Pergamon

Tetrahedron 58 (2002) 1667–1671

TETRAHEDRON

Dipiperamides A, B, and C: bisalkaloids from the white pepper *Piper nigrum* inhibiting CYP3A4 activity

Sachiko Tsukamoto, Bae-Cheon Cha and Tomihisa Ohta*

Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-0934, Japan

Received 8 November 2001; accepted 9 January 2002

Abstract—Three new bisalkaloids, dipiperamides A, B, and C, were isolated from the white pepper (*Piper nigrum* L.), along with the known piperine and piperylin. Dipiperamides A–C inhibited cytochrome P450 (CYP) 3A4 activity.

1. Introduction

Pepper (*Piper nigrum* L.) is used as a spice worldwide, and previously phytochemical studies have revealed 600 constituents classified in various structural categories, such as terpenes, steroids, lignans, flavones, and alkaloids/amides.[1] Piperine (**1**),[2] a main constituent of pepper, displayed a variety of pharmacological activities, e.g. antifungal,[3] antidiarrhoeal,[4] antiinflammatory,[5] as well as 5-lipoxygenase and cyclooxygenase-1 inhibitory activities.[6]

Cytochrome P450 (CYP) enzymes are heme-containing monooxygenases and constitute three families, CYP1, CYP2, and CYP3.[7] The majority of these enzymes have been expressed in liver microsomes, and are recognized to be responsible for drug metabolism, degradation of xenobiotics, and carcinogenesis. CYPs are also responsible for the biosynthesis of steroids, lipids, and other secondary metabolites. In human liver microsomes CYP3A4 is the most abundant enzyme; approximately 30% of the total CYP was suggested to be CYP3A4.[8] Recent investigations have shown that more than 50% of clinically used drugs are oxidized by CYP3A4.[9,10] It is reported that concomitant oral administration of several natural products affect drug metabolism in humans by inhibiting CYP3A4 activity. In particular, grapefruit juice is well known to alter the pharmacokinetics of various drugs, including cyclosporine,[11,12] midazolam,[13] dihydropyridine-type calcium channel blockers,[14] and triazolam.[15] We have succeeded in the identification of furocoumarin dimers as specific CYP3A4 inhibitors.[16–18]

Piperine (**1**) is known to elevate the serum level of drugs[19,20] and nutrients[21] by inhibiting the metabolism by CYP3A4.[22] During our research on CYP inhibitors from the diet, it was discovered that a polar fraction from an EtOAc extract of white pepper showed remarkable activity compared to piperine (**1**). Bioassay-monitored purification afforded three new alkaloids **2**–**4** whose structures were elucidated by spectroscopic methods to be piperine/piperylin-derived bisalkaloids. This paper describes the isolation, structure elucidation, and CYP inhibitory activity of these alkaloids (Chart 1).

2. Results and discussion

The white pepper (1.0 kg) was refluxed in EtOAc and acetone. The bioassay-monitored isolation of the combined extracts by silica gel chromatography afforded piperine (**1**, 33.0 g) and a more potent fraction. The purification of this active fraction by a combination of silica gel and ODS chromatography and reverse-phase HPLC afforded three new bisalkaloids, dipiperamides A (**2**, 10.4 mg), B (**3**, 16.0 mg), and C (**4**, 4.5 mg), along with the known piperylin (**5**, 3.0 g).[2]

The EI mass spectrum of alkaloid **2** showed a molecular ion peak at *m/z* 570 and the formula of $C_{34}H_{38}N_2O_6$ was established by HREIMS. The presence of 19 hydrogens and 17 carbons shown in the NMR spectra, and an intense ion peak at *m/z* 285 ($M^+/2$, 100%), suggested the symmetrical nature of **2**. The ^1H and ^{13}C NMR data of **2** (Table 1) revealed the presence of a methylenedioxyphenyl group (C-6–C-11, OCH_2O), an α,β-unsaturated carbonyl group (C-1–C-3), and a piperidine ring (C-1′–C-6′), which were almost superimposable on those of piperine (**1**)[2] except for two connected methine groups (δ_H 3.72 (td, J=8.8, 8.3 Hz, H-4) and 3.80 (t, J=8.8 Hz, H-5); δ_C 45.2 (d, C-4) and 46.6 (d, C-5)). The *E*-geometry of the double bond was substantiated by the coupling constant (J=15.2 Hz). Interpretation of the COSY and HMBC data

Keywords: bisalkaloids; pepper; cytochrome P450 3A4 inhibitor.
* Corresponding author. Tel./fax: +81-76-234-4417;
e-mail: ohta@dbs.p.kanazawa-u.ac.jp

0040–4020/02/$ - see front matter
PII: S0040-4020(02)00048-0

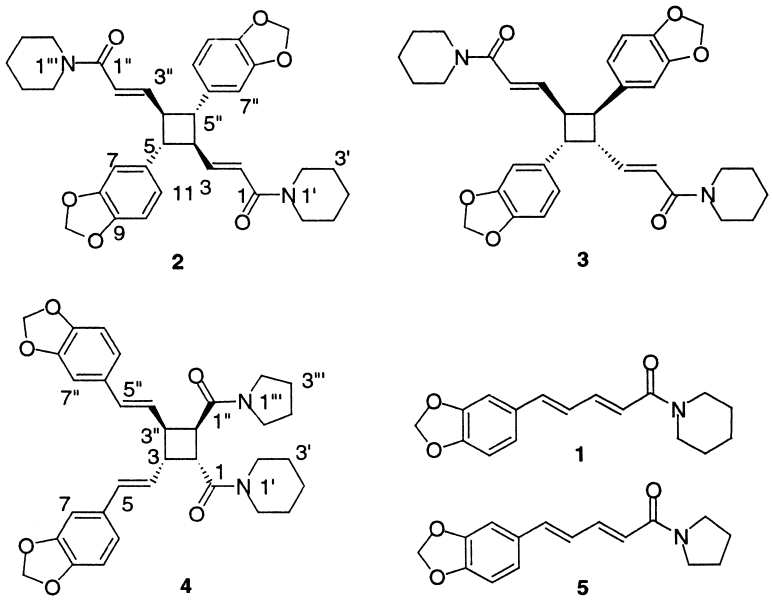


Chart 1.

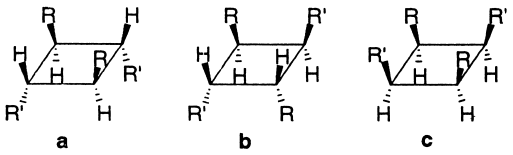
indicated that the α,β-unsaturated carbonyl and methylenedioxyphenyl units could be connected through the C_2 unit (C-4–C-5), which led to the half-unit of the molecule in which the piperidine ring could be bonded to the carbonyl carbon (C-1) through formation of an amide linkage. The multiplicity of H-4 and H-5 implied that **2** contained a cyclobutane ring (–C-4–C-5–C-4″–C-5″–) with a head-to-tail (truxillic) structure; a symmetrical dimer of piperine (**1**). Alkaloid **3** showed the same molecular formula as **2**, and the ^{1}H and ^{13}C NMR signals were almost superimposable on those of **2**, except for the upfield shift of H-4 and H-5. These spectral data suggested that **3** was also a symmetrical dimer of piperine (**1**) with the head-to-tail structure, but having a different stereochemistry. Three types of possible symmetrical dimers **a**–**c** can be deduced for **2** and **3** as shown in Scheme 1; serious steric hindrance, however, may be expected in the type **c**, which can be eliminated. In the NOE difference spectra, a NOE between H-2 and H-3″ was observed for **2** as shown in Fig. 1, but not for **3**, which indicated that the relative stereochemistry of **2** and **3** could be assigned to the types **a** and **b**, respectively. The methine hydrogens H-4 and H-5 of **2** were observed at lower field compared to **3** (δ 3.72 (H-4) and 3.80 (H-5) for **2**; δ 3.01 (H-4) and 3.15 (H-5) for **3**), which was due to the greater steric hindrance for the substituents on the cyclobutane ring in **2**, whereas the methine carbons C-4 and C-5 of **2** resonated at higher field (δ 45.2 and 46.6 for **2**; δ 50.5 and 48.5 for **3**).

The EI mass spectrum of alkaloid **4** exhibited a molecular ion peak at *m/z* 556, which matched the formula $C_{33}H_{36}N_2O_6$. The ^{1}H and ^{13}C NMR signals were typical for piperine (**1**) and piperylin (**5**), except for H-2, H-3, H-2″, and H-3″ (C-2, C-3, C-2″, and C-3″) (Table 2).

Table 1. NMR Spectral data for dipiperamides A (**2**) and B (**3**) in $CDCl_3$

	2			**3**		
	δ_H	*J* (Hz)	δ_C	δ_H	*J* (Hz)	δ_C
1, 1″			165.2 s			164.9 s
2, 2″	6.11 (2H)	d, 15.2	122.2 d	6.25 (2H)	d, 15.1	120.6 d
3, 3″	6.60 (2H)	dd, 15.2, 8.3	143.5 d	7.00 (2H)	dd, 15.1, 8.8	145.4 d
4, 4″	3.72 (2H)	td, 8.8, 8.3	45.2 d	3.01 (2H)	q, 8.8	50.5 d
5, 5″	3.80 (2H)	t, 8.8	46.6 d	3.15 (2H)	t, 8.8	48.5 d
6, 6″			133.5 s			135.4 s
7, 7″	6.72 (2H)	d, 2.0	108.3[a] d	6.73 (2H)	d, 1.5	107.1 d
8, 8″			147.8 s			147.9 s
9, 9″			146.2 s			146.4 s
10, 10″	6.74 (2H)	d, 7.8	108.2[a] d	6.74 (2H)	d, 7.8	108.3 d
11, 11″	6.67 (2H)	dd, 7.8, 2.0	120.9 d	6.67 (2H)	dd, 7.8, 1.5	119.8 d
2′, 2‴	3.26 (4H)	br s	46.9 t	3.41 (4H)	br s	46.9 t
3′, 3‴	1.45[a] (4H)	br s	26.5[a] t	1.55 (4H)	m	25.5[a] t
4′, 4‴	1.60 (4H)	br s	24.6 t	1.63 (4H)	m	24.6 t
5′, 5‴	1.50[a] (4H)	br s	25.5[a] t	1.55 (4H)	m	26.6[a] t
6′, 6‴	3.50 (4H)	br s	43.0 t	3.59 (4H)	br s	43.2 t
OCH_2O×2	5.93 (4H)	s	100.9 s	5.93 (4H)	s	101.0 s

[a] May be interchangeable.



Scheme 1.

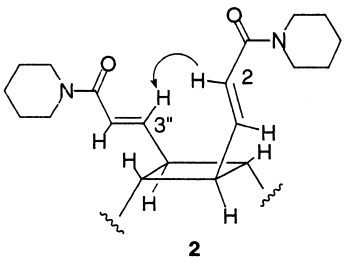


Figure 1. A NOE correlation observed for dipiperamide A (**2**).

Extensive 2D experiments led to the presence of two methylenedioxyphenyl units (A and C), a piperine ring (B), a piperylin ring (D), and two carbonyl groups in the molecule (Fig. 2). A COSY spectrum suggested correlations of δ 3.56 (t, J=8.8 Hz)/δ 3.08 (q, J=8.8 Hz)/δ 2.97 (q, J=8.8 Hz)/δ 3.58 (t, J=8.8 Hz) assignable to H-2–H-3–H-3″–H-2″ forming a cyclobutane ring. HMBC cross peaks H-2/C-4 and C-2″, H-3/C-4, H-4/C-3 and C-3″, H-5/C-3, H-2″/C-2 and C-4″, H-3″/C-4″, H-4″/C-3 and C-3″, H-5″/C-3″ implied that the units A and C were bonded to the cyclobutane ring at C-3 and C-3″. The remaining units, B and D, and the above unit could be connected through the carbonyl groups at C-2 and C-2″, respectively (Fig. 2), which was confirmed by the presence of intense ion peaks at m/z 271 and 285 corresponding to the subunits of **4** containing pyrrolidine and piperidine rings, respectively. Thus, the gross structure of **4** was established as shown. The relative stereochemistry of the cyclobutane ring was deduced by NOE difference spectra. NOE's were observed for H-3/H-2, H-5, and H-4″ and H-3″/H-4, H-2″, and H-5″, as shown in Fig. 3.

Table 2. NMR spectral data for dipiperamide C (**4**) in $CDCl_3$

	δ_H	J (Hz)	δ_C
1, 1″			170.8 s
			170.3 s
2	3.56	t, 8.8	43.6 d
2″	3.58	t, 8.8	42.6 d
3	3.08	q, 8.8	45.7 d
3″	2.97	q, 8.8	46.5 d
4	6.10	dd, 15.4, 8.8	128.6 d
4″	6.14	dd, 15.4, 8.8	128.8 d
5	6.35	d, 15.4	131.2 d
5″	6.37	d, 15.4	130.7 d
6, 6″			131.43 s
			131.38 s
7, 7″	6.88	d, 1.5	105.6 d
	6.87	d, 1.5	105.5 d
8, 8″			148.0 (2C) s
9, 9″			147.13 s
			147.08 s
10, 10″	6.72 (2H)	d, 8.0	108.3 (2C) d
11, 11″	6.75	dd, 8.0, 1.5	120.9 (2C) d
	6.74	dd, 8.0, 1.5	
OCH_2O×2	5.931 (2H)	s	101.0 (2C) s
	5.928 (2H)	s	
2′	3.48	m	45.9 t
	3.45	m	
3′	1.55 (2H)	m	26.0[a] t
4′	1.65 (2H)	m	24.5 t
5′	1.55 (2H)	m	25.7[a] t
6′	3.69	m	43.2t
	3.38	m	
2‴	3.64	m	46.6[a] t
	3.45	m	
3‴	1.82 (2H)	m	24.3 t
4‴	1.87 (2H)	m	26.8 t
5‴	3.48	m	46.7[a] t
	3.34	m	

[a] May be interchangeable.

Dipiperamides A–C (**2**–**4**) are presumably generated by [2π+2π] cycloadditions of two molecules of piperine (**1**) for **2** and **3**, and of **1** and piperylin (**5**) for **4**. Since the alkaloids (**2**–**4**) are optically inactive, which were confirmed by the flat CD curves, a cyclization enzyme may not be involved in the biosynthesis of them. In order to confirm if the bisalkaloids were artificial products during the extraction and isolation process, we extracted the white pepper with acetone at room temperature, followed by the isolation of the bisalkaloids. The bisalkaloids might be generated during the process of producing white pepper, in which the seeds of *P. nigrum* was soaked in water and dried in the sun. This was supported by the report that piplartine-dimer A, isolated from *Piper tuberculatum*, was generated by irradiation of piplartine with sunlight.[23] Although five bisalkaloids have been previously isolated from *Piper* sp.,[23–25] **2**–**4** are the first pepper bisalkaloids showing significant biological activity.

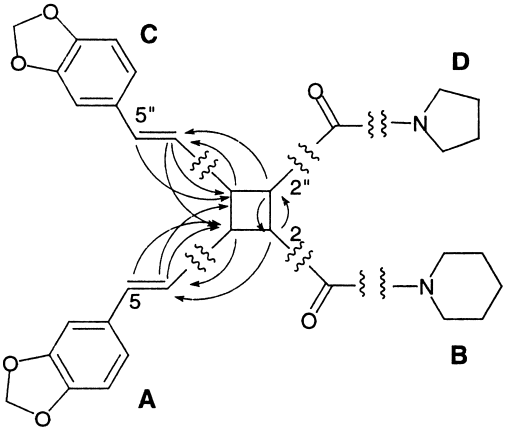


Figure 2. HMBC correlations observed for dipiperamide C (**4**).

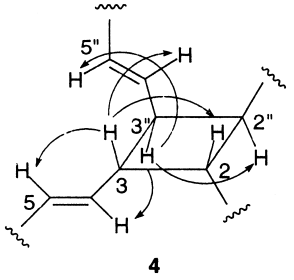


Figure 3. Selected NOE correlations observed for dipiperamide C (**4**).

Table 3. CYP3A4 inhibition of pepper metabolites

Alkaloids	IC_{50} (μM)
1	17.2
2	0.18
3	0.45
4	0.48
5	3.55
Ketoconazole[a]	0.11

[a] A typical CYP3A4 inhibitor.[27]

CYP3A4 activity was monitored by nifedipine oxidation with expressed human CYP3A4.[16] Dipiperamides A–C (**2**–**4**) exhibited the significantly potent inhibition of CYP as shown in Table 3; alkaloid **2** was one hundred times more active than the parent compound piperine (**1**). Recently, Singh et al. reported the structure–activity relationships (SAR) of piperine and its synthetic analogues for CYP inhibitory activity, and found that saturation of the side chain resulted in enhancement of CYP inhibition.[26] However, the inhibition of CYP by the new alkaloids **2**–**4** was more significant than that of the analogues reported. Since the regulation of drug metabolites is important for pharmacokinetics of drugs and their bioavailability, the activities of **2**–**4** are noteworthy concerning the SAR of CYP.

3. Experimental

3.1. General

UV spectra were measured on a SHIMADZU UV-1600 UV–visible spectrophotometer. IR spectra were recorded on a SHIMADZU IR-460 infrared spectrophotometer. Optical rotations were determined with a HORIBA SEPA-300 high sensitive polarimeter. NMR spectra were recorded on a JEOL GSX500 NMR spectrometer in $CDCl_3$. All chemical shifts were reported with respect to $CDCl_3$ (δ_H 7.26, δ_C 77.0). Mass spectra were measured on a JEOL SX-102 mass spectrometer. Expressed human CYP3A4 was purchased from Gentest Corporation.

3.2. Extraction and isolation

White pepper (*P. nigrum* L.) cultivated in Malaysia was a gift from a Japanese spice company, House Foods Corporation. The pepper (1.0 kg) was refluxed in EtOAc (1.2 L) for 1 h, and then in acetone (1.2 L) for 1 h twice. The combined extracts (65.7 g; IC_{50} value of CYP3A4 inhibition, 1.5 μg/mL) were subjected to silica gel chromatography eluting with hexane/EtOAc, EtOAc, EtOAc/MeOH (1:1), and MeOH. The fraction (35.0 g) eluted with hexane/EtOAc (1:1) afforded piperine (**1**, 33.0 g, 3.3%; IC_{50}, 4.9 μg/mL, 17.2 μM), which was crystallized in the eluent. The fraction (2.1 g) eluted with AcOEt/MeOH (1:1) and MeOH, which showed more potent CYP3A4 inhibitory activity (IC_{50}, 0.8 μg/mL) than piperine, was purified by silica gel chromatography with hexane/acetone to afford three active fractions. The first fraction (300 mg, IC_{50}, 6.6 μg/mL) was eluted with hexane/acetone (2:1) to give piperylin (**5**, 3.0 g, 0.30%; IC_{50}, 1.0 μg/mL, 3.55 μM). The alkaloids **1** and **5** were identified on the basis of their ^1H NMR spectra.[2] The second fraction (300 mg, IC_{50}, 0.4 μg/mL) eluted with hexane/acetone (2:1 and 1:1) was purified by ODS chromatography eluting with 80% MeOH/H_2O, followed by reverse-phase HPLC with 75% MeOH/H_2O to afford dipiperamide C (**4**, 4.5 mg, 4.5×10^{-4}%; IC_{50}, 0.27 μg/mL, 0.48 μM). The third fraction (640 mg, IC_{50}, 1.3 μg/mL) eluted with hexane/acetone (1:1) was subjected to silica gel chromatography with $CHCl_3$/MeOH and ODS chromatography with 70% MeOH/H_2O to afford dipiperamides A (**2**, 10.4 mg, 1.0×10^{-3}%; IC_{50}, 0.10 μg/mL, 0.18 μM) and B (**3**, 16.0 mg, 1.6×10^{-3}%; IC_{50}, 0.26 μg/mL, 0.45 μM).

3.2.1. Dipiperamide A (2). $[\alpha]_D^{25}=0°$ (*c* 0.085, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 203.5 (4.9), 238.0 (4.4, sh), 287.5 nm (4.0); IR ($CHCl_3$) ν_{max} 3000, 2940, 2850, 1700, 1650, 1600, 1490, 1440, 1360, 1250, 1230, 1040, 930 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) see Table 1. HMBC cross peaks: H-2/C-1, C-4; H-3/C-1, C-4; H-4/C-2, C-3, C-5, C-6; H-5/C-3, C-4, C-6, C-7, C-11; H-7/C-4, C-9, C-11; H-10/C-6, C-8; H-11/C-4, C-7, C-9. EIMS (%) *m/z* 570 (M^+) (4), 485 (4), 458 (6), 285 (100), 201 (80); HREIMS *m/z* 570.2731 ($C_{34}H_{38}N_2O_6$, Δ +0.1 mmu).

3.2.2. Dipiperamide B (3). $[\alpha]_D^{25}=0°$ (*c* 0.095, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 204.0 (4.8), 237.0 (4.4, sh), 273.0 (4.3, sh), 289.0 nm (4.2, sh); IR ($CHCl_3$) ν_{max} 3000, 2940, 2860, 1700, 1650, 1600, 1500, 1440, 1350, 1250, 1040, 930 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) see Table 1. HMBC cross peaks: H-2/C-1, C-4; H-3/C-1, C-5; H-4/C-2, C-3, C-5, C-6; H-5/C-3, C-4, C-6, C-7, C-11; H-7/C-6, C-9, C-11; H-10/C-8; H-11/C-7, C-9. EIMS (%) *m/z* 570 (M^+) (2%), 556 (2), 485 (3), 458 (6), 285 (100), 201 (70); HREIMS *m/z* 570.2740 ($C_{34}H_{38}N_2O_6$, Δ +1.0 mmu).

3.2.3. Dipiperamide C (4). $[\alpha]_D^{25}=0°$ (*c* 0.095, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 207.5 (4.6), 268.5 (4.3), 278.0 (4.2, sh), 308.5 (4.1), 325.0 nm (3.8, sh); IR ($CHCl_3$) ν_{max} 3000, 2930, 2880, 1720, 1710, 1700, 1650, 1620, 1500, 1460, 1440, 1340, 1250, 1230, 1040, 960, 930 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) see Table 2. HMBC cross peaks: H-2/C-3, C-4, C-2″; H-3/C-2, C-4, C-3″; H-4/C-3, C-6, C-3″; H-5/C-3, C-7, C-11; H-7, H-7″/C-9, C-11, C-9″, C-11″; H-10, H-10″/C-6, C-8, C-6″, C-8″, H-11, H-11″/C-9, C-9″; H-2‴/C-3‴, C-4‴; H-3‴/C-2‴, C-4‴, C-5‴; H-4‴/C-3‴. EIMS (%) *m/z* 556 (M^+) (25), 485 (18), 471 (27), 458 (6), 444 (8), 285 (78), 271 (68), 201 (100); HREIMS *m/z* 556.2574 ($C_{33}H_{36}N_2O_6$, Δ +0.0 mmu).

3.3. Assay of CYP inhibition

CYP activity was based on nifedipine oxidation.[16] Various amounts (0–10 μM, final concentration) of samples were incubated at 37°C for 5 min in 100 mM phosphate buffer (pH 7.4) containing 50 μM nifedipine, 5 mM glucose-6-phosphate, 0.5 mM β-$NADP^+$, 0.5 mM $MgCl_2$, and 4.3 μg/mL glucose-6-phosphate dehydrogenase. CYP3A4 (5.6 pmol) was also preincubated at 37°C for 5 min in 7 μL of the buffer and added to the sample solution. After incubation at 37°C for 1 h, the reaction was terminated by the addition of 100 μL of MeOH. After adding 3.7 μg of 6-methoxycarbonyl-5-methyl-7-(2-nitrophenyl)-4,7-dihydrofuro[3,4-*b*]pyridin-1-(3*H*)-one in DMSO (1 μL) as an internal standard, the reaction mixture was extracted with ether

(1 mL), and the ether layer was evaporated. The residue was dissolved in 100 μL of MeOH, and an aliquot (20 μL) was analyzed by reverse-phase HPLC (column, TSK-gel ODS-120T, 4.6 mm i.d.×150 mm; mobile phase, 64% MeOH/H_2O; flow rate, 1.0 mL/min; detection, UV 254 nm); retention times: 2.9 min for the internal standard, 4.0 min for the nifedipine metabolite (nifedipine pyridine), and 5.5 min for nifedipine. The value of IC_{50}, the concentration required for 50% inhibition of CYP3A4 activity, was calculated from the data of duplicate measurements.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan, the Hayashi Memorial Foundation for Female Natural Scientists, the Fugaku Trust for Medicinal Research, the Yamazaki Spice Foundation, and the Naito Foundation.

References

1. Parmar, V. S.; Jain, S. C.; Bisht, K. S.; Jain, R.; Taneja, P.; Jha, A.; Tyagi, O. D.; Prasad, A. K.; Wengel, J.; Olsen, C. E.; Boll, P. M. *Phytochemistry* **1997**, *46*, 597–673.
2. Kiuchi, F.; Nakamura, N.; Tsuda, Y.; Kondo, K.; Yoshimura, H. *Chem. Pharm. Bull.* **1988**, *36*, 2452–2465.
3. Navickiene, H. M. D.; Alecio, A. C.; Kato, M. J.; Bolzani, V.da S.; Young, M. C. M.; Cavalheiro, A. J.; Furlan, M. *Phytochemistry* **2000**, *55*, 621–626.
4. Bajad, S.; Bedi, K. L.; Singla, A. K.; Johri, R. K. *Planta Med.* **2001**, *67*, 284–287.
5. Mujumdar, A. M.; Dhuley, J. N.; Deshmukh, V. K.; Raman, P. H.; Naik, S. R. *Jpn. J. Med. Sci. Biol.* **1990**, *43*, 95–100.
6. Stohr, J. R.; Xiao, P.; Bauer, R. *J. Ethnopharmacol.* **2001**, *75*, 133–139.
7. Nelson, D. R.; Koymans, L.; Kamataki, T.; Stegeman, J. J.; Feyereisen, R.; Waxman, D. J.; Waterman, M. R.; Gotoh, O.; Coon, M. J.; Estabrook, R. W.; Gunsalus, I. C.; Nebert, D. W. *Pharmacogenetics* **1996**, *6*, 1–42.
8. Shimada, T.; Yamazaki, H.; Mimura, M.; Inui, Y.; Guengerish, F. P. *J. Pharmacol. Exp. Ther.* **1994**, *270*, 414–423.
9. Guengerish, F. P. *Adv. Pharmacol.* **1997**, *43*, 7–35.
10. Rendic, S.; DiCarlo, F. J. *Drug Metab. Rev.* **1997**, *29*, 413–580.
11. Ducharme, M. P.; Warbasse, L. H.; Edwards, D. J. *Clin. Pharmacol. Ther.* **1995**, *57*, 485–491.
12. Yee, G. C.; Stanley, D. L.; Pessa, L. J.; Dalla, C. T.; Beltz, S. E.; Ruiz, J. *Lancet* **1995**, *345*, 955–956.
13. Kupferschmidt, H. H.; Ha, H. R.; Ziegler, W. H.; Meier, P. J.; Krahenbuhl, S. *Clin. Pharmacol. Ther.* **1995**, *58*, 20–28.
14. Bailey, D. G.; Arnold, J. M.; Spence, J. D. *Clin. Pharmacokinet.* **1994**, *26*, 91–98.
15. Hukkinen, S. K.; Varhe, A.; Olkkola, K. T.; Neuvonen, P. J. *Clin. Pharmacol. Ther.* **1995**, *58*, 127–131.
16. Fukuda, K.; Ohta, T.; Oshima, Y.; Ohashi, N.; Yoshikawa, M.; Yamazoe, Y. *Pharmacogenetics* **1997**, *7*, 391–396.
17. Tassaneeyakul, W.; Guo, L.-Q.; Fukuda, K.; Ohta, T.; Yamazoe, Y. *Arch. Biochem. Biophys.* **2000**, *378*, 356–363.
18. Guo, L.-Q.; Fukuda, K.; Ohta, T.; Yamazoe, Y. *Drug Metab. Dispos.* **2000**, *28*, 766–771.
19. Zutshi, R. K.; Singh, R.; Zutshi, U.; Johri, R. K.; Atal, C. K. *J. Assoc. Phys. India* **1985**, *33*, 223–224.
20. Bano, G.; Amala, V.; Raina, R. K.; Zutshi, U.; Chopra, C. L. *Planta Med.* **1987**, *53*, 568–569.
21. Badmaev, V.; Majeed, M.; Norkus, E. *Nutr. Res.* **1999**, *19*, 381–388.
22. Wilkinson, G. R. *Adv. Drug Delivery Rev.* **1997**, *27*, 129–159.
23. Filho, R. B.; De Souza, M. P.; Mattos, M. E. O. *Phytochemistry* **1981**, *20*, 345–346.
24. Dhar, K. L.; Shah, S.; Prabhakar, A.; Sharma, R. L. *Fitoterapia* **1995**, *66*, 390–392.
25. Fujiwara, Y.; Naithou, K.; Miyazaki, T.; Hashimoto, K.; Mori, K.; Yamamoto, Y. *Tetrahedron Lett.* **2001**, *42*, 2497–2499.
26. Koul, S.; Koul, J. L.; Taneja, S. C.; Dhar, K. L.; Jamwal, D. S.; Singh, K.; Reen, R. K.; Singh, J. *Bioorg. Med. Chem.* **2000**, *8*, 251–268.
27. Guo, L.-Q.; Taniguchi, M.; Xiao, Y.-Q.; Baba, K.; Ohta, T.; Yamazoe, Y. *Jpn. J. Pharmacol.* **2000**, *82*, 122–129.