M^+); HRMS: Calcd for $C_{18}H_{18}O_3$ 282.1256, Found 282.1253.

*4.6.2.3. 3-(2-Methoxy-4-methoxymethoxyphenyl)acrylic acid (**B**).* To a stirred solution of aldehyde (**A**) [46] (1.23 g, 6.28 mmol) in toluene (15 mL) were added malonic acid (980 mg, 9.41 mmol), pyridine (0.78 mL, 9.67 mmol), and aniline (0.07 mL, 0.75 mmol), and the resulting mixture was refluxed for 24 h. After cooling, the reaction was quenched by 10% HCl. The aqueous mixture was extracted with EtOAc (15 mL × 4). The organic extracts were combined, washed with brine, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (30 g, CH_2Cl_2:MeOH = 50:1–30:1) to give **B** as a colorless solid. Yield: 97%; mp: 115–117 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.49 (3H, s), 3.88 (3H, s), 5.21 (2H, s), 6.46 (1H, d, J = 16.0 Hz), 6.60 (1H, s), 6.65 (1H, d, J = 8.5 Hz), 7.45 (1H, d, J = 8.5 Hz), 8.00 (1H, d, J = 16.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.51, 56.21, 94.23, 99.92, 107.86, 115.49, 117.10, 130.63, 142.19, 160.00, 160.64, 173.22; IR (KBr): 2723, 2602, 2519, 2299, 1697, 1610, 1576, 1504 cm^{-1}; MS (EI): m/z 194 (M^+); HRMS: Calcd for $C_{10}H_{10}O_4$ 194.0579, Found 194.0560.

4.6.3. General procedure for condensation using EDC

To a stirred solution of carboxylic acid (1 mmol) in CH_2Cl_2 (10 mL) were added EDC•HCl (1.2 mmol), DMAP (0.1 mmol), and alcohol (1.2 mmol), and the resulting mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel (15 g, hexane:acetone = 30:1–10:1) to give the corresponding ester.

*4.6.3.1. 3-(2-Methoxy-4-methoxymethoxyphenyl)acrylic acid phenethyl ester (**Ca**).* Yield: 49%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.02 (2H, t, J = 7.1 Hz), 3.49 (3H, s), 3.89 (3H, s), 4.40 (2H, t, J = 7.1 Hz), 5.20 (2H, s), 6.42 (1H, d, J = 16.1 Hz), 6.59 (1H, s), 6.64 (1H, d-like, J = 8.5 Hz), 7.24–7.34 (5H, m), 7.42 (1H, d, J = 8.5 Hz), 7.91 (1H, d, J = 16.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.26, 55.49, 56.19, 64.75, 94.24, 99.93, 107.75, 116.29, 117.40, 126.46, 128.45, 128.95, 130.17, 138.03, 140.03, 159.70, 160.24, 167.70; IR (neat): 2955, 1705, 1605 cm^{-1}; MS (EI): m/z 342 (M^+); HRMS: Calcd for $C_{20}H_{22}O_5$ 342.1467, Found 342.1466.

*4.6.3.2. 3-(2-Methoxy-4-methoxymethoxyphenyl)acrylic acid 2-(2-methoxymethoxyphenyl)ethyl ester (**Cb**).* Yield: 47%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.06 (2H, t, J = 7.2 Hz), 3.48 (6H, s), 3.87 (3H, s), 4.40 (2H, t, J = 7.2 Hz), 5.20 (2H, s), 5.23 (2H, s), 6.42 (1H, d, J = 16.1 Hz), 6.59 (1H, s), 6.64 (1H, dd, J = 8.5 Hz), 6.95 (1H, t, J = 7.3 Hz), 7.09 (1H, d, J = 7.3 Hz), 7.18–7.23 (2H, m), 7.41 (1H, d, J = 8.5 Hz), 7.89 (1H, d, J = 16.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.06, 55.45, 56.02, 56.14, 63.71, 94.24, 94.27, 99.94, 107.76, 113.75, 116.47, 117.45, 121,58, 126.80, 127.83, 130.13, 130.84, 139.84, 155.35, 159.67, 160.19, 167.74; IR (neat): 1701, 1628, 1609,1155 cm^{-1}; MS (EI): m/z 402 (M^+); HRMS: Calcd for $C_{22}H_6O_7$ 402.1679, Found 402.1685.

*4.6.3.3. 3-(2-Methoxy-4-methoxymethoxyphenyl)acrylic acid 2-(3-methoxymethoxyphenyl)ethyl ester (**Cc**).* Yield: 49%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.99 (1H, t, J = 7.1 Hz), 3.47 (3H, s), 3.49 (3H, s), 3.87 (3H, s), 4.40 (2H, t, J = 7.1 Hz), 5.17 (2H,s), 5.20 (2H, s), 6.42 (1H, d, J = 16.1 Hz), 6.59 (1H, s), 6.64 (1H, d-like, J = 8.5 Hz), 6.90–6.95 (3H, m), 7.22 (1H, t, J = 7.6 Hz), 7.42 (1H, d, J = 8.5 Hz), 7.91 (1H, d, J = 16.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.21, 55.45, 55.93, 56.17, 64.61, 94.22, 94.38, 99.90, 107.73, 114.24, 116.29, 116.89, 117.38, 122.49, 129.42, 130.18, 139.67, 140.02, 157.33, 159.69, 160.23, 167.66; IR (neat): 1713, 1606, 1573 cm^{-1}; MS (EI): m/z 402 (M^+); HRMS: Calcd for $C_{22}H_{26}O_7$ 402.1678, Found 402.1677.

*4.6.3.4. 3-(2-Methoxy-4-methoxymethoxyphenyl)acrylic acid 2-(4-methoxymethoxyphenyl)ethyl ester (**Cd**).* Yield: 41%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.96 (2H, t, J = 7.2 Hz), 3.48 (3H, s), 3.50 (3H, s), 3.87 (3H, s), 4.36 (2H, t, J = 7.1 Hz), 5.16 (2H, s), 5.20 (2H, s), 6.42 (1H, d, J = 16.2 Hz), 6.59 (1H, d, J = 2.2 Hz), 6.64 (1H, dd, J = 8.5, 2.2 Hz), 6.99 (2H, d, J = 8.5 Hz), 7.18 (2H, d, J = 8.5 Hz), 7.43 (1H, d, J = 8.5 Hz), 7.91 (1H, d, J = 16.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.38, 55.43, 55.84, 56.10, 64.86, 94.20, 94.44, 99.90, 107.74, 116.25, 117.35, 129.88, 130.09, 131.36, 139.94, 155.85, 159.65, 160.21, 167.23; IR (neat): 1703, 1628, 1609, 1151 cm^{-1}; MS (EI): m/z 402 (M^+); HRMS: Calcd for $C_{22}H_{26}O_7$ 402.1679, Found 420.1688.

*4.6.3.5. 3-(2-Methoxy-4-methoxymethoxyphenyl)acrylic acid phenpropyl ester (**Ce**).* Yield: 42%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 (2H, quint, J = 7.3 Hz), 2.75 (2H, t, J = 7.3 Hz), 3.49 (3H, s), 3.88 (3H, s), 4.21 (2H, t, J = 7.3 Hz), 5.20 (2H, s), 6.45 (1H, d, J = 16.1), 6.59 (1H, s), 6.64 (1H, d, J = 8.6 Hz), 7.19–7.31 (5H, m), 7.43 (1H, d, J = 8.6 Hz), 7.91 (1H, d, J = 16.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.39, 32.23, 55.51, 56.20, 63.57, 63.99, 94.28, 99.96, 107.77, 116.44, 117.45, 125.94, 128.40, 128.44, 130.25, 139.94, 141.37, 159.72, 160.23, 167.84; IR (neat): 1713, 1607, 1573 cm^{-1}; MS (EI): m/z 356 (M^+); HRMS: Calcd for $C_{21}H_{24}O_5$ 356.1624, Found 356.1624.

*4.6.3.6. 3-(2-Methoxy-4-methoxymethoxyphenyl)acrylic acid 3-(2-methoxymethoxyphenyl)propyl ester (**Cf**).* Yield: 49%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (2H, quint, J = 7.1 Hz), 2.77 (2H, t, J = 7.1 Hz), 3.48 (3H, s), 3.49 (3H, s), 3.87 (3H, s), 4.22 (2H, t, J = 7.1 Hz), 5.20 (2H, s), 5.21 (2H, s), 6.44 (1H, d, J = 16.1 Hz), 6.59 (1H, d, J = 2.2 Hz), 6.65 (1H, dd, J = 8.5, 2.3 Hz), 6.94 (1H, t, J = 8.5 Hz), 7.06 (1H, d, J = 7.6 Hz), 7.16 (2H, t, J = 7.6 Hz), 7.43 (1H, d, J = 8.5 Hz), 7.90 (1H, d, J = 16.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.85, 29.00, 55.47, 55.99, 56.16, 63.54, 94.25, 94.31, 99.95, 107.76, 113.80, 116.56, 117.46, 121.61, 127.22, 130.07, 130.16, 130.36, 139.77, 155.133, 159.67, 160.19, 167.84; IR (neat): 1705, 1628, 1606, 1153 cm^{-1}; MS (EI): m/z 416 (M^+); HRMS: Calcd for $C_{23}H_{28}O_7$ 416.1835, Found 416.1852.

*4.6.3.7. 3-(2-Methoxy-4-methoxymethoxyphenyl)acrylic acid 3-(3-methoxymethoxyphenyl)propyl ester (**Cg**).* Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 (2H, quint, J = 7.8 Hz), 2.72 (2H, t, J = 7.8 Hz), 3.48 (3H, s), 3.49 (3H, s), 3.89 (3H, s), 4.21 (2H, t, J = 7.8 Hz), 5.17 (2H, s), 5.20 (2H, s), 6.45 (1H, d, J = 16.1 Hz), 6.59 (1H, s), 6.64 (1H, d-like, J = 8.5 Hz), 6.86–6.89 (3H, m), 7.20 (1H, t, J = 7.8 Hz), 7.43 (1H, d, J = 8.5 Hz), 7.91 (1H, d, J = 16.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.24, 32.22, 55.49, 55.95, 56.18, 63.54, 94.25, 94.39, 99.94, 107.75, 113.67, 116.42, 117.44, 122.02, 129.37, 130.25, 139.93, 143.06, 157.35, 159.72, 160.22, 167.82; IR (neat): 2955, 1705, 1689 cm^{-1}; MS (EI): m/z 416 (M^+); HRMS: Calcd for $C_{23}H_{28}O_7$ 416.1835, Found 416.1830.

*4.6.3.8. 3-(2-Methoxy-4-methoxymethoxyphenyl)acrylic acid 3-(4-methoxymethoxyphenyl)propyl ester (**Ch**).* Yield: 67%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (2H, quint, J = 6.9 Hz), 2.69 (2H, t, J = 6.9 Hz), 3.48 (3H, s), 3.49 (3H, s), 3.88 (3H, s), 4.20 (2H, t, J = 6.9 Hz), 5.15 (2H, s), 5.20 (2H, s), 6.45 (1H, d, J = 16.1 Hz), 6.60 (1H, d, J = 2.3 Hz), 6.65 (1H, dd, J = 8.5, 2.2 Hz), 6.97 (2H, d, J = 8.5 Hz), 7.13 (2H, d, J = 8.5 Hz), 7.44 (1H, d, J = 8.5 Hz), 7.92 (1H, d, J = 16.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.52, 31.34, 55.49, 55.88, 56.17, 63.52, 94.26, 94.55, 107.77, 116.26, 116.42, 117.44, 129.34, 130.21, 134.75, 139.91, 155.47, 159.71, 160.23, 167.84; IR (neat): 1703, 1628, 1607, 1150 cm^{-1}; MS (EI): m/z 416 (M^+); HRMS: Calcd for $C_{23}H_{28}O_7$ 416.1835, Found 416.1828.

*4.6.4. General procedure for the deprotection of MOM group for **9a–d** and **10a–d***

To a stirred solution of ester (1 mmol) in THF (6 mL) was added 10% HCl (catalytic amounts), and the resulting mixture was heated at 60 °C for 24 h. After cooling, the solvent was removed under reduced pressure, and the residue was chromatographed on silica gel (15 g, hexane : acetone = 5 : 1–2 : 1) to give corresponding ester.

*4.6.4.1. 3-(4-Hydroxy-2-methoxyphenyl)acrylic acid phenethyl ester (**9a**).* Yield: 70%; mp: 113–116 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.01 (2H, t, J = 7.1 Hz), 3.86 (3H, s), 4.40 (2H, t, J = 7.1 Hz), 5.01 (1H, br), 6.38–6.40 (2H, m), 6.41 (1H, d, J = 16.3 Hz), 7.26–7.34 (5H, m), 7.38 (1H, d, J = 8.8 Hz), 7.90 (1H, d, J = 16.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.17, 55.37, 65.12, 99.10, 108.01, 114.76, 126.52, 128.43, 128.47, 128.92, 130.58, 137.81, 141.07, 159.93, 160.14, 168.90; IR (KBr): 3348, 1676, 1599 cm^{-1}; MS (EI): m/z 298 (M^+); HRMS: Calcd for $C_{18}H_{18}O_4$ 298.1205, Found 298.1196; Anal. Calcd for $C_{18}H_{18}O_4$ C, 72.47; H, 6.08. Found C, 72.29; H, 6.25.

*4.6.4.2. 3-(4-Hydroxy-2-methoxyphenyl)acrylic acid 2-(2-hydroxyphenyl) ethyl ester (**9b**).* Yield: 66%; mp: 155–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.02 (2H, t, J = 7.2 Hz), 3.86 (3H, s), 4.37 (2H, t, J = 7.2 Hz), 6.39 (1H, br), 6.42 (1H, d, J = 16.1 Hz), 6.43 (1H, s), 6.44 (1H, d, J = 9.0 Hz), 6.85–6.89 (2H, m), 7.12–7.17 (2H, m), 7.38 (1H, d, J = 9.0 Hz), 7.95 (1H, d, J = 16.1 Hz); ^{13}C NMR (100 Hz, $CDCl_3$) δ 29.46, 55.46, 62.94, 99.03, 108.13, 113.64, 113.93, 114.92, 118.89, 123.81, 127.54, 130.92, 130.55, 139.60, 155.39, 159.67, 161.48, 166.95; IR (KBr): 3385, 1674, 1599 cm^{-1}; MS (EI): m/z 314 (M^+); HRMS: Calcd for $C_{18}H_{18}O_5$ 314.1156, Found 314.1161; Anal. Calcd for $C_{18}H_{18}O_5$ C, 68.78; H, 5.77. Found C, 68.84; H, 5.59.

*4.6.4.3. 3-(4-Hydroxy-2-methoxyphenyl)acrylic acid 2-(3-hydroxyphenyl)ethyl ester (**9c**).* Yield: 80%; mp: 144–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.96 (2H, t, J = 7.1 Hz), 3.83 (3H, s), 4.39 (2H, t, J = 7.1 Hz), 5.18 (1H, br), 5.80 (1H, br), 6.38 (1H, d, J = 16.2 Hz), 6.41–6.43 (2H, m), 6.72 (1H, d-like, J = 7.7 Hz), 6.76 (1H, s-like), 6.84 (1H, d, J = 7.7 Hz), 7.18 (1H, t, J = 7.7 Hz), 7.36 (1H, d, J = 9.0 Hz), 7.91 (1H, d, J = 16.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.51, 55.49, 64.19, 99.05, 108.16, 113.32, 113.63, 113.80, 115.71, 119.45, 129.30, 130.38, 139.44, 139.76, 157.35, 159.72, 161.53, 166.96; IR (KBr): 3398, 1676, 1587 cm^{-1}; MS (EI): m/z 314(M^+); HRMS: Calcd for $C_{18}H_{18}O_5$ 314.1154, Found 314.1149; Anal. Calcd for $C_{18}H_{18}O_5$ C, 68.78; H, 5.77. Found C, 68.55; H, 5.81.

*4.6.4.4. 3-(4-Hydroxy-2-methoxyphenyl)acrylic acid 2-(4-hydroxyphenyl)ethyl ester (**9d**).* Yield: 56%; mp: 95–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.94 (2H, t, J = 7.0 Hz), 3.86 (3H, s), 4.75 (1H, br), 6.38 (1H, d, J = 16.1 Hz), 6.39–6.42 (2H, m), 6.78 (2H, d, J = 8.1 Hz), 7.38 (1H, d, J = 8.8 Hz), 7.90 (1H, d, J = 16.1 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 30.70, 55.49, 64.58, 99.04, 108.16, 113.64, 113.84, 115.14, 128.01, 129.78, 130.31, 139.66, 155.83, 159.69, 161.51, 166.94; IR (KBr): 3312, 1684, 1609, 1516 cm^{-1}; MS (EI): m/z 314 (M^+); HRMS: Calcd for $C_{18}H_{18}O_5$ 314.1154, Found 314.1157; Anal. Calcd for $C_{18}H_{18}O_5$ C, 68.78; H, 5.77. Found C, 68.69; H, 5.93.

*4.6.4.5. 3-(4-Hydroxy-2-methoxyphenyl)acrylic acid phenpropyl ester (**10a**).* Yield: 94%; mp: 89–93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 (2H, quint, $J = 7.3$ Hz), 2.75 (2H, t, $J = 7.3$ Hz), 3.87 (3H, s), 4.21 (2H, t, $J = 7.3$ Hz), 5.05 (1H, br), 6.41 (1H, d, $J = 16.1$ Hz), 6.45–6.43 (2H, m), 7.20–7.31 (4H, m), 7.39 (1H, d, $J = 9.0$ Hz), 7.90 (1H, d, $J = 16.1$ Hz); ^{13}C NMR(100 MHz, $CDCl_3$) δ 30.27, 32.14, 55.37, 63.59, 99.11, 108.01, 114.90, 115.69, 125.95, 128.38, 128.40, 130.66, 140.97, 141.17, 159.90, 160.16, 169.05; IR (KBr): 3329, 1684, 1605 cm^{-1}; MS (EI): *m*/*z* 312 (M^+); HRMS: Calcd for $C_{19}H_{20}O_4$ 312.1362, Found 312.1345; Anal. Calcd for $C_{19}H_{20}O_4$ C, 73.06; H, 6.45. Found C, 73.23; H, 6.22.

*4.6.4.6. 3-(4-Hydroxy-2-methoxyphenyl)acrylic acid 3-(2-hydroxyphenyl) propyl ester (**10b**).* Yield: 62%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04 (2H, quint, $J = 7.0$ Hz), 2.75 (2H, t, $J = 7.0$ Hz), 3.87(3H, s), 4.23 (2H, t, $J = 7.0$ Hz), 4.98 (1H, br), 5.09 (1H, br), 6.42–6.43 (2H, m), 6.44 (1H, d, $J = 16.3$ Hz), 6.77 (1H, d, $J = 7.8$ Hz), 6.88 (1H, t, $J = 7.8$ Hz), 7.10 (1H, t, $J = 7.8$ Hz), 7.14 (1H, d, $J = 7.8$ Hz), 7.40 (1H, d, $J = 9.3$ Hz), 7.92 (1H, t, $J = 16.3$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.17, 28.40, 55.49, 63.38, 99.06, 108.16, 113.68, 114.01, 114.89, 118.89, 126.96, 127.27. 129.78, 130.39, 139.59, 155.13, 159.72, 161.47, 167.10; IR (neat): 3421, 1684, 1609 cm^{-1}; MS (EI): *m*/*z* 328 (M^+); HRMS: Calcd for $C_{19}H_{20}O_5$ 328.1311, Found 328.1307.

*4.6.4.7. 3-(4-Hydroxy-2-methoxyphenyl)acrylic acid 3-(3-hydroxyphenyl)propyl ester (**10c**).* Yield: 60%; mp: 104–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 (2H, quint, $J = 7.2$ Hz), 2.69 (2H, t, $J = 7.2$ Hz), 3.83 (3H, s), 4.21 (2H, t, $J = 7.2$ Hz), 6.41 (1H, d, $J = 16.1$ Hz), 6.43–6.45 (2H, m), 6.68 (1H, d, $J = 8.9$ Hz), 6.70 (1H, s), 6.77(1H, d, $J = 7.2$ Hz), 7.15 (1H, t, $J = 7.2$ Hz), 7.36(1H, d, $J = 8.9$ Hz), 7.91(1H, d, $J = 16.1$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 29.83, 31.44, 55.49, 63.03, 99.05, 108.16, 112.85, 113.64, 113.89, 115.17, 118.92, 129.27, 130.40, 139.67, 142.58, 157.35, 159.72, 161.53, 167.08; IR (KBr): 3362, 1670, 1609 cm^{-1}; MS (EI): *m*/*z* 328 (M^+); HRMS: Calcd for $C_{19}H_{20}O_5$ 326.1311, Found 328.1306; Anal. Calcd for $C_{19}H_{20}O_5$ C, 69.50; H, 6.14. Found C, 69.51; H, 6.01.

*4.6.4.8. 3-(4-Hydroxy-2-methoxyphenyl)acrylic acid 3-(4-hydroxyphenyl)propyl ester (**10d**).* Yield: 86%; ^{1}H NMR (400 MHz, $CDCl_3$) δ1.99 (2H, quint, $J = 6.6$ Hz), 2.67 (2H, t, $J = 6.6$ Hz), 3.86 (3H, s), 4.20 (2H, t, $J = 6.6$ Hz), 4.74 (1H, br), 5.30 (1H, br), 6.42 (2H, m), 6.43 (1H, d, $J = 16.1$ Hz), 6.77 (2H, d-like, $J = 8.5$ Hz), 7.08 (2H, d-like, $J = 8.5$ Hz), 7.39 (1H, d-like, $J = 9.0$ Hz), 7.90 (1H, d, $J = 16.1$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 30.23, 30.58, 55.49, 63.01, 99.05, 108.15, 113.67, 113.93, 115.11, 129.13, 130.39, 131.15, 139. 63, 155.42, 159.72, 161.48, 167.09; IR (neat): 3383, 1684, 1607 cm^{-1}; MS (EI): *m*/*z* 328 (M^+); HRMS: Calcd for $C_{19}H_{20}O_5$ 328.1311, Found 328.1308.

Acknowledgments

This work was partly founded by grant-in-aid for Young Scientists (B) and Scientific Research (C) from the Japan Society for the Promotion of Science, Sasakawa Scientific Research Grant from Japan Science Society, and scholarship from Monash University Graduate Research School.

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