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Differentiation of Friend Leukemia Cells Induced by β-, γ-, δ- and Aza-β-lactam, and Thiaziadine Compounds

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β-, γ-, δ- and aza-β-lactam, and thiaziadine compounds induced the differentiation of Friend leukemia cells and hemoglobin was produced. Modification of the lactam rings of γ- and δ-lactam compounds with aromatic side chains and bromine atoms enhanced their differentiation-inducing-activities to Friend leukemia cells. On the other hand, 4 thiaziadine compounds (AKT-1, AKT-2, AKT-3 and AKT-4), which commonly contain a –N(CH_3)–N(CH_3)–moiety, also promoted the differentiation of Friend leukemia cells. They had specific differentiation-inducing-abilities without toxic effects to Friend leukemia cells. 5-Aza-β-lactam compounds showed differentiation-inducing-activity toward all of three types of Friend leukemia cells, two of which were dimethylsulfoxide (DMSO)-sensitive and the other of which was DMSO-resistant. But we did not find any correlation between the differentiation-inducing-activity and the chemical group at the 1-imino position. The presence of an amino bond, which commonly exist in differentiation agents, was suggested to be necessary for the induction of the differentiation of Friend leukemia cells, and this ability was enhanced with the addition of aromatic side groups.

Cells, infected with Friend virus, in culture (Friend leukemia cells) afford a useful experimental system for studying the mechanism of cell differentiation[1,2] and an approach to cancer chemotherapy.[3,4] Friend leukemia cells, which were treated with DMSO, were shown to differentiate into erythroblast and to produce hemoglobin. Recently, many chemical agents, which induce the differentiation of Friend leukemia cells, have been isolated (dimethylsulfoxide,[5] *N*,*N*-dimethylacetamide,[6] 1-methyl-2-piperidone,[7] short chain fatty acids,[8] hemin,[9] purine derivatives,[10] hexamethylene bisacetamide,[11,12] benzodiazepine,[13] actinomycin-D,[14] and proteases.[15]) These reagents can be divided into two majar classes, (1) agents which interact with the cell membrane, and (2) agents which interact with DNA. But it is not clear whether there is any correlation between the structures of the agents and the ability of inducing differentiation except in the case of hexamethylene bisacetamide (HMBA). So we investigated the correlation, if any, between the structures of β-, γ-, δ- and aza-β-lactam compounds and the ability of inducing differentiation, and we found a correlation. We describe the correlation in this paper.

MATERIALS AND METHODS

Materials. β-, γ-, δ- and aza-β-lactam and thiaziadine compounds were synthesized as described previously.

Cell lines and culture conditions: Friend leukemia cells, F5-5 and C9-6, kindly provided by Dr. Ikawa, were establshed from DDD mouse. DMSO induced differentiation of F5-5 but not C9-6 cells. C1745A cells, which are induced by DMSO, were established from DBA/2 mouse and kindly provided by Dr. Onodera. The culture media used were Ham's F-12 for F5-5 and C9-6, and Dulbecco's MEM for C1745A, each supplemented with 10% fetal calf serum. The cells were cultured at 37°C for 3～4 days in a 5% CO_2 incubator, with an initiate cell density of 2×10^4 cells/ml for F5-5 and C9-6, and of 1×10^5 cells/ml for

C1745A. To measure the differentiation-inducing-activity, cells were suspended in a final volume of 0.2 ml, to which 0.02 ml of the samples or their dilutions were added and cultured under the same conditions for 6 days. Viable cells were determined by the trypan blue dye exclusion method and counted with a Burker–Turk counter.

Determination of differentiation: The differentiation of Friend leukemia cells was assayed by the benzidine staining method.[18] On the 6th day, 0.2 ml of a cell suspension was mixed with 0.02 ml of a freshly prepared benzidine solution (10 to 1 mixture of 2% 3,3-dimethoxybenzidine in 0.5 M acetic acid and 30% hydrogen peroxide). Cells that were stained blue, indicating the presence of hemoglobin, were scored in a hemacytometer as the percentage of the total cell number.

RESULTS

β-Lactam compounds

Diphenylmethylenehydrazine, which has no lactam ring, showed no differentiation-inducing-activity and little cytotoxicity toward Friend leukemia cells at concentrations up to 50 μg/ml (Table I). Mono- and diphenylmethylene amino-β-lactams did not induce the differentiation of three kinds of Friend leukemia cells. Suitable lactam ring structures were required for the differentiation-inducing-activity since a change in the ring structure from a β-lactam containing four carbons (AKB-7) an γ-lactam (AK-1) and δ-lactam (AK-2) led to differentiation of and cytotoxicity toward Friend leukemia cells.

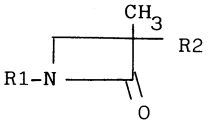
AK-64, which contains both an *N*-phenylthiocarbamoyl group as a side group and a 3-methyl group at the β-lactam ring, showed weak differentiation-inducing activity only toward F5-5, inducing only 11.5% of the cells at a concentration of 62.5 μg/ml. Whether the *N*-phenylthiocarbamoyl group or the 3-methyl group contributed to the differentiation-inducing-activity was not determined.

γ-Lactam compounds-1

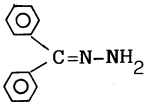
Introduction of the phenyl group at the R1

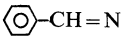
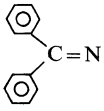
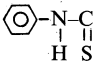
TABLE I. EFFECTS OF β-LACTAM COMPOUNDS ON THE DIFFERENTIATION OF AND CYTOTOXICITY TOWARD FRIEND LEUKEMIA CELLS, F5-5, C9-6 AND C1745A

β-lactam compounds



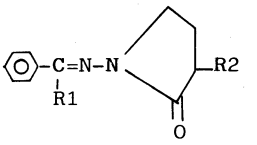
AK-8



Compound	Structure R1	Structure R2	Conc. (μg/ml)	Relative viable cells (%) F5-5	Relative viable cells (%) C9-6	Relative viable cells (%) C1745A	Benzidine positive cells (%) F5-5	Benzidine positive cells (%) C9-6	Benzidine positive cells (%) C1745A
			0	100	100	100	0	0	0.4
AK-8	—	—	6.3	96	94	115	0	0	0.5
			12.5	78	80	123	0	0	0.4
			25.0	83	75	71	0	0	0.3
			50.0	82	76	74	0	0	0.2
AK-7		Br	6.3	102	90	98	0	0	0.3
			12.5	80	87	101	0	0	0.4
			25.0	84	80	90	0	0	0.3
			50.0	50	38	43	0	0	0.5
AKB-7		Br	31.3	85	84	87	0	0	0.3
			62.5	92	66	79	0	0	0.4
			125.0	60	38	60	0	0	0.2
			250.0	47	2	35	0	0	0.2
AK-64		CH_3	31.3	118	96	—	1.3	0	—
			62.5	78	60	—	11.5	0	—
			125.0	29	18	84	0	0	0.2
			250.0	0	1	68	ND	ND	0.2

ND, not determined.

TABLE II. RELATIONSHIPS BETWEEN THE STRUCTURES OF γ-LACTAM COMPOUNDS AND THE DIFFERENTIATION-INDUCING-ACTIVITY TOWARD FRIEND LEUKEMIA CELLS, F5-5, C9-6 AND C1745A



Compound	Structure		Conc. (μg/ml)	Relative viable cells (%)			Benzidine positive cells (%)		
	R1	R2		F5-5	C9-6	C1745A	F5-5	C9-6	C1745A
			0	100	100	100	0	0	0.4
AK-1	⬡	Br	0.20	119	97	—	0	0	—
			0.39	54	11	108	5.8	0	0.2
			0.78	2	0	107	0	ND	0.2
			1.56	0	—	72	ND	—	0.3
AK-6	H	Br	0.49	117	98	98	0	0	0.2
			0.98	93	32	95	0	0	0.2
			1.95	45	4	103	0	0	0.3
			3.91	3	1	84	9.4	0	0.3
AK-43	⬡	H	15.6	93	—	—	2.2	—	—
			31.3	84	96	103	6.3	0	0.2
			62.5	101	92	96	9.6	0	0.3
			125.0	66	80	85	7.6	0	0
AK-42	H	H	31.3	107	—	—	0	—	—
			62.5	94	95	123	0	0	0.2
			125.0	87	93	88	0	0	0.2
			250.0	43	47	85	0	0	0.3

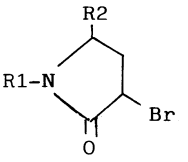
ND, not determined.

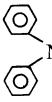
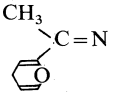
position and/or a bromine atom at the R2 position of the *N*-phenylmethylene-amino-lactam enhanced the induction of the differentiation of F5-5, but not that of C9-6 or C1745A, as shown in Table II. AK-42, which lacks one phenyl group and a bromine atom, was inactive as an inducer of differentiation of F5-5 and its cytotoxicity was also extremely weak toward all three kinds of Friend leukemia cells. Introduction of a phenyl group into AK-42 (AK-43) led to differentiation-inducing-activity, at a relatively high concentration, toward F5-5. Introduction of a bromine atom into AK-42 (AK-6) changed its biological properties, both its differentiation-inducing and cytotoxic activities being augmented. AK-1, which contains both a diphenyl group and a bromine atom, showed the most potent differentiation-inducing and cytotoxic activities toward F5-5 among the compounds listed in Table II. The concentration required for the differentiation was only 0.39 μg/ml for AK-1, being one-tenth that for AK-6.

γ-Lactam compounds-2

The requirement of side groups of γ-lactam compounds for differentiation-inducing activity was examined. Three kinds of side groups, which bind at the nitrogen atom of the γ-lactam ring affected the biological potency against F5-5 and that weakly against C1745A (Table III). The distance between the aromatic group and the lactam ring showed no relation to the ability of differentiation induction, since compounds possessing CH_2–, N– and C=N– bridges promoted the differentiation of F5-5. However, the levels of differentiation-inducing-activity differend. AK-85, in which an *N,N*-diphenyl amino group (*N*-bridge) directly binds to the lactam ring, was more toxic than

TABLE III. DIFFERENTIATION-INDUCING AND CYTOTOXIC ACTIVITIES OF γ-LACTAM COMPOUNDS TOWARD FRIEND LEUKEMIA CELLS, F5-5, C9-6 AND C1745A



Compound	Structure R1	Structure R2	Conc. (μg/ml)	Relative viable cells (%) F5-5	Relative viable cells (%) C9-6	Relative viable cells (%) C1745A	Benzidine positive cells (%) F5-5	Benzidine positive cells (%) C9-6	Benzidine positive cells (%) C1745A
			0	100	100	100	0	0	0
AK-5		H	4.88	107	97	—	0	0	—
			9.77	111	62	70	1.5	0	0.5
			19.50	78	29	92	11.7	0	0.4
			39.10	11	6	56	18.0	0	0.2
AK-21		CH_3	1.95	119	98	105	0	0	0.3
			3.91	104	61	79	3.8	0	0.5
			7.81	69	26	71	23.0	0	0.4
			15.60	2	0	33	16.7	ND	2.0
AK-85		H	0.24	89	120	105	0	0	0.4
			0.49	111	104	111	2.5	0	0.5
			0.98	77	82	91	3.0	0	0.4
			1.95	9	22	77	0	0	3.9
AK-78		H	0.98	100	92	99	0	0	0.2
			1.95	105	74	91	6.2	0	0.4
			3.91	93	18	111	3.3	0	0.3
			7.81	1	0	71	ND	ND	0.5

ND, note determined.

the other compounds listed in Table III.

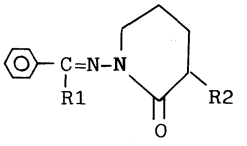
δ-Lactam compound

AK-2, which contains two phenyl groups and a bromine atom showed two distinct features as to the differentiation-inducing-activity, as shown in Table IV. The number of benzidine positive in F5-5 cells induced by AK-2 was six times higher than that by AK-1. Secondly, it showed differentiation-inducing activity not only toward F5-5 but also C9-7 to 0.78 μg/ml and weakly toward C1745A at 3.13 μg/ml. AK-44, which lacks a phenyl group and a bromine atom, was completely inactive as an inducer. It showed a cytotoxic effect on the three kinds of Friend leukemia cells at a concentration of 125 μg/ml. Introduction of bromide atoms into AK-44 (AK-41) led to both differentiation of and cytotoxicity toward F5-5 and C9-6 but not C1745A. AK-41 induced a similar number of benzidine positive cells to that with AK-2 only at an approximately 20 times higher concentration than that of AK-2. Benzaldehyde 5-chlorohexanoylhydrazone, which is a linear analogue of that lactam ring, did not induce differentiation of any of the three kinds of Friend leukemia cells at 500 μg/ml.

Aza-β-lactam compounds

Five kinds of aza-β-lactam compounds, as shown in Table V, initiated hemoglobin biosynthesis of both DMSO-sensitive cells (F5-5 and C1745A) and DMSO resistant cells (C9-6). Substitution of *n*-butyl (AK-9) for benzyl (AK-10) and cyclohexyl (AK-8) at the R1 position had little effect on the differentiation-inducing-activity.

TABLE IV. RELATIONSHIPS BETWEEN THE STRUCTURES OF δ-LACTAM COMPOUNDS AND THE DIFFERENTIATION-INDUCING-ACTIVITY TOWARD FRIEND LEUKEMIA CELLS, F5-5, C9-6 AND C1745A



Compound	Structure		Conc. (μg/ml)	Relative viable cells (%)			Benzidine positive cells (%)		
	R1	R2		F5-5	C9-6	C1745A	F5-5	C9-6	C1745A
			0	100	100	100	0	0	0.5
AK-2	⬡	Br	0.20	112	103	—	0	0	—
			0.39	105	108	68	6.0	0	0.3
			0.78	71	92	80	35.7	4.4	0.2
			1.56	25	22	55	28.3	20.0	0.3
			3.13	0	0	29	ND	ND	3.6
AK-41	H	Br	3.9	111	96	—	0	0	—
			7.8	99	71	77	3.3	0	0.2
			15.6	96	38	105	7.3	0	0.3
			31.3	33	18	71	28.7	13.1	0.3
AK-44	H	H	31.3	84	84	69	0	0	0.2
			62.5	62	58	62	0	0	0.2
			125.0	50	49	47	0	0	0.3
			250.0	5	62	50	0	0	0.1

ND, not determined.

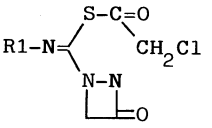
Thiaziadine compounds

Thiaziadine compounds, as shown in Table VI, induced the differentiation of DMSO-sensitive F5-5 and C1745A, but not of DMSO-resistant C9-6 cells (Table VI). A relatively high number of benzidine positive cells was observed when AKT-1, AKT-2 and AKT-3 were used at 15.6 to 125 μg/ml, a concentration range in which the growth of Friend leukemia cells was only slightly inhibited. These results suggested that thiaziadine compounds affected Friend leukemia cells in a manner similar to in the case of DMSO. As to structure–activity relationships, there were the following three findings. (1) The *N*-methyl group at the R3 position was necessary for enhancement of the activity since AK-24 induced one-tenth the extent of differentiation induced by AK-79. (2) Introduction of a phenyl group at the R2 position reduced the ability of the compounds to induce differentiation. However, the biological potency was unaltered when the R2 substituent was a methyl or ethyl group. (3) Substitution of cyclohexyl (AK-79) for benzyl (AKT-1) at the R1 position had little influence on differentiation-inducing-activity.

DISCUSSION

More than thirty kinds of hetero cyclic compounds were examined as to their ability to induce the differentiation of Friend leukemia cells. Consequently, a typical construction among the inducers was found in this study which was thought to be required for the interaction with DNA and the express the biological properties of Friend leukemia cells, *i.e.*, the differentiation along the erythroid pathway. The common structural moiety in the inducers was an amide bond, and benzyl or phenyl groups attaching to –C=N– or =N– were found to be effective side groups. β- and γ-lactams toward differentiation-inducing-activity toward F5-5 but not toward C9-6 or

TABLE V. RELATIONSHIPS BETWEEN THE STRUCTURES OF AZA-β-LACTAM COMPOUNDS AND THE DIFFERENTIATION-INDUCING-ACTIVITY TOWARD FRIEND LEUKEMIA CELLS, F5-5, C9-6 AND C1745A



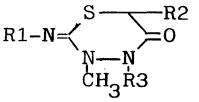
Compound	Structure R1	Conc. (μg/ml)	Relative viable cells (%) F5-5	C9-6	C1745A	Benzidine positive cells (%) F5-5	C9-6	C1745A
		0	100	100	100	0	0	0.2
AK-67	CH_3	31.3	90	85	—	0	0	—
		62.5	93	56	79	0	0	0.2
		125.0	85	35	62	1.7	8.2	6.5
		250.0	16	24	37	16.8	0	13.0
AK-9	n-C_4H_9	0.78	101	94	108	0	0	0.4
		1.56	107	90	101	10.3	0	0.2
		3.13	93	81	106	30.8	4.7	0.5
		6.25	3	1	67	0	ND	15.2
AK-68		15.6	—	89	—	—	0	—
		31.3	91	46	86	0	1.9	0.3
		62.5	81	22	70	0	7.2	0.2
		125.0	13	9	22	0	0	7.4
AK-10		0.78	91	92	100	0	0	0.4
		1.56	93	91	112	0	1.7	0.2
		3.13	103	91	108	23.8	5.4	0.3
		6.25	55	37	109	35.0	6.5	5.8
AK-8		0.78	110	103	118	0	0	0.4
		1.56	110	92	124	4.2	0	0.2
		3.13	91	78	112	9.6	3.0	0.3
		6.25	94	33	72	21.0	5.8	5.2

ND, not determined.

C1745A, while the related linear forms did not. Furthermore, δ-lactams were 10 times more potent in inducing hemoglobin biosynthesis in not only F5-5 but also C9-6 and C1745A than γ-lactams. Reuben *et al.* reported that polyethylene bis-acetamides are better inducers than structurally related diamines.[11,12] Tanaka *et al.* found that *N*-methyl pyrrolidone, 2-pyrrolidinone and piperidone, which contain an amide bond in the five or six membered ring structure, showed differentiation-inducing-activity of the Friend leukemia cell, C1745A.[7] The benzodiazepine structure–activity relationships showed that the benzodiazepines that induced the differentiation of Friend leukemia cells, C1745A, also possess an amidelinkage.[13] These results showed that a common amide bond must be arranged in suitable conformation to react with the target sites. The active compounds found in this study may react mainly with DNA sites for the differentiation of Friend leukemia cells. Recently, Nomura and Oishi reported the presence of two target sites involved in the differentiation of Friend leukemia cells at the cell membrane and DNA.[19,20] The ring structure consisting of six atoms and an amide bond was considered to be more suitable for altering the configurations of proteins, DNA and protein-DNA complexes.

The structural requirements as to the side group in differentiation induction of Friend leukemia cells were not specific, however, modifications of the side group changed the

TABLE VI. RELATIONSHIPS BETWEEN THE STRUCTURES OF THIAZIADINE COMPOUNDS AND DIFFERENTIATION-INDUCING-ACTIVITY TOWARD FRIEND LEUKEMIA CELLS, F5-5, C9-6 AND C1745A



Compound	Structure			Conc. (μg/ml)	Relative viable cells (%)			Benzidine positive cells (%)		
	R1	R2	R3		F5-5	C9-6	C1745A	F5-5	C9-6	C1745A
				0	100	100	100	0	0	0.3
AKT-1	⬡	H	CH_3	15.6	120	—	82	6.3	—	11.8
				62.5	116	98	94	17.3	0	19.0
				125.0	102	98	99	13.3	0	39.8
				250.0	19	42	63	15.6	0	61.0
AKT-2	⬡	CH_3	CH_3	15.6	123	—	76	3.3	—	0.3
				31.3	121	92	96	6.1	0	9.7
				62.5	123	83	98	14.6	0	34.9
				125.0	28	56	79	16.4	0	28.6
AKT-3	⬡	C_2H_5	CH_3	15.6	103	—	103	8.1	0	16.2
				31.3	123	96	98	17.2	0	21.5
				62.5	118	79	106	26.1	0	55.5
				125.0	15	49	80	15.5	0	45.4
AKT-4	⬡	⬡	CH_3	31.3	99	—	—	1.7	—	—
				62.5	45	—	68	10.4	—	0.3
				125.0	19	85	61	0	0	0.4
				250.0	13	97	77	0	0	0.2
AK-24	⬡	H	H	125.0	105	116	113	3.6	0	0.4
				250.0	120	92	78	3.8	0	0.2
				500.0	105	70	85	0	0	0.1
AK-79	⬡	H	CH_3	15.6	104	60	115	12.9	0	0.3
				31.3	104	54	107	31.9	0	18.4
				62.5	78	26	106	36.2	0	23.2
AK-80	⬡	⬡	CH_3	31.3	61	10	84	0.7	0	0.2
				62.5	69	0	100	2.7	0	0.4
				125.0	72	—	98	0	—	0.3

ND, not determined.

differentiation-inducing-activity. As far as γ- and δ-lactams are concerned, diphenyl methylene was much more potent than monophenyl methylene. In the case of thiaziadines or aza-lactams, substitution of the phenyl group enhanced the activity. A more hydrophilic property of the side group may be suitable for the interaction of drug with cell of DNA sites.

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