Eur. J. Med. Chem. 34 (1999) 1077–1083

Short communication

Structure-activity relationships of quaternary protoberberine alkaloids having an antimalarial activity

Kinuko Iwasa[a]*, Yumi Nishiyama[a], Momoyo Ichimaru[a], Masataka Moriyasu[a], Hye-Sook Kim[b], Yusuke Wataya[b], Takao Yamori[c], Turuo Takashi[d], Dong-Ung Lee[e]

[a]*Kobe Pharmaceutical University, 4-19-1 Motoyamakita, Higashinada-ku, Kobe 658-8558, Japan*
[b]*Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700-8530, Japan*
[c]*Division of Experimental Chemotherapy, Cancer Chemotherapy Center, 1-37-1 kamiikebukuro, Toyoshima-ku, Tokyo 170-8455, Japan*
[d]*Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 yayoi, bunkyo-ku, Tokyo 113-0000, Japan*
[e]*Department of Biochemistry, College of Natural Science, Dongguk University, Kyongju Kyongbuk 780-714, Korea*

(Received 20 April 1999; revised 28 June 1999; accepted 30 June 1999)

Abstract – Seventeen quaternary protoberberine alkaloids related to berberine **1** were tested for antimalarial activity in vitro against *Plasmodium falciparum* and structure-activity relationships are proposed. The activity of the protoberberine alkaloids was influenced by the type of the oxygen substituents on rings A, C and D and the position of the oxygen functions on ring D. The position of the oxygen functions on ring D and the type of the oxygen substituents at the C-13 position (ring C) strongly influenced the activity. Shifting the oxygen functions at C-9 and C-10 to C-10 and C-11 on ring D resulted in a significant increase in the activity. Compounds bearing a methylenedioxy function at C-2 and C-3 (ring A) or C-9 and C-10 (ring D) showed higher activity than those which have methoxy groups at the same positions. Introduction of a methoxy group into the C-1 position (ring A) decreased the activity. Replacement of a hydroxy group at C-2 or C-3 (ring A) by a methoxy group led to a reduction in the activity. Displacement of a hydroxy function at C-13 (ring C) by the oxygen substituents such as OMe, OEt, OCOOEt, and $OCON(Me)_2$ reduced the activity. In the same replacement at C-9 (ring D), the activity depended upon the type of the oxygen function. Six protoberberines displayed more potent activity than berberine **1**. The activity decreased in the order: **10**, **11**, **17** and **18** > **7** and **8** > **1**. © 1999 Éditions scientifiques et médicales Elsevier SAS

in vitro antimalarial activity / structure-activity relationships / protoberberinium salts

1. Introduction

In Japan, and other Asian countries, berberine **1** and the extracts of *Phellodendri cortex* (Obaku in Japanese) and *Coptidis rhizoma* (Oren in Japanese) are used in the treatment of diarrhoea and other gastrointestinal diseases. Berberine and its relatives exhibit several types of biological activity [1]. We found that some of the 8- and 13-alkylderivatives of berberine **1** and palmatine **2** possessed antimicrobial and antimalarial activities [2–6]. We have recently shown that a 13-hydroxy derivative of **1** had similar antimalarial activity to that of **1** [6].

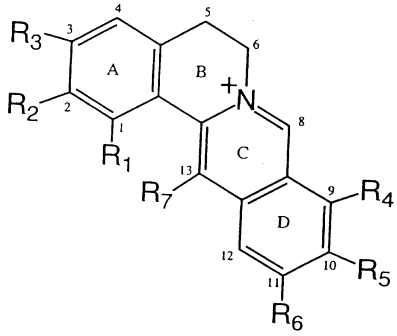
In the present study, the antimalarial activity of 17 protoberberinium salts, in which the type and/or position of the oxygen substituents such as hydroxy, methylenedioxy, methoxy, ethoxycarbonyloxy, and *N*, *N*-dimethylcarbamoyloxy functions on the aromatic rings A, C and D are different from **1** or **2** (*table I*), were examined using the selectivity indices as an indication of the activity. The results on the study of the structure-antimalarial activity relationships of the tested alkaloids are discussed.

2. Chemistry

The protoberberinium salts **3**–**13** and **17**–**20** have been prepared or isolated from natural sources previously [2, 5, 7, 8]. Palmatrubine **14** was prepared according to the method applied to the synthesis of **5** [2]. The carboethoxy

*Correspondence and reprints

Table I. Protoberberinium salts.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
1	H	OCH_2O		OMe	OMe	H	H
2	H	OMe	OMe	OMe	OMe	H	H
3	H	OCH_2O		OMe	OMe	H	Me
4	H	OMe	OMe	OMe	OMe	H	Me
5	H	OCH_2O		OH	OMe	H	H
6	OMe	OCH_2O		OMe	OMe	H	H
7	H	OCH_2O		OCH_2O		H	H
8	H	OCH_2O		OCH_2O		H	Me
9	H	OMe	OMe	OCH_2O		H	Me
10	H	OCH_2O		H	OMe	OMe	H
11	H	OCH_2O		H	OMe	OMe	Me
12	H	OMe	OH	OMe	OMe	H	H
13	H	OH	OMe	OMe	OMe	H	Me
14	H	OMe	OMe	OH	OMe	H	H
15	H	OCH_2O		OCOOEt	OMe	H	H
16	H	OCH_2O		$OCON(Me)_2$	OMe	H	H
17	H	OCH_2O		OMe	OMe	H	OH
18	H	OCH_2O		OMe	OMe	H	O^-
19	H	OCH_2O		OMe	OMe	H	OMe
20	H	OCH_2O		OMe	OMe	H	OEt
21	H	OCH_2O		OMe	OMe	H	OCOOEt
22	H	OCH_2O		OMe	OMe	H	$OCON(Me)_2$

Table III. Mass spectral data and melting points of the protoberberinium salts (**15**, **16**, **21** and **22**).

Compound	M.p. °C (dec)	Formula	LSIMS *m/z* $[M - Cl]^+$	HR-LSIMS calcd.	HR-LSIMS found
15	160–166	$C_{22}H_{20}NO_6$	394	394.1289	394.1310
16	210–214	$C_{22}H_{21}N_2O_5$	393	393.1449	393.1458
21	155–158	$C_{23}H_{22}NO_7$	424	424.1395	424.1404
22	194–200	$C_{23}H_{23}N_2O_6$	423	423.1556	423.1565

and *N*,*N*-dimethylcarbamoyl derivatives of berberrubine **5** and 13-hydroxyberberine **17** (**15** and **21**, and **16** and **22**, respectively) were prepared by the reaction with ethyl chloroformate and *N*,*N*-dimethylcarbamoyl chloride, respectively, in the presence of triethylamine or pyridine. ^{1}H-NMR (*table II*), LSIMS (*table III*), and HR-LSIMS (*table III*) data were consistent with the assigned structures.

3. Results and discussion

Protoberberinium salts **6**–**22** were tested in vitro against human malaria *Plasmodium falciparum* FCR-3. The antimalarial activity of each compound was determined as a percentage reduction. The compound concentration required to inhibit cell growth by 50% was expressed as IC_{50} (*table IV*). From the evaluation of the toxicity of the compounds for mammalian cells, the concentration causing a 50% reduction of cell growth (GI_{50}) of mouse mammary FM3A cells, a model of the host, was determined (*table IV*). The GI_{50}/IC_{50} ratios for the compounds were calculated as selectivity indices (*table IV*). These ratios were used as an evaluation of antimalarial activity. The results are presented in *table IV*.

Table II. ^{1}H-NMR[a] data of the protoberberinium salts **14–16**, **21** and **22**.

	C-9 or C-13 $OCOOCH_2CH_3$		C-9 or C-13 $OCON(CH_3)_2$		H-5	H-6	2-OMe	3-OMe	10-OMe	9-OMe	OCH_2O	H-4	H-1	H-11	H-12	H-13	H-8
14					3.25	4.87	4.00	3.92	4.05			7.00	7.58	7.96	7.71	8.65	9.73
					2H, t (6.0)	2H, t (6.0)	3H, s	3H, s	3H, s			1H, s	1H, s	1H, d (9.0)	1H, d (9.0)	1H, s	1H, s
15	4.40	1.43			3.26	4.94			4.11		6.11	6.97	7.69	8.24*	8.21*	8.82	9.79
	2H, q (7.0)	3H, t (7.0)			2H, t (6.5)	2H, t (6.5)			3H, s		2H, s	1H, s	1H, s	1H, d (9.0)	1H, d (9.0)	1H, s	1H, s
16			3.09	3.31	3.26	4.95			4.09		6.11	6.97	7.68	8.19*	8.18*	8.80	9.73
			3H, s	3H, s	2H, t (6.5)	2H, t (6.5)			3H, s		2H, s	1H, s	1H, s	1H, d (9.0)	1H, d (9.0)	1H, s	1H, s
21	4.31	1.31			3.21	4.95			4.13*	4.24*	6.12	7.03	7.67	8.19*	8.01*		9.88
	2H, q (7.0)	3H, t (7.0)			2H, t (6.0)	overlap			3H, s	3H, s	2H, s	1H, s	1H, s	1H, d (9.0)	1H, d (9.0)		1H, s
22			2.98	3.42	3.20	4.92			4.12*	4.22*	6.11	7.00	7.63	8.17	7.96		9.84
			3H, s	3H, s	2H, t (6.0)	overlap			3H, s	3H, s	2H, s	1H, s	1H, s	1H, d (9.0)	1H, dd (9.0, 0.5)		1H, d (0.5)

[a]Coupling constants (Hz in parentheses). *Assignments may be interchanged.

Table IV. In vitro antimalarial activity of protoberberinium salts **1**–**22**.

	50% growth inhibition (μM)		Selectivity indexes	Distribution of selectivity indexes		
	Plasmodium falciparum	mouse mammary cells	mouse mammary cells/ *Plasmodium falciparum*	human tumour cells/ *Plasmodium falciparum*		
	FCR-3	FM3A		the number (selectivity indexes: GI_{50}[a]/IC_{50})		
	IC_{50}	GI_{50}	GI_{50}/IC_{50}	< 150	150–300	> 300
1	0.27[b]	> 12[b]	> 44[b]	27	7	4 (> 370)
2	6.4[b]	> 27[b]	> 4.0b	38		
3	2.1[b]	> 11[b]	> 5.2[b]	38		
4	7.0[b]	> 25[b]	> 3.5[b]	38		
5	2.2[b]	5.0[b]	2.3[b]	38		
6	0.60	12	20			
7	0.25	14	56	23	7	2 (300–400) 6 (> 400)
8	0.26	> 14	> 54			
9	0.50	> 14	> 28			
10	0.18	> 19	>106	6	7	1 (300–400) 5 (400–550) 19 (> 634)
11	0.29	> 15	> 52			
12	0.67	> 17	> 25			
13	1.0	> 16	> 16			
14	0.90	5.0	5.6			
15	2.2	4.0	2.0			
16	1.9	> 41	> 22			
17	0.56	> 38	> 68			38 (> 454)
18	0.78	> 41	> 53			
19	2.4	20	12			
20	0.084	0.16	2			
21	0.68	17	25			
22	5.3	37	7			
Quinine	0.11	100	910	20 (< 38)		17 (> 1100)

[a]GI_{50} values presented in *table V*. [b]These data have been previously reported [6].

Introduction of a methoxy group at the C-1 position (ring A) of **1** caused a decrease in antimalarial activity (compare **6** with **1**) (*table IV*). Replacement of methoxy groups at the C-9 and C-10 positions (ring D) of **1** or **3** by a methylenedioxy group increased the activity (compare **7** with **1** or **8** with **3**) (*table IV*) and the same replacement at the C-2 and C-3 positions (ring A) of **9** resulted in an increase in the activity (compare **9** with **8**) (*table IV*).

Shifting methoxy groups at C-9 and C-10 of **1** or **3** to C-10 and C-11 caused a significant increase in the activity (compare **10** with **1** or **11** with **3**) (*table IV*). Displacement of a methoxy group at C-3 (ring A) of **2** by a hydroxy function increased the activity and the same replacement at the C-2 position (ring A) of **4** also increased the activity (compare **12** with **2** and **13** with **4**) (*table IV*). Substitution of a methoxy group at C-9 (ring D) of **2** by a hydroxy group increased the activity (compare **14** with **2**) (*table IV*), though the same displacement at C-9 of **1** resulted in a decrease in the activity (compare **5** with **1**) as shown in the previous data [6]. Replacement of a hydroxy group at C-9 (ring D) of **5** by OCOOEt did not notably change its activity (compare **15** with **5**) (*table IV*), whereas replacing a hydroxy function at C-9 of **5** by $OCON(Me)_2$ caused an increase in the activity (compare **16** with **5**) (*table IV*).

We have found that the inhibitory effect of 13-hydroxyberberine **17** was comparable to that of berberine **1** [6]. However, the inhibitory effects of phenolbetaine form **18** have been reported to be much less than that of **1** [9]. Thus, a comparison of the inhibitory effect between **17** and **18** was carried out. As a result, both **17** and **18** displayed a similar inhibitory effect in vitro. Substitution of a hydroxy group at the C-13 position of **17** by OMe or OEt or OCOOEt or $OCON(Me)_2$ showed a reduction in the activity (compare **19**–**22** with **17**) (*table IV*).

Among the tested salts, **7**, **8**, **10**, **11**, **17** and **18** exhibited much more potent activity (selectivity index over 50) than berberine **1**.

The selectivity indexes (*table IV*) of **1**, **7**, **10** and **17** were also determined using the 50% growth-inhibitory concentration (GI_{50}, in table *V*) derived from the results [10] of in vitro cytotoxity tests on 38 human tumour

Table V. Cytotoxicities of protoberberinium salts **1**–**5**, **7**, **10** and **17** against various human cell lines.

Human tumour cell line	Compounds / Cytotoxicity (GI_{50} in μM)[a]								
	1	**2**	**3**	**4**	**5**	**7**	**10**	**17**	Quinine
Breast									
HBC-4	4.4	76	5.1	33	35	19	17	b	b
BSY-1	5.2	27	2.8	12	23	10	7.1	b	35
HBC-5	30	b	29	99	39	11	79	b	b
MCF-7	24	b	10	50	20	12	27	b	55
MDA-MB-231	50	b	47	b	56	39	b	b	12
CNS									
U251	6.9	55	5.7	76	28	39	35	b	b
SF-268	7.7	48	14	91	30	27	27	b	b
SF-295	4.8	b	1.8	b	27	b	b	b	b
SF-539	23	b	17	81	25	37	b	b	37
SNB-75	30	b	30	b	22	5.9	42	b	b
SNB-78	35	b	37	55	26	52	b	b	b
Colon									
HCC2998	20	b	16	96	51	38	44	b	76
KM-12	4.3	71	4.6	35	27	7.6	33	b	b
HT-29	41	b	31	b	43	79	b	b	41
WiDr	42	b	34	b	35	53	b	b	
HCT-15	b	b	b	b	50	b	b	b	b
HCT-116	30	b	27	b	32	37	b	b	43
Lung									
NCI-H23	8.2	b	7.1	62	48	18	81	b	87
NCI-H226	8.1	58	14	40	19	36	b	b	29
NCI-H522	5.8	28	4.3	28	37	12	16	b	100
NCI-H460	12	b	16	b	11	19	b	b	86
A549	41	b	77	b	22	48	b	b	b
DMS273	5.9	b	4.2	40	25	20	56	b	52
DMS114	3.9	31	2.1	25	42	6.0	20	b	85
Melanoma									
LOX-IMVI	33	b	25	b	32	40	b	b	31
Ovarian									
OVCAR-3	6.2	46	5.2	34	44	21	29	b	b
OVCAR-4	31	b	22	b	47	36	77	b	b
OVCAR-5	39	b	29	b	31	31	b	b	72
OVCAR-8	17	b	14	b	40	32	91	b	b
SK-OV-3	b	b	b	b	49	b	b	b	b
Renal									
RXF-631L	59	b	64	b	26	b	b	b	b
ACHN	b	b	b	b	17	b	b	b	79
Stomach									
St-4	b	b	83	b	36	b	b	b	30
MKN1	76	b	42	b	38	93	b	b	74
MKN7	26	b	32	84	31	4.2	39	b	38
MKN28	14	95	13	76	27	11	b	b	79
MKN45	28	b	28	b	35	24	34	b	b
MKN74	16	53	16	b	31	9.8	96	b	71

[a]The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition. GI_{50} values were not presented previously [10].
[b]GI_{50} value is > 100 μM.

cell lines (seven lung, six colon, six CNS, six stomach, five ovarian, five breast, two renal, and one melanoma), the hosts for FM3A cells. The selectivity indexes of inactive **2–5** and the antimalarial drug quinine were also determined as the control experiments. 13-Hydroxyberberine **17** and compound **10** displayed a

significant activity, coptisine **7** less potent activity, berberine **1** much less activity, and compounds **2**–**5** inactivity.

4. Conclusion

From the structure-activity point of view, some features can be pointed out. On the basis of the results derived from examinations with protoberberinium salts, it appears that the position and the type of the oxygen substituents on rings A, C and D had an influence on the antimalarial activity. The activity was strongly influenced by the position of the oxygen functions on ring D and the type of the oxygen substituents at the C-13 position (ring C). Compounds with the oxygen functions at C-10 and C-11 on ring D displayed more activity than those which bear the same substituents at C-9 and C-10. Compounds bearing a methylenedioxy function at C-2 and C-3 or C-9 and C-10 on ring A or D showed higher activity than those which have methoxy groups at the same position. Introduction of a methoxy group into the C-1 position on ring A caused a decrease in the activity. Replacement of a hydroxy group at C-2 or C-3 on ring A by a methoxy group led to a reduction in the activity. Displacement of a hydroxy function at C-13 on ring C by an OMe, OEt, OCOOEt or $OCON(Me)_2$ function decreased the activity. In the same replacement at C-9 on ring D, the activity depended on the type of the oxygen substituents. Among the potent protoberberinium salts under investigation, the activity decreased in the order: **10**, **11**, **17** and **18** > **7** and **8** > **1**. The most active compound may be comparable with the antimalarial drug quinine in activity. Studies on in vivo antimalarial activity of the most potent alkaloids are in progress.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Yanako Micromelting Point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian VXR-500 (500 MHz) spectrometer using tetramethylsilane as an internal standard and CD_3OD as solvent. Mass spectra were determined on a Hitachi M-4100 instrument. The secondary ion mass spectra (LSIMS) were measured using glycerol as matrix. Preparative HPLC and HPLC analyses were performed on a Hitachi M-6250 Intelligent Pump (6 mL/min) and a Hitachi M-6200 Intelligent Pump (1 mL/min), respectively, and a Hitachi L-4000 UV detector (280 nm). Analyses were made on a Cosmosil $5C_{18}$-AR reversed-phase column (20 i.d. × 250 mm or 4.6 i.d. × 150 mm) eluting with H_2O (0.05% TFA)/MeOH (0.05% TFA), A/B, initial (30% of B), 20 min (100% of B).

Berberine **1** and palmatine **2** were purchased. Protoberberinium salts **3** [7], **4** [7], **5** [2], **6** [5], **7** [5], **9**–**11** [5], and **17**–**20** [5] had previously been prepared. Corysamine **8** was obtained by oxidation of tetrahydrocorysamine [11] isolated from *Corydalis incisa* Pers.

Columbamine **12** [8] and dehydrocorybulbine **13** [8] were natural products isolated from *C. turtschaninovii* Besser forma *yanhusuo* Y.H. Chou and C.C. Hsu.

5.1.1. Preparation of palmatrubine **14**

Palmatine (1 g) was heated at 240–250 °C in a dry oven under vacuum (20–30 mm Hg) for 10 min. The crude product was recrystallized from EtOH to give **14** (870 mg, 90%), m.p. 288–295 °C (dec.); LSIMS *m/z* 338. For ^{1}H-NMR data see *table II*.

5.1.2. Preparation of the ethoxycarbonyl derivative of berberrubine **15**

A solution of ClCOOEt (1.2 g) in $CHCl_3$ (3 mL) was added dropwise to a mixture of berberrubine **5** (300 mg) [2] and $(Et)_3N$ (900 mg) in $CHCl_3$ (50 mL). The mixture was stirred at room temperature for 30 min and evaporated in vacuo. $(Me)_2CO$ was added to the residue and the resulting crystals were collected, which recrystallized from MeOH-$(Me)_2CO$ to give **15** (229 mg, 64%). For ^{1}H-NMR, LSIMS, and HR-LSIMS data and melting point see *tables II* and *III*.

5.1.3. Preparation of N,N-dimethylcarbamoyl derivative of berberrubine **16**

A solution of $(Me)_2NCOCl$ (0.7 mL) in $CHCl_3$ (2 mL) was added dropwise to a mixture of berberrubine **5** (200 mg) and $(Et)_3N$ (0.5 mL) in $CHCl_3$ (50 mL). The mixture was stirred at room temperature for 5 h. To the reaction mixture $(Me)_2NCOCl$ (0.5 mL) was further added and the mixture was allowed to stir at room temperature overnight. $(Me)_2NCOCl$ (0.5 mL) was added to the solution which was further stirred overnight. Solvent was removed under reduced pressure and $(Me)_2CO$ was added to the residue. The resulting crystals were recrystallized from MeOH-$(Me)_2CO$ to give **16** (196 mg, 82%). For ^{1}H-NMR, LSIMS, and HR-LSIMS data and melting point see *tables II* and *III*.

5.1.4. Preparation of ethoxycarbonyl derivative of 13-hydroxyberberine **21**

A solution of ClCOOEt (0.7 mL) in $CHCl_3$ (2 mL) was added dropwise to a mixture of 13-hydroxyberberine **17** (200 mg) [5] and $(Et)_3N$ (0.5 mL) in $CHCl_3$ (20 mL). The

mixture was stirred at room temperature for 1 h and evaporated in vacuo. $(Me)_2CO$ was added to the residue and the resulting crystals were recrystallized from H_2O-MeOH to give **21** (100 mg, 46%). For ^{1}H-NMR, LSIMS, and HR-LSIMS data and melting point see *tables II* and *III*.

5.1.5. Preparation of N,N-dimethylcarbamoyl derivative of 13-hydroxyberberine **22**

A solution of $(Me)_2NCOCl$ (0.5 mL) in $CHCl_3$ (2 mL) was added dropwise to a mixture of 13-hydroxyberberine **17** (50 mg) and $(Et)_3N$ (0.5 mL) in $CHCl_3$ (20 mL). The mixture was stirred at room temperature overnight. The reaction was followed by HPLC. To the solution 3 drops of pyridine was added and the mixture was allowed to stir at room temperature for 3 days. The solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC to give **22** (20 mg, 36%). For ^{1}H-NMR, LSIMS, and HR-LSIMS data and melting point see *tables II* and *III*.

5.2. In vitro antimalaria screening

5.2.1. Parasites and mammalian cells

Plasmodium falciparum (ATCC 30932, FCR-3 strain) was used in our study. *P. falciparum* was cultivated by a modification of the method of Trager and Jensen [12]. Mouse mammary tumour FM3A cells (wild-type, subclone F28-7) in culture were used as a control for mammalian cell cytotoxicity [13].

5.2.2. Drug testing

The following procedures were used for assaying antimalarial activity [6, 14, 15].

Five microlitres of each solution were added to individual wells of a 24 well dish. Erythrocytes with 0.3% parasitaemia were added to each well containing 995 μL of culture medium to give a final haematocrit level of 3%. The plates were incubated at 37 °C for 72 h in a 5% CO_2/5% O_2/90% N_2 incubator. To evaluate the antimalarial activity of the test compound, a total of 1×10^4 erythrocytes/L of thin blood film were examined under microscopy. The IC_{50} value refers to the concentration of the compound necessary to inhibit the increase in parasite density at 72 h by 50% of control.

5.2.3. Toxicity to mammalian cells

FM3A cells grew with a doubling time of about 12 h. Prior to exposure to drugs, cell density was adjusted to 5×10^4 cells/mL. A cell suspension of 995 μL was dispensed into the test plate, and compounds were added to individual wells of a 24 well dish. The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 48 h. Cell numbers were measured using a microcell counter CC-130 (Toa Medical Electric Co., Japan). The GI_{50} value refers to the concentration of the compound necessary to inhibit the increase in cell density at 48 h by 50% of control. Selectivity refers to the mean of the GI_{50} value for FM3A cells per the mean of the IC_{50} value for *P. falciparum*.

5.2.4. Selective toxicity using human tumour cells

The in vitro cytotoxicity assay was carried out according to the method [10, 16] of a modified National Cancer Institute protocol [17]. We screened eight compounds, **1**–**5**, **7**, **10**, and **17** against a panel of 38 human cancer cell lines (seven lung, six CNS, six colon, six stomach, five breast, five ovarian, two renal, and one melanoma). The 50% growth-inhibitory concentration (GI_{50}) for any single cell line is an index of cytotoxicity or cytostasis. The selective toxicity was estimated from the GI_{50}/IC_{50} ratio between the malaria parasites and human tumour cells which served as a model host.

Acknowledgements

This work was supported in part by a Grants-in-Aid for Scientific Research on Priority Areas (08281105) from the Ministry of Education, Science, Culture and Sports, Japan.

References

[1] Bhukuni D.S., Jain S., in: Brossi A. (Ed.), The Alkaloids Vol. 28, Academic Press, New York, 1986, pp. 95–171.

[2] Iwasa K., Kamigauchi M., Ueki M., Taniguchi M., Eur. J. Med. Chem. 31 (1996) 469–478.

[3] Iwasa K., Kamigauchi M., Sugiura M., Nanba H., Planta Med. 63 (1997) 196–198.

[4] Iwasa K., Lee D.U., Kang S.I., Wiegrebe W., J. Nat. Prod. 61 (1998) 1150–1153.

[5] Iwasa K., Nanba H., Lee D.U., Kang S.I., Planta Med. 64 (1998) 1–4.

[6] Iwasa K., Kim H.S., Wataya Y., Lee D.U., Eur. J. Med. Chem. 33 (1998) 65–69.

[7] Iwasa K., Kondoh Y., Kamigauchi M., J. Nat. Prod. 58 (1995) 379–391.

[8] 27th Symposium on Natural Drug Analysis, Higashiosaka, Osaka, (1998); p. 69.

[9] Jonathan L.V., Daniel L.K., J. Med. Chem. 31 (1988) 1084–1087.

[10] Yamori T., Jpn. J. Cancer Chemother. 25 Supplement II (1998) 373–381.

[11] Nonaka G., Okabe H., Nishioka I., Takao N., J. Pharm. Soc. Jpn. 93 (1973) 87–93.

[12] Trager W., Jensen J.B., Science 193 (1976) 673–675.

[13] Yoshioka A., Tanaka S., Hiraoka O., Koyama Y., Hirota Y., Ayusawa D., Seno T., Garrett C., Wataya Y., J. Biol. Chem. 262 (1987) 8235–8241.

[14] Kim H.S., Miyake H., Arai M., Wataya Y., Parasitol. Int. 47 (1998) 59–67.

[15] Takaya Y., Kurumada K., Takeuji Y., Kim H.S., Shibata Y., Ikemoto N., Wataya Y., Oshima Y., Tetrahedron Lett. 39 (1998) 1361–1364.

[16] Yamori T., Jpn. J. Cancer Chemother. 24 (1997) 129–135.

[17] Weinstein J.N., Myers T.G., O'Connor P.M., Friend S.H., Fornace J.R.A.J., Kohn K.W. et al., Science 275 (1997) 343–349.