Synthesis and Antitumor Activities of Water-Soluble Benzoylphenylureas

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Water-soluble benzoylphenylurea derivatives were synthesized as candidate prodrugs and their antitumor activities were examined *in vivo* against P388 leukemia. Some of the prodrugs were highly soluble in water and showed good antitumor activities against P388 leukemia cells in mice when injected intravenously.

Key words benzoylphenylurea; antitumor agent; prodrug; amino acid

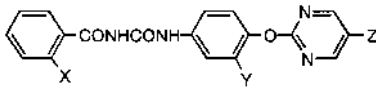
Antitumor agents which prevent the formation of the mitotic spindle during cell division by interfering with the tubulin–microtubules system have attracted considerable attention. It is known that they are classified into two different types. One of them is taxol, which promotes the assembly of microtubules and inhibits microtubule depolymerization.[1,2] The other type include vinca alkaloids and colchicine, which inhibit tubline polymerization and cause microtubule depolymerization.[3,4] Benzoylphenylurea derivatives belong to the second type and show high antitumor activities.[5,6] However, they are almost insoluble in water and in most organic solvents. Therefore, they have the disadvantage of being difficult to formulate and have relatively low bioavailability.[7] In order to develop a benzoylphenylurea compound as an antitumor drug, it is therefore necessary to improve its solubility. In a previous paper,[6] we reported the synthesis and antitumor activities of prodrugs of benzoylphenylureas, which have high solubility in various organic solvents. We synthesized those compounds to increase the bioavailability of benzoylphenylureas for its use as an oral antitumor agent. On the other hand, we continue to look for antitumor agents which have higher solubility in water and can be injected intravenously. In the course of study of the metabolism of *N*-[4-(5-bromo-2-pyrimidinyloxy)-3-chlorophenyl]-*N'*-(2-nitrobenzoyl)urea (**1**) (coded HO-221: Table 1),[5,8] compound **2**, a main metabolite of HO-221, was shown to have good antitumor activity. Furthermore, related 2-aminobenzoyl derivatives of **2** (**3**, **4**) also showed antitumor activities similar to that of **2**. In the next step, we tried to synthesize water-soluble derivatives of benzoylphenylureas by condensing the amino acid moiety with a 2-amino group.

Chemistry The catalytic reduction of **1** did not give the desired compound, but amino compounds (**2**—**4**) were obtained by using reduced iron from **1** or corresponding nitro compounds (Chart 1). The introduction of amino acids into the amino group could be achieved by the coupling reaction of amino compounds (**2**—**4**) with *N*-*tert*-butyloxy (Boc) amino acids. We chose 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSCI·HCl) as a coupling reagent, and trifluoroacetic acid was used for removing the protective group (Boc). Then, the substituted 2-aminobenzoylphenylurea trifluoroacetates were converted to pharmaceutically acceptable salts. In the case of *N*,*N*-dimethylglycyl compounds (**6**, **16**, **18**), a protective group and trifluoroacetic acid were not used because there was no need to use the protective group.

Structural assignment was carried out by ^{1}H-nuclear magnetic resonance (^{1}H-NMR) and elemental analysis. The structures, melting points and ^{1}H-NMR spectral data of substituted 2-aminobenzoylphenylurea hydrochlorides or methanesulfonate are summarized in Table 2.

Antitumor Activity Antitumor activities and the solubility of water-soluble benzoylphenylureas are summarized in Table 3. As all benzoylphenylurea hydrochlorides or

Table 1. Structures and Antitumor Activities of Benzoylphenylureas



Compd.	X	Y	Z	Antitumor activity i.p.[a)] Dose (mg/kg)	Antitumor activity i.p.[a)] *T*/*C* (%)	mp (°C)	^{1}H-NMR (DMSO-d_6) δ
1 (HO-221)	NO_2	Cl	Br	12.5	173	234—236	
2	NH_2	Cl	Br	12.5	207	191—195	6.61 (1H, t, *J*=8 Hz), 6.67 (2H, br s), 6.84 (1H, d, *J*=8 Hz), 7.31 (1H, t, *J*=8 Hz), 7.47 (1H, d, *J*=9 Hz), 7.61 (1H, dd, *J*=9, 2 Hz), 7.77 (1H, d, *J*=7 Hz), 8.01 (1H, d, *J*=2 Hz), 8.90 (2H, s), 10.71 (1H, br s), 10.91 (1H, s)
3	NH_2	CH_3	Br	25 12.5	260 226	189—193	2.06 (3H, s), 6.52—6.56 (3H, m), 6.77 (1H, d, *J*=8 Hz), 7.11 (1H, d, *J*=9 Hz), 7.25 (1H, t, *J*=8 Hz), 7.47—7.48 (2H, m), 7.70 (1H, d, *J*=8 Hz), 8.78 (2H, s), 10.55 (1H, br s), 10.75(1H, s)
4	NH_2	CF_3	Cl	6.25	171	177—182	6.54 (1H, t, *J*=8 Hz), 6.61 (2H, br s), 6.78(1H, d, *J*=8 Hz), 7.25 (1H, t, *J*=8 Hz), 7.48 (1H, d, *J*=9 Hz), 7.71 (1H, d, *J*=8 Hz), 7.88 (1H, dd, *J*=9, 2 Hz), 8.12 (1H, d, *J*=2 Hz), 8.79 (2H, s), 10.66 (1H, br s), 10.93 (1H, s)

a) Intraperitoneal injection.

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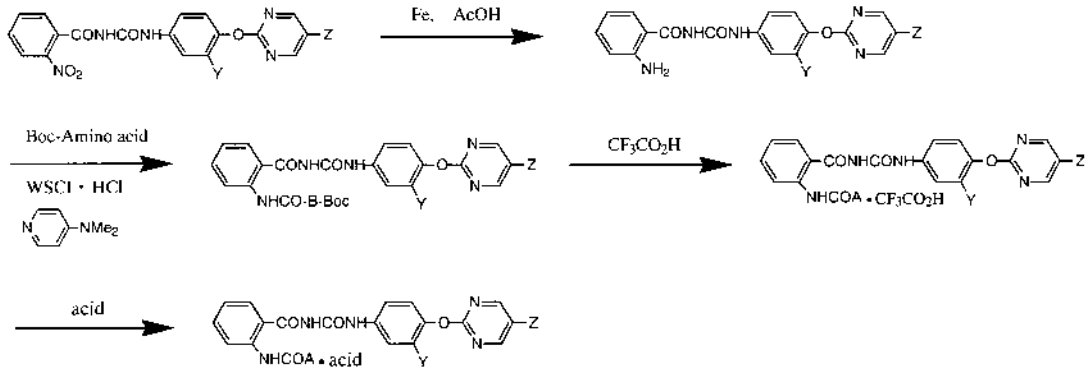
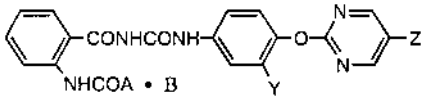


Chart 1

Table 2. Structures of Substituted 2-Aminobenzoylphenylureas



Compd.	A	B	Y	Z	mp (°C)	^{1}H-NMR (DMSO-d_6) δ
5	CH_2NH_2	HCl	Cl	Br	201—203	3.80 (2H, br s), 7.30 (1H, t, J=8 Hz), 7.40 (1H, d, J=9 Hz), 7.51—7.67 (4H, m), 7.93 (1H, d, J=2 Hz), 8.24 (3H, br s), 8.83 (2H, s), 10.59 (1H, s), 10.74 (1H, s), 11.13 (1H, s)
6	$CH_2N(CH_3)_2$	HCl	Cl	Br	164—169	2.83 (3H, s), 2.84 (3H, s), 4.16 (2H, br s), 7.33 (1H, t, J=7 Hz), 7.39 (1H, d, J=9 Hz), 7.48—7.53 (2H, m), 7.59 (1H, t, J=8 Hz), 7.64 (1H, d, J=9 Hz), 7.92 (1H, d, J=2 Hz), 8.83 (2H, s), 10.04 (1H, br s), 10.95 (1H, s), 11.16 (1H, s)
7	$CH(CH_3)NH_2$ [a,b]	HCl	Cl	Br	210—240 (dec.)	1.48 (3H, d, J=7 Hz), 4.15 (1H, br s), 7.31 (1H, t, J=8 Hz), 7.39 (1H, d, J=9 Hz), 7.48—7.64 (4H, m), 7.92 (1H, d, J=2 Hz), 8.41 (3H, br s), 8.82 (2H, s), 10.83 (1H, s), 10.88 (1H, s), 11.04 (1H, s)
8	$CH(CH_3)NH_2$ [c,d]	HCl	Cl	Br	>224 (dec.)	1.48 (3H, d, J=7 Hz), 4.15 (1H, br s), 7.31 (1H, t, J=7 Hz), 7.40 (1H, d, J=9 Hz), 7.49—7.67 (4H, m), 7.93 (1H, d, J=2 Hz), 8.42 (3H, br s), 8.84 (2H, s), 10.84 (1H, s), 10.90 (1H, s), 11.05 (1H, s)
9	$CH((CH_2)_2SCH_3)NH_2$ [b]	HCl	Cl	Br	170—177	2.08 (3H, s), 2.08—2.20 (2H, m), 2.66 (2H, t, J=8 Hz), 4.17 (1H, br s), 7.31 (1H, t, J=8 Hz), 7.40 (1H, d, J=9 Hz), 7.50—7.62 (4H, m), 7.90 (1H, d, J=2 Hz), 8.49 (3H, br s), 8.83 (2H, s), 10.83 (1H, s), 11.00 (1H, s), 11.15 (1H, s)
10	$CH_2NHCOCH_2NH_2$	HCl	Cl	Br	255—263	3.70 (2H, d, J=6 Hz), 4.00 (2H, br s), 7.24 (1H, br t, J=8 Hz), 7.41 (1H, d, J=9 Hz), 7.51 (1H, dd, J=9, 2 Hz), 7.58 (1H, br t, J=8 Hz), 7.81 (1H, br d, J=8 Hz), 7.94 (1H, d, J=2 Hz), 8.00 (1H, br d, J=8 Hz), 8.14 (3H, br s), 8.84 (2H, s), 9.02 (1H, br s), 10.61 (1H, br s), 10.76 (1H, br s), 11.15 (1H, s)
11	CH_2NH_2	HCl	CF_3	Cl	184—190	3.81 (2H, br s), 7.30 (1H, t, J=8 Hz), 7.48 (1H, d, J=9 Hz), 7.57 (1H, t, J=8 Hz), 7.64—7.68 (2H, m), 7.86 (1H, dd, J=9, 2 Hz), 8.12 (1H, d, J=2 Hz), 8.31 (3H, br s), 8.79 (2H, s), 10.68 (1H, s), 10.88 (1H, s), 11.13 (1H, s)
12	CH_2NH_2	HCl	Cl	Cl	196—199	3.80 (2H, br s), 7.29 (1H, t, J=8 Hz), 7.39 (1H, d, J=9 Hz), 7.52—7.67 (4H, m), 7.93 (1H, d, J=3 Hz), 8.29 (3H, br s), 8.78 (2H, s), 10.65 (1H, s), 10.77 (1H, s), 11.12 (1H, s)
13	CH_2NH_2	HCl	CH_3	Br	187—192	2.05 (3H, s), 3.80 (2H, br s), 7.10 (1H, d, J=9 Hz), 7.28 (1H, t, J=8 Hz), 7.45—7.47 (2H, m), 7.56 (1H, t, J=8 Hz), 7.63—7.68 (2H, m), 8.54 (3H, br s), 8.78 (2H, s), 10.63 (1H, s), 10.70 (1H, s), 11.04 (1H, s)
14	$CH(CH_3)NH_2$ [b]	HCl	CH_3	Br	195—200	1.45 (3H, d, J=7 Hz), 2.06 (3H, s), 4.13 (1H, br s), 7.11 (1H, d, J=8 Hz), 7.31 (1H, t, J=7 Hz), 7.42—7.59 (4H, m), 7.63 (1H, d, J=8 Hz), 8.36 (3H, br s), 8.78 (2H, s), 10.66 (1H, s), 10.78 (1H, s), 10.96 (1H, s)
15	$CH(CH_3)NH_2$ [d]	HCl	CH_3	Br	>300	1.47 (3H, d, J=8 Hz), 2.06 (3H, s), 4.11 (1H, br s), 7.11 (1H, d, J=8 Hz), 7.31 (1H, t, J=8 Hz), 7.43—7.59 (4H, m), 7.63 (1H, d, J=8 Hz), 8.34 (3H, br s), 8.78 (2H, s), 10.67 (1H, s), 10.76 (1H, br s), 10.98 (1H, br s)
16	$CH_2N(CH_3)_2$	HCl	CH_3	Br	119—132	2.07 (3H, s), 2.84 (6H, s), 4.17 (2H, s), 7.12 (1H, d, J=9 Hz), 7.30—7.58 (5H, m), 7.66 (1H, d, J=8 Hz), 8.79 (2H, s), 10.26 (1H, br s), 10.65 (1H, s), 11.06 (1H, s), 11.13 (1H, br s)
17	CH_2NHCH_3	HCl	CH_3	Br	194—197 (dec.)	2.06 (3H, s), 2.57 (3H, s), 3.94 (2H, s), 7.11 (1H, d, J=8 Hz), 7.30 (1H, t, J=7 Hz), 7.44—7.65 (5H, m), 8.78 (2H, s), 9.12 (2H, br s), 10.62 (1H, s), 10.74 (1H, br s), 11.06 (1H, s)
18	$CH_2N(CH_3)_2$	CH_3SO_3H	CH_3	Br	119—127	2.07 (3H, s), 2.34 (3H, s), 2.84 (6H, s), 4.14 (2H, s), 7.12 (1H, d, J=8 Hz), 7.34 (1H, t, J=7 Hz), 7.41—7.65 (5H, m), 8.78 (2H, s), 9.38 (1H, br s), 10.57 (1H, s), 10.67 (1H, s), 11.08 (1H, s)

a) $[\alpha]_D^{20}$ −11.4° (c=2.01, DMSO). *b*) Synthesized by using an L-amino acid as a starting material. *c*) $[\alpha]_D^{20}$ +14.3° (c=2.17, DMSO). *d*) Synthesized by using a D-amino acid as a starting material.

Table 3. Antitumor Activities and Solubilities of Substituted 2-Aminobenzoylphenylureas

Compd.	Antitumor activity i.v.[a]		Solubility in test solution (%)[b]			
	Dose (mg/kg)[c]	*T/C* (%)	A	B	C	D
5	25	319	0.12	0.005	0.21	
	12.5	179				
6	25	243	0.25	0.055	>0.40	
	12.5	151				
7	25	234	0.28	0.032	0.37	
	12.5	170				
8	25	195	0.20	0.014	0.27	
9	25	252	0.12	0.003	0.20	
10	25	343	0.18	0.007	0.21	
	12.5	190				
11	12.5	238	0.14	0.007	0.19	
	6.25	160				
12	25	252	0.16	0.016	0.15	
	12.5	186				
13	25	243	>0.23	0.038	0.39	
	6.25	150				
14	25	290	0.76	0.18	0.98	
	12.5	176				
16	25	255	1.90	0.30	2.00	
	12.5	136				
17	20	>150	0.50	0.012		
	12.5	>130				
18	20	275	>20.0	0.60		>23.0
	10	133				

a) Intravenous injection. *b*) Test solution A: Distilled water, B: Physiological saline, C: 10% polyethylene glycol #400 aqueous solution, D: 5% glucose. *c*) When two values are listed, the upper one is the optimum dose at which the maximum *T/C* value is shown and the lower one is the minimum dose at which the *T/C* value is 130% or more. When the optimum dose equals the minimum dose, only one value is listed.

methanesulfonate, which possess an amino acid moiety, were substantially dissolved in distilled water or physiological saline, we could inject all these compounds intravenously. As to the substituent (Y), compounds (**13**, **14**, **16**) (Y=CH_3) showed higher solubility than compounds (**5**—**7**) (Y=Cl). Among all the derivatives, the methanesulfonate compound (**18**) showed the highest solubility. These water-soluble benzoylphenylureas showed good antitumor activities when injected intravenously, and the effect of the structure of the amino acid moiety was small. In addition, their dosage levels were almost the same as those in the intraperitoneal injection of corresponding 2-aminobenzoylphenylureas (**2**—**4**). These results suggest that water-soluble benzoylphenylureas which possess an amino acid moiety can efficiently regenerate the parent 2-aminobenzoylphenylureas *in vivo*. In fact, we observed that **16** disappeared and **2** appeared in blood after **16** was intravenously administered to mice. Furthermore, the IC_{50} (μM) against various human tumor cell lines (leukemia, non-small cell lung, small cell lung, colon cancer, central nervous system (CNS)-cancer, melanoma, ovarian cancer, and renal cancer) of **16** and **2** was 2.5—46.9 and 0.01—0.04, respectively.

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL JNM-GSX400 spectrometer with tetramethylsilane as an internal standard, and the abbreviations of signal patterns are as follows: s, singlet; d, doublet; t, triplet; dd, double doublet; m, multiplet; br, broad.

Preparation Example of 2-Aminobenzoylphenylureas. *N*-(2-Aminobenzoyl)-*N*′-[4-(5-bromo-2-pyrimidinyloxy)-3-chlorophenyl]urea (2) Iron powder (0.57 g, 10 mmol) was gradually added to a solution of **1** (1.0 g, 2 mmol) in AcOH (30 ml) at 80 °C. The reaction mixture was stirred at 80 °C for 30 min, then poured into water. The insoluble product was collected by filtration, dried *in vacuo* and purified by column chromatography on silica gel (hexane : EtOAc=7 : 3) to give **2** (0.4 g, 43.2%), mp 196—200 °C. *Anal*. Calcd for $C_{18}H_{13}BrClN_5O_3$: C, 46.73; H, 2.83; N, 15.14. Found: C, 46.44; H, 2.98; N, 14.91. ^{1}H-NMR (DMSO-d_6) δ: 6.61 (1H, t, J=8 Hz), 6.67 (2H, br s), 6.84 (1H, d, J=8 Hz), 7.31 (1H, t, J=8 Hz), 7.47 (1H, d, J=9 Hz), 7.61 (1H, dd, J=9, 2 Hz), 7.77 (1H, d, J=7 Hz), 8.01 (1H, d, J=2 Hz), 8.90 (2H, s), 10.71 (1H, br s), 10.91 (1H, s).

Preparation Example of 2-Aminoacylaminobenzoylphenyureas. *N*-[4-(5-Bromo-2-pyrimidinyloxy)-3-chlorophenyl]-*N*′-(2-glycylamino)benzoylurea Hydrochloride (5) WSCI·HCl (9.11 g, 48 mmol) and **2** (20.0 g, 43 mmol) were added to a solution of 4-dimethylaminopyridine (5.80 g, 47 mmol) in anhydrous CH_2Cl_2 (1000 ml), successively. The mixture was stirred at room temperature for 15 min. *N*-Boc-glycine (8.33 g, 48 mmol) was added to the mixture, and the mixture was stirred at room temperature for 40 h. Insoluble substance was removed by filtration and was washed with CH_2Cl_2. The filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (CH_2Cl_2 : EtOAc=9 : 1) to give *N*-[4-(5-bromo-2-pyrimidinyloxy)-3-chlorophenyl]-*N*′-(2-Boc-glycylamino)benzoylurea (3.5 g, 13.1%), mp 145—192 °C.

This benzoylurea (5.08 g, 8.2 mmol) was reacted with trifluoroacetic acid (48 ml) at room temperature for 1.5 h with stirring. An excess amount of trifluoroacetic acid was evaporated under reduced pressure, and Et_2O was added to the residue. The mixture was stirred at room temperature for 1 h, then the precipitated product was collected by filtration to give *N*-[4-(5-bromo-2-pyrimidinyloxy)-3-chlorophenyl]-*N*′-(2-glycylamino)benzoylurea trifluoroacetate (3.35 g, 64.5%), mp 212—245 °C (dec.).

Excess hydrogen chloride gas was introduced into a solution of the above trifluoroacetate (1.37 g, 2.2 mmol) in *N*,*N*-dimethylformamide (DMF, 3 ml) and MeOH (12 ml) at 0 °C. The reaction mixture was allowed to stand at room temperature for 5 min, then the precipitated product was collected by filtration and washed with MeOH. The product was dried *in vacuo* to give **5** (1.08 g, 88.3%) as a white powder, mp 201—203 °C. *Anal*. Calcd for $C_{20}H_{17}BrC_{l2}N_6O_4$: C, 43.19; H, 3.08; N, 15.11. Found: C, 42.92, H, 3.21, N, 15.03. ^{1}H-NMR (DMSO-d_6) δ: 3.79 (1H, s), 3.81 (1H, s), 7.30 (1H, t, J=8 Hz), 7.40 (1H, d, J=9 Hz), 7.51—7.67 (4H, m), 7.93 (1H, d, J=2 Hz), 8.24 (3H, br s), 8.83 (2H, s), 10.59 (1H, s), 10.74 (1H, s), 11.13 (1H, s).

Preparation of *N*-[4-(5-Bromo-2-pyrimidinyloxy)-3-chlorophenyl]-*N*′-[2-(*N*,*N*-dimethylglycyl)amino]benzoylurea Hydrochloride (6) WSCI·HCl (11.0 g, 57 mmol) and **2** (23.7 g, 51 mmol) were added to a solution of 4-dimethylaminopyridine (7.06 g, 58 mmol) in anhydrous CH_2Cl_2 (900 ml), successively. The mixture was stirred at room temperature for 15 min. *N*,*N*-Dimethylglycine (5.94 g, 58 mmol) was added to the mixture, and the mixture was stirred at room temperature for 40 h. Insoluble substance was removed by filtration and was washed with CH_2Cl_2. The filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (CH_2Cl_2 : hexane : EtOAc=1 : 1 : 1) to give *N*-[4-(5-bromo-2-pyrimidinyloxy)-3-chlorophenyl]-*N*′-[2-(*N*,*N*-dimethylglycyl)-amino]benzoylurea (2.86 g, 10.2%), mp 192—193 °C.

Excess hydrogen chloride gas was introduced into a solution of the above benzoylurea (0.5 g, 0.9 mmol) in DMF (2 ml) and CH_2Cl_2 (10 ml) at 0 °C. The reaction mixture was allowed to stand at room temperature for 2 h, and CH_2Cl_2 was evaporated. Then, Et_2O was added to the residue, and precipitated product was collected by filtration and dried *in vacuo* to give **6** (0.42 g, 79.9%) as a white powder, mp 164—169 °C. *Anal*. Calcd for $C_{22}H_{21}BrCl_2N_6O_4$: C, 45.23; H, 3.62; N, 14.38. Found: C, 45.01; H, 3.90; N, 14.05. ^{1}H-NMR (DMSO-d_6) δ: 2.83 (3H, s), 2.84 (3H, s), 4.15 (1H, s), 4.16 (1H, s), 7.33 (1H, t, J=7 Hz), 7.39 (1H, d, J=9 Hz), 7.48—7.53 (2H, m), 7.59 (1H, t, J=8 Hz), 7.64 (1H, d, J=9 Hz), 7.92 (1H, d, J=2 Hz), 8.83 (2H, s), 10.04 (1H, br s), 10.95 (1H, s), 11.16 (1H, s).

Preparation of *N*-[4-(5-Bromo-2-pyrimidinyloxy)-3-methylphenyl]-*N*′-[2-(*N*,*N*-dimethylglycyl)amino]benzoylurea Methanesulfonane (18) WSCI·HCl (16.9 g, 88 mmol) and **3** (30.0 g, 68 mmol) were added to a solution of 4-dimethylaminopyridine (10.77 g, 88 mmol) in anhydrous CH_2Cl_2 (1000 ml), successively. The mixture was stirred at room temperature for 15 min. *N*,*N*-Dimethylglycine (9.1 g, 88 mmol) was added to the mixture, and the mixture was stirred at room temperature for 18 h and refluxed for 23 h. Insoluble substance was removed by filtration and was washed with CH_2Cl_2. The filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (CH_2Cl_2 : hexane : EtOAc=5 : 1 : 4). The eluate was concentrated under reduced pressure, and recrystallized from CH_2Cl_2 and hexane to give *N*-[4-(5-bromo-2-pyrimidinyloxy)-3-methylphenyl]-*N*′-[2-(*N*,*N*-dimethylglycyl)amino]ben-

zoylurea (10.9 g, 30.4%), mp 186—192 °C.

The above benzoylurea (1.0 g, 1.9 mmol), methanesulfonic acid (0.173 g, 1.8 mmol) and distilled water (300 ml) were stirred at room temperature for 2 h. The mixture was fitered and the filtrate was lyophilized to give **18** (0.89 g, 79.3%) as a white powder, mp 119—127 °C. *Anal*. Calcd for $C_{24}H_{27}BrN_6O_7S$: C, 46.23; H, 4.37; N, 13.48. Found: C, 46.42; H, 4.60; N, 13.21. ^{1}H-NMR (DMSO-d_6) δ: 2.07 (3H, s), 2.34 (3H, s), 2.84 (6H, s), 4.14 (2H, s), 7.12 (1H, d, J=8 Hz), 7.34 (1H, t, J=7 Hz), 7.41—7.65 (5H, m), 8.78 (2H, s), 9.38 (1H, br s), 10.57 (1H, s), 10.67 (1H, s), 11.08 (1H, s).

Solubility in Water, Physiological Saline, Polyethylene Glycol Aqueous Solution and 5% Glucose About 2 mg of each of the compounds was accurately weighed and was completely dissolved in 2 ml *N*,*N*-dimethylacetamide. The content was transferred into a 10 ml volumetric flask, and its volume was made constant with CH_3CN. The resulting solution (3 ml) was placed in a 50 ml volumetric flask, and its volume was adjusted to a constant with CH_3CN to prepare a standard solution. Then, the proper amount of each of the compounds was weighed and placed in an agate mortar. Test liquid (1.5 ml) was added to the compound, and the content was mixed for 5 min. A suspension thus obtained was transferred into a 1.5 ml microtube, and was centrifuged at 15000 rpm. for 10 min. The supernatant liquid was placed in a 1 ml microtube and was centrifuged again under the same conditions. The supernatant liquid (0.1 ml) thus obtained was diluted with 0.9 ml of CH_3CN to prepare a measuring sample. The above standard solution and the measuring sample were analyzed by HPLC, and their solubilities were measured by external standard method.

Formulation Method Compound (0.125 part by weight) was dissolved in 5 parts by the weight of *N*,*N*-dimethylacetamide and 5 parts by the weight of polyoxyethylene sorbitan monooleate, then 90 parts by the weight of physiological saline was added thereto to form a solution formulation (in the case of compounds **1**—**4**, the mixture is a suspension formulation) in an agate mortar.

Biological Testing Method Anititumor activities were tested by means of the protocols used for routine screening at the National Cancer Institute (Bethesda, MD, U.S.A.). To BDF1 mice, P388 leukemia cells were intraperitoneally inoculated in an amount of 1×10^6 cells/mouse. A formulated compound was intraperitoneally or intravenously administered to mice on days 1, 5 and 9 after the inoculation. Groups of five mice per dose level of the test compound were used, with one control group of five mice. The mice were observed for 50 d for survival or death. The antitumor activity of the compounds was expressed as follows:

$$\frac{\text{median survival time of treated group}}{\text{median survival time of control}}\times 100\ (T/C)$$

Median survival times of the control group ranged from 9.0 to 10.6 d. Any sample with a T/C value that exceeded 125% was evaluated as antitumor-active.

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