Synthesis and Antitumor Activity of Novel Pyrimidinyl Pyrazole Derivatives

Hiroyuki NAITO,[a] Masamichi SUGIMORI,[b] Ikuo MITSUI,[b] Yoshihide NAKAMURA,[c] Michio IWAHANA,[a] Mineko ISHII,[a] Kenji HIROTANI,[a] Eiji KUMAZAWA,[a] and Akio EJIMA*,[a]

New Product Research Laboratories IV,[a] and Basic Technology Research Laboratory,[b] Daiichi Pharmaceutical Co., Ltd., 16–13, Kita-kasai 1-chome, Edogawa-ku, Tokyo 134–8630, Japan and Technical Department,[c] Fuji Chemical Industries, Ltd., 530 Chokeiji, Takaoka, Toyama 933–0951, Japan. Received May 21, 1999; accepted August 24, 1999

Novel pyrimidinyl pyrazole derivatives were synthesized and examined for cytotoxic and antitumor activity. Mannich reaction was employed to construct this scaffold. Among the compounds synthesized, a series of propene derivatives exhibited a potent cytotoxic activity against some tumor cell lines including multidrug resistant cell lines due to the overexpression of P-glycoprotein. The vinyl bond moiety in the scaffold was believed to be required for the cytotoxic activity. Among them, compound 14g, when administered intraperitoneally, showed potent antitumor activity against the malignant ascites caused by intraperitoneal inoculation of P388 cells in mice. This compound also showed high activity against a solid tumor Meth A mouse fibrosarcoma when administered both intraperitoneally and orally.

Key words pyrazole; cytotoxic activity; antitumor activity

As part of our ongoing program of random screening to search for new pharmacophores as antitumor agents, we discovered that the known pyrazole derivatives **1**—**8**,[1] **13d**—**e**,[2] which have been prepared as neuroleptics,[3] and a new propanone derivative **13b** showed moderate *in vitro* cytotoxic activity (Table 1). Encouraged by this result, we synthesized novel compounds based on this scaffold and explored their potential as chemotherapeutic agents by investigating their activity in P-glycoprotein-mediated multidrug-resistant cells and a solid tumor model. In this report, we describe in detail the synthesis of these novel 4-substituted 1-(pyrimidinyl)-pyrazole derivatives and their profile of antitumor activity.

Chemistry The new substituted piperazine **11a** was prepared from aniline derivative **9** and bis(2-chloroethyl)amine hydrochloride (**10**). Pyrimidinyl pyrazole propanone derivatives **13a**—**c** were prepared by Mannich reaction of acetyl pyrazoles **12a** and **12b**[2] with suitably substituted phenyl piperazines **11a**—**c** in the presence of paraformaldehyde. These propanone derivatives **13a**—**c** and known derivatives **13d**—**i**[2] were reduced with $NaBH_4$ and dehydrated in the presence of *p*-toluenesulfonic acid (*p*-TsOH) to give propene derivatives **14a**—**i**. Demethylation of **13c** and **13i** by treatment with BBr_3 gave **15a** and **15b**, respectively. Propanones **15a** and **15b** were converted to the propene derivatives **16a** and **16b** using the same procedure as for the preparation of **14** from **13**. Compound **18** without a pyrimidine substituent was derived from **13i** by hydrolysis with 47% HBr, reduction with $NaBH_4$, and dehydration by *p*-TsOH, because the bond between the pyrazole and pyrimidine rings was easily cleaved by strong acid. Compounds **24a**—**c** bearing a bulky substituent in the R^7 or R^8 position were prepared starting from diketones **19a**—**c**. The compounds **19a**—**c** were treated with $CH(OEt)_3$ and Ac_2O, and cyclized with pyrimidinyl hydrazine **20**[2] to give ketones **21a**—**c**. Compound **21c** was used in the following step as a mixture with 4-benzoyl-1-(2-pyrimidinyl)-5-methylpyrazole (**22**) without purification. The ketones **21a**—**c** were treated with 1-(3-chlorophenyl)piperazine·HCl (**11c**) to obtain propanone derivatives **23a**—**c**, which were reduced by $NaBH_4$, and dehydrated as described above to give **24a**—**c**.

Biological Activity and Structure–Activity Relationship (SAR) Studies The cytotoxic activity (GI_{50} values) of randomly screened compounds against P388 leukemia cells and PC-6 human lung carcinoma cells is shown in Table 1. 5-Fluorouracil (5-FU) was used as a reference compound. Known methylene derivatives **1**—**8**[1] and propanone derivatives[2] **13b**, **d**, **e** showed only weak or moderate *in vitro* cytotoxic activity. The derivatives **2**, **5**, and **8** bearing a longer chain $(CH_2)_3$ showed more potent activity than the derivatives bearing CH_2 or $(CH_2)_2$. When the number of carbon atoms in the methylene group was three, the activity of the propanones **13b**, **d**, **e** was clearly better than that shown by the propane type **2**, **5**, and **8**, respectively. Furthermore, propene derivatives **14b**, **d**, **e** showed enhanced activity compared with the corresponding propanones **13b**, **d**, **e** (Table 2). This encouraging trend was pursued by synthesizing further derivatives with a vinyl group. The effect on activity of changing substituents in the phenyl ring was also investigated. Compounds **14b** and **14f** bearing a halogen atom at the *o*-position of the phenyl ring showed potent *in vitro* cytotoxic activity compared with the *o*-methyl derivative **14e**, while the activity of non-substituted derivative **14d** was similar to that of **14e**. The *m*-chloro derivative **14g** showed similar and potent activity to that of *o*-chloro derivative **14f**, but the *p*-fluoro substituent of **14h** dramatically decreased the *in vitro* activity. 4-Methyl-6-methoxypyrimidine **14i** showed decreased activity while 4-methyl-6-hydroxypyrimidine **16b** retained activity. The pyrimidine ring is important for activity, because the activity of **18** without a pyrimidine substituent is considerably lower than **14e**. Introduction of a bulky group in the pyrazole 5-position and/or vinyl moiety caused a decrease in cytotoxic activity (**24a**—**c**).

The *in vivo* antitumor activity of compounds **14b**, **d**—**g**, which showed strong cytotoxic activity *in vitro*, was evaluated against murine P388 leukemia by intraperitoneal administration. The observed percentage of increase in life span (ILS) is shown in Table 2. The non-substituted derivative **14d** and its methyl derivative **14e**, which showed moderate cyto-

* To whom correspondence should be addressed.

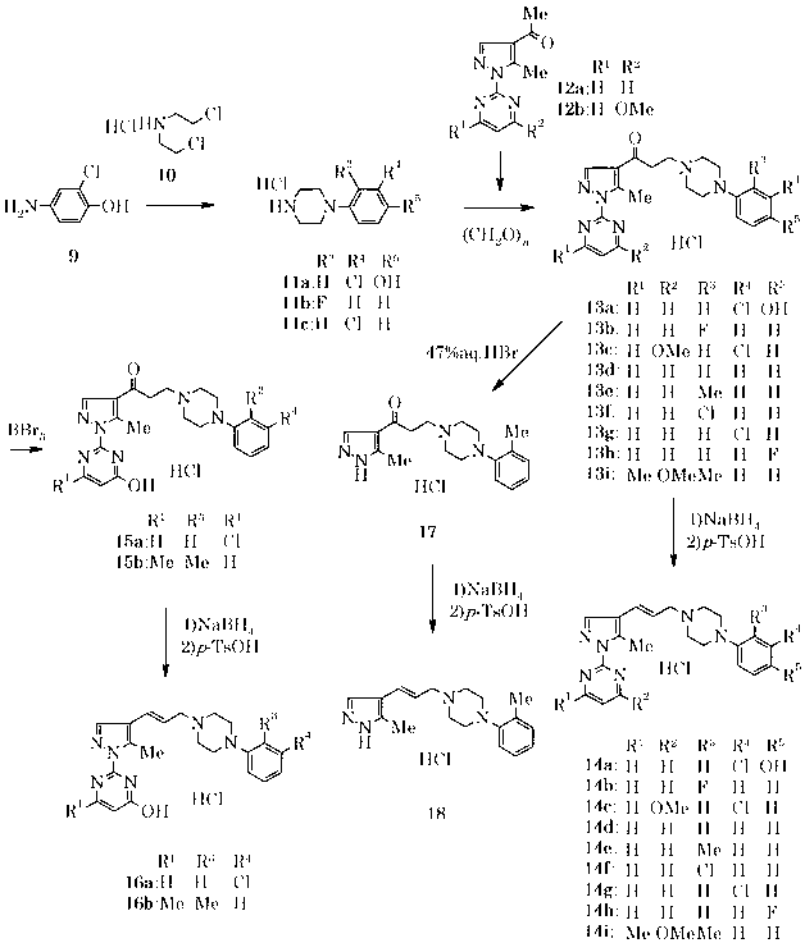


Chart 1

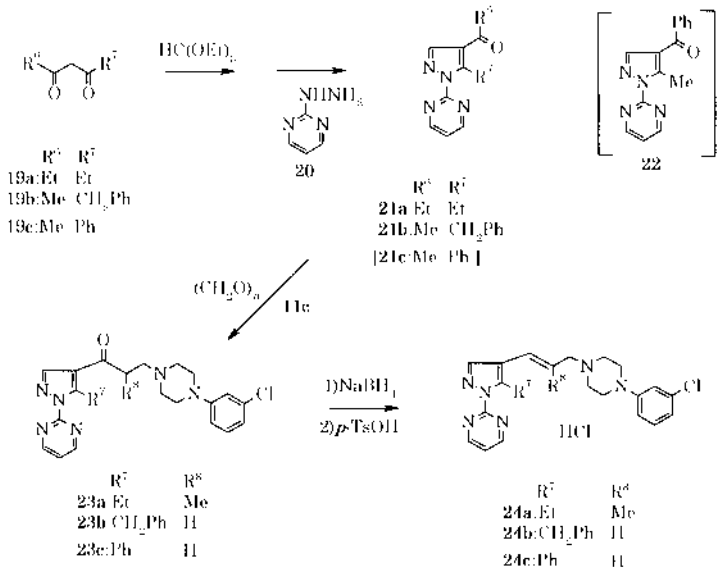
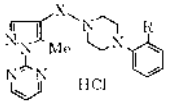


Chart 2

toxic activity *in vitro*, only slightly prolonged life span. The halogen-substituted derivatives **14b**, **f**—**g** significantly enhanced longevity compared with **14d** and **14e**. Thus, there appears to be a close correlation between *in vitro* cytotoxic activity and *in vivo* antitumor activity. Among these compounds, the *m*-chloro derivative **14g** exhibited the most potent antitumor effect *in vivo* with an ILS value of 69%, very similar to that of 5-FU.

The appearance of drug resistant cells in patients is known to be one of the biggest obstacles when antitumor agents are

Table 1. *In Vitro* Cytotoxic Activity of Pyrazole Derivatives



Compd.	R	X	GI_{50} (μg/ml)[a]	
			P388	PC-6
1	F	$-CH_2CH_2-$	>50	42.9
2	F	$-CH_2CH_2CH_2-$	21.6	4.24
13b	F	$-COCH_2CH_2-$	0.837	1.92
3	H	$-CH_2-$	>50	>50
4	H	$-CH_2CH_2-$	35.8	47.7
5	H	$-CH_2CH_2CH_2-$	24.1	22.8
13d	H	$-COCH_2CH_2-$	1.77	3.39
6	Me	$-CH_2-$	30.2	43.5
7	Me	$-CH_2CH_2-$	24.7	20.1
8	Me	$-CH_2CH_2CH_2-$	9.75	17.4
13e	Me	$-COCH_2CH_2-$	1.85	3.47
	5-FU		0.057	0.460

P388: Murine leukemia cell line. PC-6: Human non-small cell lung cancer cell line. *a*) See experimental.

Table 2. *In Vitro* Cytotoxic Activity and *in Vivo* Antitumor Activity

Compd.	GI_{50} (μg/ml)[a]		*In vivo* (P388)[b]
	P388	PC-6	ILSmax% (i.p.-i.p.)
14a	NT	0.106	
14b	0.052	0.028	38 (100×2 mg/kg)[c]
14c	0.166	0.073	
14d	0.375	0.153	10 (140×2 mg/kg)
14e	0.571	0.199	20 (105×2 mg/kg)
14f	0.057	0.016	47 (163×2 mg/kg)
14g	0.037	0.034	69 (77×2 mg/kg)
14h	14.5	26.4	
14i	4.12	4.78	
16a	0.520	0.608	
16b	0.196	0.641	
18	4.720	9.730	
24a	7.860	9.612	
24b	1.502	3.146	
24c	1.679	5.986	
5-FU	0.057	0.460	79 (100x2 mg/kg)

a, *b*) See experimental. *c*) Drugs were administered intraperitoneally twice on days 1 and 5. NT: Not tested.

Table 3. *In Vitro* Cytotoxic Activity against Multidrug Resistant Cell Lines[a]

Compd.	GI_{50} (μg/ml)			GI_{50} (μg/ml)		
	PC-6	PC-6/VCR	Rate[b]	SBC-3	SBC-3/ADM	Rate
14g	0.034	0.034	1.0	0.051	0.072	1.42
ADM	0.0075	0.277	36.9	0.0049	0.088	17.7
VCR	0.0009	0.199	212	0.0030	0.472	165

PC-6: Human non-small cell lung cancer cell line. SBC-3: Human small cell lung cancer cell line. PC-6/VCR: VCR-resistant PC-6. SBC-3/ADM: ADM-resistant SBC-3. *a*) See experimental. *b*) (GI_{50} for resistant cell line)/(GI_{50} for the parent cell line).

Table 4. Antitumor Activity of **14g** against Meth A Mouse Fibrosarcoma[a]

Compd.	Dose[b] (mg/kg)	s.c.-i.p.			Dose[e] (mg/kg)	s.c.-*p.o.*		
		IR (%)	BWLmax (%)[c]	D/U[d]		IR (%)	BWLmax (%)	D/U
14g	43×5	62.1	4.2	0/7	60×5	88.2	1.4	0/7
	30×5	32.5	1.3	0/7	42×5	70.6	3.0	0/7
	21×5	−1.6	<0	0/7	29×5	51.0	1.7	0/7
5-FU	40×5	66.8	27.0	6/7	60×5	79.7	25.4	3/7
	20×5	26.0	9.2	0/7	40×5	40.3	14.5	0/7
					30×5	45.5	14.3	0/7

a) See experimental. *b*) Compounds were administered intraperitoneally for five days consecutively. *c*) Maximum rate of BWL (%). *d*) Number of mice that died of toxicity/number of mice used. *e*) Compounds were administered orally for five days consecutively.

used for chemotherapy.[4] Therefore, the activity of **14g** was also evaluated in the multidrug resistant (MDR) cell lines PC-6/VCR[5] and SBC-3/ADM,[6] which have been reported to overexpress P-glycoprotein. The extent of activity against resistant cell lines compared with the parent cell line is shown by the resistance rate (Table 3). Although adriamycin (ADM) and vincristine (VCR) showed high cross-resistance, **14g** did not show significant cross-resistance, as indicated by the rates of 1.0 and 1.42, respectively, against the two cell lines. The tumor growth-inhibition rate (IR) of **14g** was also evaluated both intraperitoneally (i.p.) and orally (*p.o.*) against a solid tumor Meth A mouse fibrosarcoma (Table 4). The activity of 5-FU (IRmax=26.0% (i.p.) and 40.3% (*p.o.*), respectively) was weak in spite of the considerable body weight losses of 9.2% and 14.5%. Compound **14g** exhibited potent antitumor activity with IR values of 62.1% (43×5 mg/kg, i.p.) and 88.2% (60×5 mg/kg, *p.o.*), and was superior to 5-FU. This result suggests that **14g** is well-absorbed. The main side effects observed at these doses were induction of catalepsy and decrease of body temperature without body weight loss.

In conclusion, compound **14g** showed potent cytotoxic activity against P388 and PC-6 cancer cell lines including P-glycoprotein-mediated MDR cell lines *in vitro* and high antitumor activity against a solid tumor when administered orally. The pyrazole derivatives reported here represent a new

Table 5. Physical and Spectral Data for Pyrimidinylpyrazole Derivatives

Compd. No.	Yield (%)	mp (°C)	^{1}H-NMR (DMSO-d_6) δ	IR (cm^{-1})	Formula	FAB-MS (m/z)	*Anal.* Calcd (Found)		
							C	H	N
14a	28	151—156	2.62 (3H, s), 2.9—3.2 (4H, m), 3.4—3.8 (4H, m), 3.9—4.0 (2H, m), 6.1—6.3 (1H, m), 6.82 (2H, d, J=15.4 Hz), 6.7—7.0 (3H, m), 7.53 (1H, t, J=4.9 Hz), 8.09 (1H, s), 8.93 (2H, d, J=4.9 Hz)	1566 1510	$C_{21}H_{23}ClN_6O$ ·2HCl ·0.25H_2O	410 (M^+, ^{35}Cl)[a] 412 (M^++2, ^{37}Cl)	51.65 (51.69	5.26 5.27	17.21 17.11)
14b	50	210—215	2.62 (3H, s), 3.0—3.3 (4H, m), 3.4—3.7 (4H, m), 3.9—4.1 (2H, m), 6.24 (1H, dt, J=15.4, 7.3 Hz), 6.84 (1H, d, J=15.4 Hz), 7.0—7.3 (4H, m), 7.54 (1H, t, J=4.9 Hz) 8.09 (1H, s), 8.93 (2H, d, J=4.9 Hz)	1660 1574	$C_{21}H_{23}FN_6$ ·1.5HCl ·0.5H_2O	378 (M^+)[b]	57.05 (57.14	5.81 5.88	19.01 18.88)
14c	52	181—184	2.67 (3H, s), 3.1—3.3 (4H, m), 3.5—3.6 (2H, m), 3.8—4.0 (4H, m), 4.00 (3H, s), 6.23 (1H, dt, J=15.6, 7.3 Hz), 6.83 (1H, d, J=15.6 Hz), 6.87 (1H, d, J=7.8 Hz),6.92 (1H, d, J=4.9 Hz), 6.97 (1H, dd, J=7.8, 2.0 Hz), 7.05 (1H, s), 7.26 (1H, t, J=7.8 Hz), 8.07 (1H, d, J=4.9 Hz), 8.58 (1H, s)	1638 1574	$C_{22}H_{25}ClN_6O$ ·1.5HCl ·1.5H_2O	424 (M^+, ^{35}Cl)[a] 426 (M^++2, ^{37}Cl)	52.15 (52.34	5.87 5.62	16.59 16.59)
14d	26	197—201	2.62 (3H, s), 3.0—3.2 (4H, m), 3.5—3.7 (2H, m), 3.7—3.9 (2H, m), 3.9—4.0 (2H, m), 6.24 (1H, dt, J=15.4, 7.3 Hz), 6.83 (1H, d, J=15.4 Hz), 7.01 (2H, d, J=7.8 Hz), 7.27 (2H, t, J=7.8 Hz), 7.54 (1H, t, J=4.4 Hz), 7.87 (1H, t, J=7.8 Hz), 8.08 (1H, s), 8.92 (2H, d, J=4.4 Hz)	1660 1574	$C_{21}H_{24}N_6$ ·2HCl ·0.25H_2O	361 (M^++1)	57.60 (57.78	6.10 6.17	19.19 18.99)
14e	21	210—216	2.27 (3H, s), 2.63 (3H, s), 3.0—3.1 (2H, m), 3.1—3.3 (4H, m), 3.5—3.6 (2H, m), 3.9—4.0 (2H, m), 6.23 (1H, dt, J=15.6, 7.3 Hz), 6.84 (1H, d, J=15.6 Hz), 7.02 (1H, t, J=7.8 Hz), 7.06 (1H, d, J=7.8 Hz), 7.19 (1H, t, J=7.8 Hz), 7.20 (1H, d, J=7.8 Hz), 7.54 (1H, t, J=4.9 Hz), 8.10 (1H, s), 8.92 (2H, d, J=4.9 Hz)	1662 1576	$C_{22}H_{26}N_6$ ·HCl·H_2O	375 (M^++1)	61.60 (61.46	6.81 6.66	19.59 19.58)
14f	35	245—250	2.62 (3H, s), 3.0—3.3 (4H, m), 3.4—3.7 (4H, m), 3.9—4.1 (2H, m), 6.25 (1H, dt, J=15.6, 7.3 Hz), 6.85 (1H, d, J=15.6 Hz), 7.1—7.2 (1H, m), 7.2—7.3 (1H, m), 7.3—7.5 (2H, m), 7.5—7.6 (1H, m), 8.08 (1H, s), 8.92 (2H, d, J=4.9 Hz)	1576 1476	$C_{21}H_{23}ClN_6$ ·HCl ·0.5H_2O	395 (M^++1, ^{35}Cl) 397 (M^++3, ^{37}Cl)	57.28 (57.35	5.72 5.93	19.08 18.91)
14g	39	186—191	2.62 (3H, s), 3.0—3.3 (4H, m), 3.5—3.6 (2H, m), 3.8—4.0 (4H, m), 6.23 (1H, dt, J=15.6, 7.3 Hz), 6.82 (1H, d, J=15.6 Hz), 6.87 (1H, dd, J=8.3, 2.0 Hz), 6.96 (1H, dd, J=8.3, 2.0 Hz), 7.05 (1H, t, J=2.0 Hz), 7.27 (1H, t, J=8.3 Hz), 7.53 (1H, t, J=4.9 Hz), 8.10 (1H, s), 8.92 (2H, d, J=4.9 Hz)	1660 1574	$C_{21}H_{23}ClN_6$ ·HCl·H_2O	395 (M^++1, ^{35}Cl) 397 (M^++3, ^{37}Cl)	56.13 (56.16	5.83 5.81	18.70 18.69)
14h	23	205—215	2.62 (3H, s), 3.0—3.3 (4H, m), 3.5—4.1 (6H, m), 6.23 (1H, dt, J=15.4, 7.3 Hz), 6.83 (1H, d, J=15.4 Hz), 7.0—7.3 (4H, m), 7.54 (1H, t, J=4.9 Hz), 8.09 (1H, s), 8.93 (2H, d, J=4.9 Hz)	1660 1576	$C_{21}H_{23}FN_6$ ·2HCl ·0.5 H_2O	379 (M^++1)	54.78 (54.67	5.69 5.47	18.25 18.21)
14i	30	206—212	2.27 (3H, s), 2.45 (3H, s), 2.66 (3H, s), 3.0—3.3 (6H, m), 3.5—3.6 (2H, m), 3.98 (3H, s), 3.9—4.0 (2H, m), 6.24 (1H, dt, J=15.6, 7.5 Hz), 6.81 (1H, s), 6.84 (1H, d, J=15.6 Hz), 7.02 (1H, t, J=7.3 Hz), 7.05 (1H, d, J=7.3 Hz), 7.19 (1H, t, J=7.3 Hz), 8.05 (1H, s)	1662 1576	$C_{24}H_{30}N_6O$ ·2.5HCl ·H_2O	419 (M^++1)	54.63 (54.38	6.59 6.53	15.93 15.98)
16a	53	197—201	2.64 (3H, s), 3.0—3.3 (4H, m), 3.5—3.6 (2H, m), 3.8—4.0 (4H, m), 6.26 (1H, dt, J=15.6, 7.4 Hz), 6.39 (1H, d, J=8.3 Hz), 6.82 (1H, d, J=15.6 Hz), 6.87 (1H, d, J=8.3 Hz), 6.97 (1H, d, J=8.3 Hz), 7.05 (1H, s), 7.26 (1H, t, J=8.3 Hz), 8.09 (1H, d, J=4.9 Hz), 8.15 (1H, s)	1627 1481	$C_{21}H_{23}ClN_6O$ ·2.5HCl ·1.25H_2O	410 (M^+, ^{35}Cl)[a] 412 (M^+, ^{37}Cl)	48.08 (47.89	5.38 5.24	16.02 16.03)
16b	20	220—225	2.27 (3H, s), 2.31 (3H, s), 2.64 (3H, s), 3.0—3.2 (4H, m), 3.2—3.4 (4H, m), 3.5—3.6 (2H, m), 3.9—4.1 (2H, m), 6.25 (1H, dt, J=15.6, 6.8 Hz), 6.33 (1H, br s), 6.83 (1H, d, J=15.6 Hz), 6.9—7.1 (2H, m), 7.1—7.2 (1H, m), 8.12 (1H, s)	1676 1560	$C_{23}H_{28}N_6O$ ·2.5HCl ·1.1H_2O	405 (M^++1)	53.59 (53.80	6.39 6.30	16.30 16.01)
18	22	173—178	2.27 (3H, s), 2.33 (3H, s), 3.1—3.3 (6H, m), 3.4—3.8 (4H, m), 3.9—4.0 (2H, m), 6.10 (1H, dt, J=16.1, 7.8 Hz), 6.73 (1H, d, J=16.1 Hz), 6.9—7.1 (2H, m), 7.18 (1H, t, J=7.8 Hz), 7.97 (1H, s)	1564 1496	$C_{18}H_{24}N_4$ ·3HCl ·2.75H_2O	296 (M^+)[a]	47.48 (47.53	7.19 6.89	12.30 12.40)
24a	19	113—116	1.12 (3H, t, J=7.3 Hz), 2.13 (3H, s), 3.09 (2H, q, J=7.3 Hz), 3.1—3.5 (6H, m), 3.8—4.0 (4H, m), 6.65 (1H, s), 6.86 (1H, d, J=7.8 Hz), 6.97 (1H, d, J=7.8 Hz), 7.06 (1H, s), 7.27 (1H, t, J=7.8 Hz), 7.56 (1H, t, J= 4.9 Hz), 7.97 (1H, s), 8.95 (2H, d, J=4.9 Hz)	1620 1574	$C_{23}H_{27}ClN_6$ ·2HCl ·0.75H_2O	423 (M^++1, ^{35}Cl) 425 (M^++3, ^{37}Cl)	54.23 (54.05	6.04 5.85	16.50 16.30)
24b	28	167—171	3.0—3.2 (4H, m), 3.52 (2H, d, J=7.5 Hz), 3.8—4.0 (4H, m), 4.62 (2H, s), 6.28 (1H, dt, J=15.6, 7.5 Hz), 6.88 (1H, d, J=7.5 Hz), 6.89 (1H, d, J=15.6 Hz), 6.98 (1H, d, J=7.5 Hz), 7.00 (2H, d, J=7.5 Hz), 7.06 (1H, s), 7.11 (1H, t, J=7.5 Hz), 7.19 (2H, t, J=7.8 Hz), 7.27 (1H, t, J=7.8 Hz), 7.47 (1H, t, J=4.9 Hz), 8.19 (1H, s), 8.85 (2H, d, J=4.9 Hz)	1660 1572	$C_{27}H_{27}ClN_6$ ·HCl	471 (M^++1, ^{35}Cl) 473 (M^++3, ^{37}Cl)	63.91 (63.69	5.56 5.58	16.56 16.52)
24c	14	140—144	2.5—2.7 (4H, m), 3.13 (2H, d, J=6.4 Hz), 3.1—3.4 (4H, m), 6.17 (1H, dt, J=16.1, 6.2 Hz), 6.26 (1H, d, J=16.1 Hz), 6.79 (2H, dt, J=8.3, 2.0 Hz), 6.87 (1H, t, J=2.0 Hz), 7.13 (1H, t, J=4.9 Hz), 7.16 (1H, d, J=8.3 Hz), 7.2—7.3 (2H, m), 7.3—7.5 (3H, m), 8.04 (1H, s), 8.59 (2H, d, J=8.3 Hz)	1566 1488	$C_{26}H_{25}ClN_6$	457 (M^++1, ^{35}Cl) 459 (M^++3, ^{37}Cl)	68.34 (68.35	5.51 5.56	18.39 18.31)

a) FD-MS. *b*) EI-MS.

pharmacophore, and have not previously been reported as antitumor agents. Although the muscle relaxation effect associated with catalepsy[7] was observed when **14g** was administered to mice, this is understandable. It is for reason that the parent compounds had previously been found to show central nervous system (CNS) effects and to act as tranquilizers.[8] Synthesis of further analogues based on this scaffold to minimize CNS side effects is in progress. Compound **14g** was also found to inhibit tubulin polymerization. Detailed studies of the mechanism and action of these compounds on tumor cells will be reported separately.

Experimental

Melting points were determined on a Yanaco MP-500D apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JEOL JIR-5300 or Horiba FT-720 spectrometer. ^{1}H-NMR spectra were recorded on a JEOL JNM-EX400 (400 MHz) instrument and the chemical shifts are given in δ values. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; dt, doublet of triplet; br, broad; m, multiplet. Mass spectra (MS) were recorded on a JEOL JMS-HX110 or a JMS-AX505W mass spectrometer. Elemental analyses were performed using a Perkin-Elmer Series II CHNS/O 2400 instrument. Column chromatography was performed with Silica gel 60 F_{254} (70—230 mesh) (Merck). Sodium sulfate was employed as a drying agent.

1-(3-Chloro-4-hydroxyphenyl)piperazine·HCl (11a) A mixture of 2-chloro-4-aminophenol (**9**) (1.29 g, 8.99 mmol) and bis(2-chloroethyl)amine hydrochloride (**10**) (1.60 g, 8.99 mmol) in *n*-BuOH (15 ml) was refluxed for 20 h. Anhydrous sodium carbonate (0.95 g, 8.96 mmol) was added to the mixture. After being stirred for 8 h, the reaction mixture was cooled and the precipitate obtained was filtered. The precipitate was suspended with H_2O and extracted with $CHCl_3$. The organic phase was washed with H_2O, dried, and evaporated *in vacuo*. Et_2O and 1 N HCl/EtOH (2.5 ml) were added to the residue, and the precipitate obtained was filtered to give **11a** (630 mg, 28%), **11a**: mp 207—210 °C. Field desorption MS (FD-MS) *m/z*: 212 (M^+, ^{35}Cl), 214 (M^++2, ^{37}Cl). IR (KBr) cm^{-1}: 1598, 1516. ^{1}H-NMR (DMSO-d_6) δ: 3.0—3.5 (8H, m), 6.8—6.9 (1H, m), 6.9—7.0 (2H, m), 9.30 (2H, br s), 9.81 (1H, br s). *Anal*. Calcd for $C_{10}H_{13}ClN_2O\cdot HCl\cdot 0.25H_2O$: C, 47.35; H, 5.76; N, 11.04. Found: C, 47.27; H, 5.55; N, 10.81.

Pyrimidinylpyrazole Propanone Derivatives (13a—c). Illustrative Procedure A mixture of pyrazole **12a**[2] (285 mg, 1.41 mmol), amine **11a** (300 mg, 1.20 mmol), and paraformaldehyde (600 mg) in EtOH (30 ml) was heated to reflux, and additional paraformaldehyde (2.4 g) was added in small portions over 24 h. After being stirred for 48 h, the reaction mixture was cooled, and the precipitate obtained was filtered to give **13a** (244 mg, 44%). Compounds **11b** and **11c** were treated with **12a** and **12b**[2] in the manner described above to give **13b** and **13c**, respectively.

13a: mp 170—175 °C (dec.). IR (KBr) cm^{-1}: 1687, 1575. ^{1}H-NMR (DMSO-d_6) δ: 2.81 (3H, s), 2.9—3.2 (4H, m), 3.2—3.8 (8H, m), 6.7—7.0 (3H, m), 7.67 (1H, t, J=4.9 Hz), 8.42 (1H, s), 9.01 (1H, d, J=4.9 Hz), 9.65 (1H, s). High resolution (HR)-MS (FAB) Calcd for $C_{21}H_{24}ClN_6O_2$ $(M+H)^+$: 427.1649 (Cl^{35}), 429.1628 (Cl^{37}). Found: 427.1635, 429.1648.

13b: (42%), mp 198—202 °C (dec.). FAB-MS *m/z*: 395 (M^++1). IR (KBr) cm^{-1}: 1681, 1552. ^{1}H-NMR (DMSO-d_6) δ: 2.82 (3H, s), 3.1—3.8 (12H, m), 7.0—7.3 (4H, m), 7.66 (1H, t, J=4.9 Hz), 8.43 (1H, s), 9.01 (2H, d, J=4.9 Hz), 11.0 (1H, br s). *Anal*. Calcd for $C_{21}H_{23}FN_6O\cdot HCl\cdot 0.25H_2O$: C, 57.93; H, 5.67; N, 19.30. Found: C, 58.20; H, 5.64; N, 19.38.

13c: (30%), mp 194—197 °C (dec.). FD-MS *m/z*: 440 (M^+, ^{35}Cl), 442 (M^++2, ^{37}Cl). IR (KBr) cm^{-1}: 1674, 1596. ^{1}H-NMR (DMSO-d_6) δ: 2.84 (3H, s), 3.1—3.3 (4H, m), 3.4—4.2 (8H, m), 4.00 (3H, s), 6.88 (1H, d, J=8.3 Hz), 6.99 (1H, d, J=8.3 Hz), 7.07 (1H, d, J=5.6 Hz), 7.09 (1H, s), 7.27 (1H, t, J=8.3 Hz) , 8.41 (1H, s), 8.67 (1H, d, J=5.6 Hz). *Anal*. Calcd for $C_{22}H_{25}ClN_6O_2\cdot HCl\cdot 0.75H_2O$: C, 53.88; H, 5.65; N, 17.12. Found: C, 53.88; H, 5.52; N, 17.24.

Pyrimidinylpyrazole Propene Derivatives (14a—i). Illustrative Procedure Sodium borohydride (280 mg, 7.37 mmol) was added in small portions to a solution of **13a** (230 mg, 0.50 mmol) in EtOH (10 ml) and tetrahydrofuran (THF) (10 ml). The mixture was stirred at room temperature until TLC indicated completion of the reaction. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The organic layer was washed with H_2O, dried, and evaporated *in vacuo*. To this residue were added THF (20 ml), dioxane (30 ml), and *p*-TsOH·H_2O (170 mg), and the resulting mixture was heated to reflux for 1.5 h. The mixture was diluted with saturated $NaHCO_3$ solution and extracted with $CHCl_3$. The organic layer was washed with H_2O, dried, and evaporated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$–MeOH, 30 : 1), diluted with 1 N HCl/EtOH, and evaporated *in vacuo*. The residue was recrystallized from EtOH to give **14a** (68 mg, 28%). Compounds **13b**, **c** and **13d**—**i**[2] were treated in the manner described above to give **14b**—**i**, respectively. The physical data for these compounds and yields are shown in Table 5.

1-[5-Methyl-1-(4-hydroxy-2-pyrimidinyl)-4-pyrazolyl]-3-[4-(3-chlorophenyl)-1-piperazinyl]-1-propanone·HCl (15a) A solution of **13c** (950 mg, 2.0 mmol) in CH_2Cl_2 (150 ml) was added to a solution of BBr_3 (600 mg, 2.4 mmol) in CH_2Cl_2 (8 ml) at 0 °C, and the resulting mixture was stirred at room temperature for 45 h. The mixture was diluted with CH_2Cl_2 and the organic layer was washed with saturated $NaHCO_3$ solution and H_2O, dried, and evaporated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$–MeOH, 10 : 1), diluted with 1 N HCl/EtOH and evaporated *in vacuo*, then was recrystallized from EtOH to give **15a** (167 mg, 16%), mp 177—181 °C (dec.). FAB-MS *m/z*: 427 (M^++1, ^{35}Cl), 429 (M^++3, ^{37}Cl). IR (KBr) cm^{-1}: 1686, 1634, 1596. ^{1}H-NMR (DMSO-d_6) δ: 2.84 (3H, s), 2.9—3.4 (6H, m), 3.4—4.0 (6H, m), 6.64 (1H, d, J=5.5 Hz), 6.87 (1H, d, J=8.3 Hz), 6.98 (1H, d, J=8.3 Hz), 7.08 (1H, s), 7.26 (1H, t, J=8.3 Hz), 8.31 (1H, d, J=5.5 Hz), 8.42 (1H, s).

1-[5-Methyl-1-(4-hydroxy-6-methyl-2-pyrimidinyl)-4-pyrazolyl]-3-[4-(2-methylphenyl)-1-piperazinyl]-1-propanone·HCl (15b) Compound **13i** (470 mg, 1 mmol) was treated in the same manner as described for **15a** to give **15b** (150 mg, 28%), mp 208—211 °C (dec.). FAB-MS *m/z*: 421 (M^++1). IR (KBr) cm^{-1}: 1678, 1610, 1558. ^{1}H-NMR (DMSO-d_6) δ: 2.28 (3H, s), 2.38 (3H, s), 2.83 (3H, s), 3.0—3.4 (6H, m), 3.4—3.7 (6H, m), 6.56 (1H, s), 6.9—7.1 (2H, m), 7.1—7.3 (2H, m), 8.39 (1H, s).

Hydroxypyrimidinylpyrazole Propene Derivatives (16a—b) Compounds **15a** and **15b** were treated in the same manner as described for **14a**—**i** to give **16a** and **16b**, respectively. The physical data for these compounds and yields are shown in Table 5.

1-[1*H*-5-Methyl-4-pyrazolyl]-3-[4-(2-methylphenyl)-1-piperazinyl]-1-propanone·HCl (17) A solution of **13i** (1.0 g, 2.1 mmol) in 47% HBr (10 ml) was heated to reflux for 2 h. The solution was neutralized with aqueous 15% NaOH solution and extracted with AcOEt. The organic layer was washed with H_2O, dried, and evaporated *in vacuo*. To the residue was added 1 N HCl/EtOH, and the solution was evaporated *in vacuo* and recrystallized from EtOH–Et_2O to give **17** (602 mg, 82%). FD-MS *m/z*: 312 (M^+). IR (KBr) cm^{-1}: 1690, 1496. ^{1}H-NMR (DMSO-d_6) δ: 2.28 (3H, s), 2.45 (3H, s), 3.0—3.3 (6H, m), 3.3—3.6 (4H, m), 3.6—3.7 (2H, m), 7.00 (1H, d, J=7.2 Hz), 7.05 (1H, d, J=7.2 Hz), 7.18 (2H, t, J=7.2 Hz), 8.26 (1H, s).

1-[1*H*-5-Methyl-4-pyrazolyl]-3-[4-(2-methylphenyl)-1-piperazinyl]-1-propene·HCl (18) Compound **17** was treated in the manner described for **14a** to give **18**. The physical data and yields are shown in Table 5.

5-Ethyl-4-propionyl-1-(2-pyrimidinyl)pyrazole (21a) A mixture of 3,5-heptadione (**19a**) (5.11 g, 39.9 mmol) and triethyl orthoformate (7.96 ml, 47.9 mmol) in Ac_2O (4.52 ml, 47.9 mmol) was heated to reflux for 50 min. After removal of the solvent, the residue was distilled at 117—125 °C under 4 mmHg to give 4-ethoxymethylene-3,5-heptadione (3.0 g, 41%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.06 (3H, t, J=7.3 Hz), 1.10 (3H, t, J=7.3 Hz), 1.40 (3H, t, J=7.3 Hz), 2.67 (2H, q, J=7.3 Hz), 2.74 (2H, q, J=7.3 Hz), 4.22 (2H, q, J=7.3 Hz), 7.63 (1H, s). A mixture of this oil (1.5 g, 8.14 mmol) and 2-pyrimidinylhydrazine (**20**)[2] (0.941 g, 8.55 mmol) in EtOH (11 ml) was stirred at 60 °C for 3 h. After removal of the solvent, the residue was chromatographed on silica gel (AcOEt–hexane, 1 : 1) to give **21a** (1.49 g, 79%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H, t, J=7.3 Hz), 1.28 (3H, t, J=7.3 Hz), 2.89 (2H, q, J=7.3 Hz), 3.50 (2H, q, J=7.3 Hz), 7.34 (1H, t, J=4.9 Hz), 8.10 (1H, s), 8.86 (2H, d, J=4.9 Hz).

4-Acetyl-5-benzyl-1-(2-pyrimidinyl)pyrazole (21b) A mixture of 1-phenyl-2,4-pentadione (**19b**) (10 g, 56.7 mmol) and triethyl orthoformate (14.2 ml, 85.1 mmol) in Ac_2O (16.1 ml, 170 mmol) was heated to reflux for 1 h. After removal of the solvent, the residue was chromatographed on silica gel (AcOEt–hexane, 2 : 1) to give 3-hydroxymethylene-1-phenyl-2,4-pentadione (7.34 g, 63%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 2.53 (3H, s), 4.22 (2H, s), 7.2—7.4 (5H, m), 10.10 (1H, s). A mixture of the oil (1.0 g, 4.90 mmol) and **20** (0.539 g, 4.89 mmol) in EtOH (8 ml) was stirred at 60 °C for 6.5 h. After removal of the solvent, the residue was chromatographed on silica gel (AcOEt–hexane, 1 : 1) to give **21b** (0.23 g, 17%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 2.55 (3H, s), 5.07 (2H, s), 7.0—7.4 (6H, m), 8.17 (1H, s), 8.77 (2H, d, J=4.4 Hz).

4-Acetyl-5-phenyl-1-(2-pyrimidinyl)pyrazole (21c) A mixture of 1-phenyl-1,3-butanedione (**19c**) (10 g, 61.7 mmol) and triethyl orthoformate (5.26 ml, 61.7 mmol) in Ac_2O (11.6 ml, 123 mmol) was heated to reflux for

50 min. After removal of the solvent, the residue was chromatographed on silica gel (AcOEt–hexane, 1 : 3) to give 2-ethoxymethylene-1-phenyl-1,3-butanedione (3.23 g, 24%). ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H, t, J=7.3 Hz), 2.22 (3H, s), 4.08 (2H, q, J=7.3 Hz), 7.0—8.0 (6H, m). A mixture of the above compound (1.0 g, 4.58 mmol) and **20** (0.505 g, 4.58 mmol) in EtOH (8 ml) was stirred at 60 °C for 6 h. After removal of the solvent, the residue was chromatographed on silica gel (AcOEt–hexane, 1 : 1) to give a mixture of 4-acetyl-5-phenyl-1-(2-pyrimidinyl)pyrazole (**21c**) and 4-benzoyl-5-methyl-1-(2-pyrimidinyl) pyrazole (**22**) (1.04 g, 86%). The ratio of **21c** and **22** was about 1 : 3 based on NMR. This mixture was used in the next Mannich reaction without further purification. FAB-MS m/z: 265 (M^++1). **21c**: ^{1}H-NMR ($CDCl_3$) δ: 2.18 (3H, s), 7.21 (1H, J=4.8 Hz), 7.3—7.7 (5H, m), 8.27 (1H, s), 8.62 (2H, d, J=4.8 Hz). **22**: ^{1}H-NMR ($CDCl_3$) δ: 3.00 (3H, s), 7.3—7.7 (4H, m), 7.8—7.9 (2H, m), 7.96 (1H, s), 8.88 (2H, d, J=4.8 Hz).

Pyrimidinylpyrazole Propanone Derivatives (23a—c) Compounds **21a**, **21b**, and **21c** were treated with **11c** in the manner described for **13a** and were chromatographed on silica gel ($CHCl_3$–MeOH, 30 : 1) to give **23a**, **23b**, and **23c**, respectively.

23a: Yield 48%, oil. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, d, J=7.3 Hz), 1.28 (3H, t, J=7.3 Hz), 2.4—3.0 (6H, m), 3.12 (4H, t, J=4.9 Hz), 3.4—3.5 (1H, m), 3.49 (2H, q, J=7.3 Hz), 6.74 (1H, d, J=7.5 Hz), 6.77 (1H, d, J=7.5 Hz), 6.82 (1H, s), 7.13 (1H, t, J=7.5 Hz), 7.35 (1H, dt, J=4.6, 1.4 Hz), 8.14 (1H, s), 8.87 (2H, d, J=4.6 Hz).

23b: Yield 35%, amorphous. ^{1}H-NMR ($CDCl_3$) δ: 2.6—2.7 (4H, m), 2.8—3.2 (4H, m), 3.11 (2H, t, J=7.3 Hz), 3.1—3.3 (4H, m), 5.07 (2H, s), 6.7—7.2 (5H, m), 7.3—7.5 (5H, m), 8.22 (1H, s), 8.77 (2H, d, J=4.9 Hz).

23c: Yield 31%, oil. ^{1}H-NMR ($CDCl_3$) δ: 2.3—2.6 (4H, m), 2.7—2.9 (4H, m), 3.1—3.2 (4H, m), 6.7—6.9 (3H, m), 7.15 (1H, t, J=8.3 Hz), 7.23 (1H, t, J=4.9 Hz), 7.3—7.5 (5H, m), 8.28 (1H, s), 8.63 (2H, d, J=4.9 Hz).

Pyrimidinylpyrazole Propene Derivatives (24a—c) Compounds **23a**, **23b**, and **23c** were treated in the manner described for **14a** to give **24a**, **24b**, and **24c**, respectively. The physical data for these compounds and yields are shown in Table 5.

***In Vitro* Cytotoxicity** To examine the direct growth-inhibitory effects of test compounds against PC-6 human non-small cell lung cancer cell line, SBC-3 human small cell lung cancer cell line, these resistant cell lines (PC-6/VCR[5], SBC-3/ADM[6]), and P388 murine leukemia cell line, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed and the concentration giving a growth inhibition of 50% (GI_{50}) was calculated according to the published procedure.[5]

Evaluation of Therapeutic Effect *in Vivo* P388 cells (1×10^6) were inoculated i.p. into CDF1 mice (6 mice per group) on day 0. Compounds and 5-FU were suspended in BTC salt solution (0.9% benzyl alcohol, 0.4% Tween 80, 0.5% carboxymethyl cellulose, 0.9% NaCl) and given i.p. on days 1 and 5. The ILS was calculated using the following formula: ILS (%)=[(median survival time of treated group)/(median survival time of control group)—1]×100. Meth A murine fibrosarcoma cells (1×10^6) were implanted into the right flank of BALB/c mice (day 0). Compound **14g** and 5-FU were administered i.p. or *p.o*. on days 7—11 consecutively. Tumor weights were measured on day 17. The IR was calculated using the formula: IR (%)=(1—TWt/TWc)×100 (%), where TWt represents the mean tumor weight of a treated group and TWc represents that of the control group. To evaluate the intensity of the side effects of compounds, the rate of body weight loss (BWL) was utilized as a parameter of toxicity. The maximum value of BWL was designated as BWLmax, and BWLmax less than zero indicated no body weight loss.

References

1) Ueno K., Moroi R., Kojima H., Chiba T., Japan Kokai Tokkyo Koho, JP 74 11705 (1974) [*Chem. Abstr*., **81**, 91517*k* (1974)].
2) Ueno K.,Ohmura Y., Moroi R., Akashi A., Arimoto M., Kasahara A., Ger. Patent, DE 2038503 [*Chem. Abstr*., **75**, 49121*c* (1971)].
3) Sato M., Arimoto M., Ueno K., Kojima H., Yamasaki T., Sakurai T., Kasahara A., *J. Med. Chem*., **21**, 1116—1120 (1978).
4) *a*) Lehnert M., *European J. Cancer*, **32A**, 912—920 (1996); *b*) Colin de Verdiere A., Dubernet C., Nemati F., Poupon M. F., Puisieux F., Couvreur P., *Cancer Chemother. Pharmacol*., **33**, 504—508 (1994).
5) Mitsui I., Kumazawa E., Hirota Y., Aonuma M., Sugimori M., Ohsuki S., Uoto K., Ejima A., Terasawa H., Sato K., *Jpn. J. Cancer Res*., **86**, 776—782 (1995).
6) Kiura K., Ohnoshi T., Tabata M., Shibayama T., Kimura I., *Acta Med. Okayama*, **47**, 191—197 (1993).
7) Asper H., Baggiolini M., Burki H. R., Lauener H., Ruch W., Stille G., *European J. Pharmacol*., **22**, 287—294 (1973).
8) Tachizawa H., Sudo K., Sano M., *European J. Pharmacol*., **59**, 245—251 (1979).