Novel Antitumor 2-Cyanoaziridine-1-carboxamides

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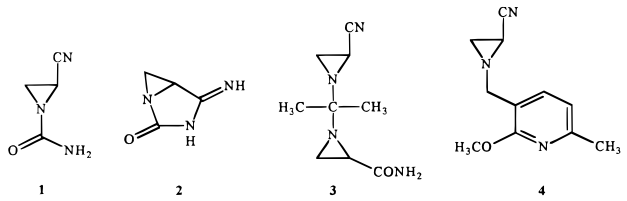
A set of 20 2-cyanoaziridine-1-carboxamides was synthesized from 2-cyanoaziridine and appropriate isocyanates. These compounds were active against a variety of solid and hematological tumor cells in culture, including strains resistant to doxorubicin and mitoxantrone. Their potencies in these assays correlated with the lipophilicity of substituents. The *N*-phenyl derivative was more potent and equally effective to imexon, a cyclized 2-cyanoaziridine-1-carboxamide of clinical interest, against cloned fresh human tumors.

Hundreds of aziridines have been synthesized and screened as potential antineoplastic agents.[1,2] Most of these compounds are substituted on the aziridine nitrogen with groups such as alkyl, aralkyl, aryl, heterocyclic, acyl, alkanoic, carbamate, and dithiocarbamic acid.[1] Clinically significant agents have two or more aziridine rings linked through nitrogen to a phosphorus (thio-TEPA) or a quinone ring (diazaquone).[3,4] The aziridine ring also occurs in complex natural products such as mitomycin C, in which the aziridine nitrogen is unsubstituted.[5] Generally, aziridine antitumor agents act by alkylating nucleophilic atoms on guanine residues in DNA.[3]

In 1975, Bicker reported a new type of carcinostatic aziridine, 2-cyanoaziridine-1-carboxamide (**1**).[6,7] This compound was active against PIE 2-3 sarcoma in Wistar rats, and it had low toxicity. Especially interesting was the observation that it increased the number of leukocytes, rather than decreasing them.[6] In contrast to known aziridines, it showed no alkylating activity toward 4-(4-nitrobenzyl)pyridine.[7] The *N*-phenyl derivative **13** of **1** (see Table 1 for structure) was then synthesized and found active in the PIE 2-3 sarcoma model over a 10-fold dose range.[8] This finding led to the suggestion that **1** and **13** acted indirectly by an effect on immunological mechanisms. Consequently, they prepared some cyanoaziridines including **1**, its *N*-methyl derivative **6**, and its *N*-(4-sulfamylphenyl) derivative **22** (whose structures are shown in Table 1) and evaluated them for oral immunostimulatory activity as measured by increase in leukocyte counts in rats.[9]

When 2-cyanoaziridine-1-carboxamide **(1)** was treated with KOH in methanol, it underwent cyclization to 4-imino-3-diazabicyclo[3.1.0]hexan-2-one (**2**), which is named imexon.[10] Imexon solutions in water slowly revert partially to **1**. Thus, the chemistry and biological activity of imexon is closely related to that of cyanoaziridines. Imexon was active against a variety of transplanted syngeneic tumors in rodents.[11] It also was active against human lymphoma, melanoma, and prostate cancer cell lines in SCID mice.[12] Phase I clinical trials conducted in Europe in 1985 established that imexon was well-tolerated and produced minimal nausea and vomiting even in the absence of prophylactic antiemetics.[13] In one of these trials, objective responses or stabilization of the disease was found in patients with lung cancer, breast cancer, or liver cancer.[14] More recently, it was reported that in fresh human tumor cells in clonogenic assay, imexon was selectively cytotoxic to multiple myeloma.[15]



Many other derivatives of 2-cyanoaziridine have been prepared and screened for antitumor activity. Among them, azimexon (**3**) and ciamexon (**4**) received initial clinical study, but they were not successful. Azimexon is a prodrug that decomposes very quickly in water to acetone, 2-cyanoaziridine, and aziridine-2-carboxamide.[6] The German patent literature contains claims to numerous N-substituted 2-cyanoaziridines. These substituents include esters of carboxylic acids, acylcarboxamides, sulfonylcarboxamides, and phosphorylcarboxamides.[16−19] Some of the compounds have alkyl or phenyl substituents at position 3, and others have the cyano group replaced by carboxamide or carboxylic acid ester. No data was given on the claimed antitumor and immunomodulatory activity.

Despite the quantity and variety of 2-cyanoaziridine derivatives already prepared, we found in the literature only two 2-cyanoaziridine-1-carboxamides, **1** and **13**, with simple substituents such as alkyl or aryl on the amide nitrogen, that had been tested in antitumor assays. Based on the reported activities of these compounds, it appeared desirable to investigate further examples of this structural type. We were especially interested to find if the addition of lipophilic groups to the amide nitrogen of **1** would result in increased cytotoxicity. Consequently, the series of N-substituted 2-cyanoaziridine-1-carboxamides shown in Table 1 was synthesized and tested against a panel of tumor cells in culture.

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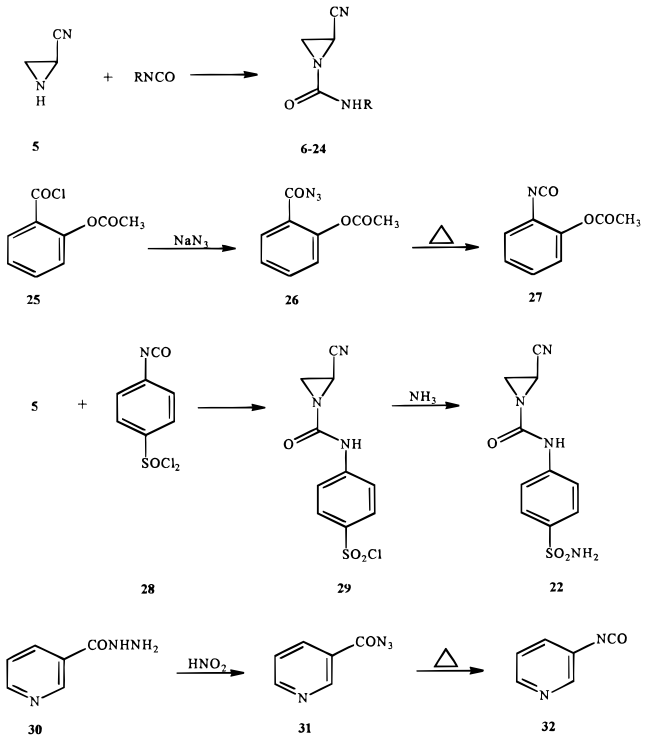
Scheme 1



Chemistry

2-Cyanoaziridine (**5**) was prepared according to the published precedure, which involves treating ethyl 2,3-dibromopropionitrile with ammonia in methanol followed by adding triethanolamine and heating.[20] Conversion of **5** into the desired N-substituted 1-carboxamide derivatives **6**−**21**, **23**, and **24** was effected simply by stirring it with the appropriate isocyanate in toluene or benzene at ice-bath temperature. For cases **6**−**21** the isocyanates were commercially available (Scheme 1). In certain other cases, the isocyanates had to be synthesized. Thus, 2-acetylphenyl isocyanate (**27**) was prepared by treating 2-acetoxybenzoyl chloride (**25**) with sodium azide, and the resulting acid azide (**26**) was heated in benzene under nitrogen at 70−75 °C. 3-Pyridyl isocyanate (**32**) was made from nicotinic acid hydrazide (**30**) by way of nicotinic azide (**31**) according to the published method.[21] In the synthesis of *N*-(4-sulfamylphenyl) analogue **22**, 2-cyanoaziridine (**5**) was condensed with 4-chlorosulfonylphenyl isocyanante **28** and the resulting intermediate **29** was treated with liquid ammonia (Scheme 1). The yields and properties of these products are given in Table 1.

Biology and QSAR

Relative potencies of the N-substituted 2-cyanoaziridine-1-carboxamides in a panel of tumor cell cultures are compared in Table 2. This panel includes the following lines of human tumor cells: sensitive and imexon-resistant lines of multiple myeloma;[22] ovarian carcinoma resistant to standard drugs;[23] melanoma with a slow growth fraction;[24] breast carcinoma including sensitive, doxorubicin-resistant (P-glycoprotein-positive),[25] and mitoxantrone-resistant (P-glycoprotein-negative)[26] lines; sensitive and multidrug-resistant colon carcinoma;[27,28] and multidrug-resistant lung carcinoma (P-glycoprotein-negative).[24] It also has sensitive[29] and multidrug-resistant (P-glycoprotein-positive)[33] murine L1210 leukemia cells.

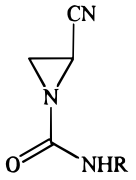
Table 2 shows clearly that imexon is selective for multiple myeloma and that it is more potent than 2-cyanoaziridine-1-carboxamide (**1**) as expected from previous publications. Addition of lipophilic groups to the amide nitrogen significantly increased the cytotoxicity and decreased the selectivity so that in many cases the compounds were roughly equipotent across the spectrum of tumor cell types. Even the methyl group showed this effect. Among the alkyl-substituted compounds, there was a trend to greater potency as the lipophilicity increased, except that the bulky *tert*-butyl group has much reduced potency. Presumably, it is too large to fit into whatever receptor is critical in cell death. The *N*-aryl groups, including naphthyl, significantly increase cytotoxicity. Compounds with 4-fluorophenyl (**14**), 4-nitrophenyl (**16**), 4-carbethoxyphenyl (**19**), and naphthyl (**23**) have IC_{50} values less than 10 μM for all of the tumor cell types. A statistically significant correlation (99% confidence limit) was found between antitumor potency for sensitive multiple myeloma and the lipophilicity of substituents as represented by their contributions (π) to the octanol−water partition coefficients, using simple linear regression and the program Sigmastat.[31] The data for this correlation is given in Table 3. For all 17 compounds with definite IC_{50} values, the equation is $\log(1/C) = 4.25 + 0.325\pi$ ($r^2 = 0.39$, $F = 9.44$, $Es_i = 0.23$, $Es_v = 0.11$, where Es_i is the standard deviation of the intercept and Es_v is the standard deviation of the variable). A plot of this equation is given in Figure 1. When the bulky *tert*-butyl-containing analogue **9** is removed, the equation becomes $\log(1/C) = 4.30 + 0.330\pi$ ($r^2 = 0.49$, $n = 16$, $F = 13.4$, $Es_i = 0.20$, $Es_v = 0.09$).

Assays of antitumor agents against fresh human tumor-colony-forming cells provide results that predict better the responses obtained in treating cancer patients than those obtained with long established human cell lines.[32] For this reason, the *N*-phenyl derivative **13** was tested against a variety of fresh human tumors obtained at the Arizona Cancer Center using established methodology.[33] Table 4 gives a comparison of the potency of **13** with that of imexon in eight different tumor cell types from 44 patients. This table shows that **13** at a concentration of 0.2 μM is about as effective as imexon at 1.0 μM. Both compounds have similar profiles of activity at these concentrations, with myeloma cells being the most sensitive and breast carcinoma, melanoma, and ovarian carcinoma cells also being inhibited significantly. Compound **13** appears to be superior against the sarcoma cells.

Conclusions

2-Cyanoaziridine-1-carboxamides with alkyl, aryl, and other substituents on the amide nitrogen have greater potency than the N-unsubstituted parent compound against tumor cells in culture. This greater potency correlates with the lipophilicity of the substituents, which suggests that cell penetration may be an important factor in cytotoxicity. Some of them have greater potency and a broader spectrum of activity against tumor cells including activity against resistant tumor cell lines. The one analogue tested in fresh human tumor cells, *N*-phenyl derivative **13**, was similar in

Table 1. Properties of New 2-Cyanoaziridine-1-carboxamides[a]



no.	R	yield, %	solvent impurity	mp, °C	^{1}H NMR signals, δ (ppm from TMS)
6	CH_3[i]	94		98−100	2.47 (d, 1, J = 3 Hz), 2.57 (d, 1, J = 6 Hz), 2.8 (d, 3, J = 5 Hz), 3.05 (dd, 1, J = 6, 3 Hz), 6.18 (br, s 1, NH)[b]
7	C_2H_5[j]	63		58−62	1.1 (t, 3, 6 Hz), 2.4 (d, 1, J = 3 Hz), 2.50 (d, 1, J = 6 Hz), 2.97 (dd, 1, J = 6, 3 Hz), 3.3 (q, 2, J = 6 Hz), 6.1 (br s, 1, NH)[b]
8	n-C_4H_9	92	0.1H_2O	98−102	1.0 (t, 3), 1.4 (m, 2), 1.6 (m, 2), 2.5 (d, 1, J = 3 Hz), 2.55 (d, 1, J = 6 Hz), 3.0 (dd, 1, J = 6, 3 Hz), 3.2 (m, 2), 6.1 (br s, 1, NH)[b]
9	t-C_4H_9	81		46−48	1.4 (s, 9), 2.84 (d, 1, J = 3 Hz), 2.86 (d, 1, J = 6 Hz), 3.0 (dd, 1, J = 6, 3 Hz), 5.8 (br s, 1, NH)[c]
10	c-C_6H_{11}	64		98−102	1.2−1.5 (m, 5), 1.6−2.1 (m, 5), 2.45 (d, 1, J = 3 HZ), 2.53 (d, 1, J = 6 Hz), 3.015 (dd, 1, J = 6, 3 Hz), 3.6 (m, 1), 5.8 (br s, 1, NH)[b]
11	$C_6H_5CH_2$	25		42−44	2.36 (d, 1, J = 3 Hz), 2.46 (d, 1, J = 6 Hz), 2.93 (dd, 1, J = 6, 3 Hz), 4.4 (d, 2), 6.8 (br s, 1, NH), 7.4 (m, 5)[b]
12	$C_2H_5OCOCH_2$	50	0.75CH_3OH[d]	oil	1.22 (t, 3, J = 7 Hz), 2.49 (d, 1, J = 3 Hz), 2.56 (d, 1, J = 6 Hz), 3.07 (dd, J = 6, 3 Hz), 3.91 (d, 2), 4.15 (q, 2, J = 7 Hz), 6.74 (t, 1, NH)[b]
13	C_6H_5[k]	71		88−90	2.65 (d, 1, J = 3 Hz), 2.69 (d, 1, J = 6 Hz), 3.57 (dd, 1, J = 6, 3 Hz), 7.05 (t, 1), 7.45 (d, 2), 7.60 (d, 2), 10.2 (br s, 1 NH)[c]
14	4-FC_6H_4	54		99−100	2.55 (d, 1, J = 3 Hz), 2.68 (d, 1, J = 6 Hz), 3.20 (dd, 1, J = 6, 3 Hz), 7.0 (d, 2, J = 9 Hz), 7.5 (d, 2, J = 9 Hz), 10.2 (br s, 1 NH)[c]
15	4-$CF_3C_6H_4$	91		166−168	2.62 (d, 1, J = 3 Hz), 2.74 (d, 1, J = 6 Hz), 3.32 (dd, 1, J = 6, 3 Hz), 7.54 (d, 2, J = 9 Hz), 7.74 (d, 2, J = 9 Hz), 10.2 (br s, 1 NH)[c]
16	4-$NO_2C_6H_4$	89	0.1H_2O	>230 dec	2.77 (d, 1, J = 3 Hz), 2.81 (d, 1, J = 6 Hz), 3.69 (dd, 1, J = 6, 3 Hz), 7.8 (d, 2, J = 9 Hz), 8.2 (d, 2, J = 9 Hz), 10.8 (br s, 1 NH)[c]
17	2,4-$C1_2C_6H_3$	50		110−114	2.70 (d, 1, J = 3 Hz), 2.71 (d, 1, J = 6 Hz), 3.57 (dd, 1, J = 6, 3 Hz), 7.44 (d, 1, dd, J = 3 Hz, J = 6 Hz), 7.57 (d, 1, J = 6 Hz), 7.68 (d, 3 Hz), 10.0 (br s, 1, NH)[c]
18	3,4-$C1_2C_6H_3$[e]	76		132−134	2.71 (d, 1, J = 3 Hz), 2.73 (d, 1, J = 6 Hz), 3.62 (dd, 1, J = 6, 3 Hz), 7.5 (dd, 1, J = 9, 3 Hz), 7.6 (d, 1, J = 9 Hz), 7.9 (d, 1, J = 3 Hz), 10.6 (br s, 1, NH)[c]
19	4-$C_2H_5OCOC_6H_4$	90		162−165	1.3 (t, 3, J = 6 Hz), 2.72 (d, 1, J = 3 Hz), 2.76 (d, 1, J = 6 Hz), 3.64 (dd, 1, J = 6, 3 Hz), 4.3 (q, 2, J = 6 Hz), 7.69 (d, 2, J = 9 Hz), 7.73 (d, 2, J = 9 Hz), 10.63 (br s, 1 NH)[c]
20	3-$CH_3COC_6H_5$[f]	74		110−112	2.6 (s, 3), 2.71 (d, 1, J = 3 Hz), 2.74 (d, 1, J = 6 Hz), 3.63 (dd, 1, J = 6, 3 Hz), 7.5 (t, 1, J = 9 Hz), 7.7 (d, 1, J = 9 Hz), 7.85 (d, 1, J = 9 Hz), 8.1 (s, 1), 10.5 (br s, NH)[b]
21	2-$CH_3CO_2C_6H_4$	10		101−102	2.38 (s, 3), 2.55 (br s, 1), 2.64 (br s, 1), 3.20 (br s, 1), 7.15 (br s, 2), 7.2−7.6 (br s, 1), 7.68 (br s, 1), 7.96 (br s, 1, NH)[c,h]
22	4-$H_2NSO_2C_6H_4$[g]	39		170−174	2.72 (d, 1, J = 3 Hz), 2.74 (d, 1, J = 6 Hz), 3.7 (dd, 1, J = 6, 3 Hz), 7.26 (s, 2, NH_2), 7.69 (d, 2), 7.73 (d, 2), 10.6 (br s, 1, NH)[c]
23	1-$C_{10}H_7$	56		98−100	2.6 (br s, 1), 2.7 (br s, 1), 3.2 (br s, 1), 7.4 (br s, 1), 7.5 (m, 3), 7.7 (br s, 1), 7.8 (br s, 2), 8.1 (br s, NH)[b,h]
24	3-C_5H_4N	10	0.2H_2O	205 dec	2.72 (d, 1, J = 3 Hz), 2.76 (d, 1, J = 6 Hz), 3.65 (dd, 1, J = 6, 3 Hz), 7.36 (dd, J = 3 Hz, 9 Hz, 1), 7.97 (dd, J = 3, 6 Hz, 1), 8.28 (dd, J = 3, 6 Hz), 8.71 (s, 1), 10.5 (br s, 1 NH)[c]

[a] Analytical results were within ±0.40% of theoretical values for all elements (C, H, N, C1, S, and F), except as shown in subsequent footnotes. In some examples, water or the solvent impurities indicated in the table had to be added to reconcile the calculated and found values for these elements. [b] The solvent was $CDC1_3$. [c] The solvent was DMSO-d_6. [d] The product was eluted from TLC plate scrapings by CH_3OH. [e] N: calcd, 16.40; found, 15.68. [f] N: calcd, 18.32; found, 17.97. [g] N: calcd, 21.04; found, 19.60. [h] The expected doublets were not resolved and appeared as broad singlets. [i] MS (EI) 125 (M^+). [j] MS (EI) 139 (M^+). [k] MS (EI) 187 (M^+).

activity to imexon and more potent. These results suggest that further studies on **13** as well as other analogues described herein are desirable.

Concerning the possibility that N-substituted aziridine-1-carboxamides are cytotoxic because they cyclize to N-substituted imexon analogues, we have made no in vivo studies; however, such cyclizations appear unlikely because a strongly alkaline solution was required for the formation of imexon from **1**.

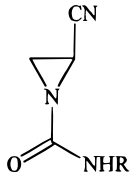
Experimental Section

Melting points were recorded on a Mel-Temp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker 250 WM spectrometer, and absorptions are reported downfield from Me_4Si (δ values in ppm). Mass spectra were recorded on a Varian-MAT311 spectrometer. Elemental analyses were performed by Desert Analytics, Inc., Tucson, AZ.

Preparation of 2-Cyanoaziridine-1-carboxamides (General Method). To an ice-cooled mixture of 2-cyanoaziridine (**5**) and toluene was added an ice-cold solution of an isocyanate (1.05 equiv) in toluene at a rate to keep the temperature below 5 °C. The mixture was stirred for 1 h in an ice bath and then placed in a refrigerator overnight. The resulting precipitate was collected, washed with toluene, and dried under vacuum to give the product. Table 1 gives the yields and properties of the products (**6**−**24**).

2-Cyanoaziridine-1-[*N*-[ethoxycarbonyl)methyl]carboxamide] (**12**) was a colorless oil that did not crystallize on cooling. It was dissolved in cold chloroform and diluted with cold

Table 2. Antitumor Activities of 2-Cyanoaziridine-1-carboxamides



		IC$_{50}$ against human and mouse tumors (μM)[a]											
		8226 myeloma		OVCAR3	A375	L1210 leukemia		MCF7 breast			WiDr colon		A-549
no.	R	sens[b]	res[c]	ovarian	melan	sens	MDR	sens	dox	mitox	sens	res	lung
imexon		17	115	640	324	612	477	>18	>18	>18	>72	>72	>72
1	H	288	432	>90	>90	>90	>90	>54	>54	>54	>280	>280	>280
6	CH_3	26	26	14	17	8.7	21	27	17	18	21	23	22
7	C_2H_5	22	26	14	36	14	14	29	20	10	4	7	18
8	n-C_4H_9	17	17	12	15	12	12	>12	6.7	>12	14	18	18
9	t-C_4H_9	120	216	186	418	132	457	>300	190	120	180	>300	180
10	c-C_6H_{11}	36	98	31	35	7.4	16	13	8.9	9.8	2.4	12	10
11	$CH_2C_6H_5$	9.0	11	5.0	11	3.8	10	7.4	6.0	3.4	5.3	10	7.4
12	$CH_2COOC_2H_5$	25	30		51	13	13	15	17	11	31	33	24
13	C_6H_5	5.3	8.0	6.8	11	1.2	11	2.9	2.6	4.3	5.0	12	9
14	4-FC_6H_4	5.0	5.9	5.9	3.4	7.3	5.9	1.9	2.4	2.7	2.1	9.0	6.2
15	4-$CF_3C_6H_4$	8.2	7.8	5.9	11.8	7.8	7.1	1.5	1.4	1.3	1.3	5.8	4.3
16	4-$O_2NC_6H_4$	9.1	8.6	6.5	3.9	2.6	2.6	3	3	3	4.3	3.7	3.9
17	2,4-$C1_2C_6H_3$	4.7	9.4	35	>39	207	33	0.9	1.1	0.35	2.2	1.9	2.5
18	3,4-$C1_2C_6H_3$	1.1	2.7	5.6	5.9	2.6	7.2	4.6	7.2	4.4	7.0	14	7.5
19	4-$C_2H_5O_2CC_6H_4$	3	3	7.7	5.8	9.7	9.7	2.3	2.1	3.3	2.5	4.2	2.9
20	2-$CH_3CO_2C_6H_4$	>39	>39	>39	>39	>39	>39	>16	>16	>16	40	>41	>41
21	3-$CH_3COC_6H_4$	8.7	9.6	10	7.9	9.6	7.9	3.6	3.2	3.5	3.3	9	5.6
22	4-$C_6H_4SO_2NH_2$	>38	>38	188	188	>38	>38	>15	>15	>15	>38	>38	>38
23	1-naphthyl	8.0	8.9	5.9	4.2	7.2	2.5	2.0	1.7	2.0	2.4	2.5	2.5
24	3-pyridyl	12	15	10	12	11	16	4.2	6.6	5.4	6	18	16

[a] Cytotoxicity against L1210 leukemia was determined by the MTT assay (Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Schoemaker, R. H.; Boyd, M. R. Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay. *Cancer Res.* **1988**, *48*, 589−601), and the SRB assay (Skehan, P.; Strong, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New Colorimetric Assay for Anticancer-Drug Screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112) was used for the other tumor cell lines. [b] Sens indicates a drug-sensitive cell line, and res indicates a resistant cell line. [c] This cell line was made specifically resistant to imexon.

Table 3. Correlation of Antimyeloma Potency of 2-cyanoaziridine-1-carboxamides with π[a]

compd	IC$_{50}$	log(1/*C*)	π
1	288	3.54	0
6	26	4.59	0.50
7	22	4.66	1.02
8	17	4.77	2.00
9	120	3.92	1.98
10	36	4.44	2.51
11	9.0	5.05	2.50
12	25	4.60	1.01
13	5.3	5.27	2.00
14	5.0	5.30	2.14
15	8.2	5.09	2.88
16	9.1	5.04	1.72
17	4.7	5.33	3.42
18	1.1	5.96	3.42
21	8.7	5.06	1.45
23	8.0	5.10	3.32
24	12	4.95	0.32

[a] Antitumor data (IC$_{50}$ against sensitive myeloma) is taken from Table 2. π values were taken from Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology;* Wiley-Interscience: New York, 1979, or calculated using Clog P software.

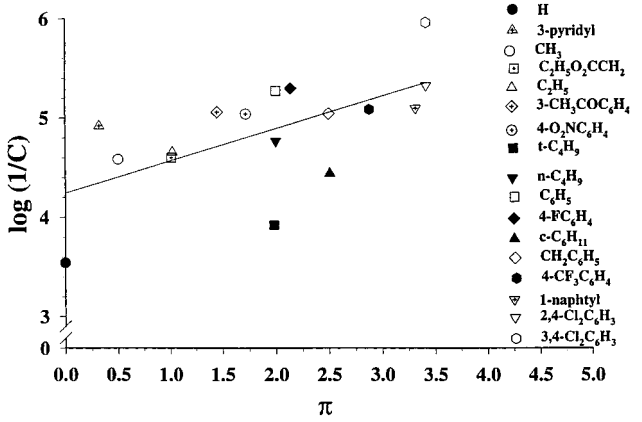


Figure 1. Correlation between substituent lipophilicity (π) and toxicity to multiple myeloma cells for 2-cyanoaziridine-1-carboxamides.

hexane. The mixture was stirred briefly, then the solvent was decanted, and the oil was dried under vacuum.

Preparation of 2-Cyanoaziridine-1-[*N*-(2-acetoxyphenyl)carboxamide] (21). 2-Acetylbenzoic acid azide (**26**) was prepared by treating 2-acetoxybenzoyl chloride (**25**) with 1.1 equiv of sodium azide in acetone and water at 0−5 °C for 24 h. It had a peak at 2245 cm^{-1} in the IR spectrum. Without further purification, it was heated in benzene at 70−75 °C under nitrogen for 2 h to give 2-acetylphenyl isocyanate (**27**). This crude isocyanate was converted into **21** by the general procedure, and the overall yield was 10%. The properties of **21** are given in Table 1.

Preparation of 2-Cyanoaziridine-1-[*N*-(4-sulfamylphenyl)carboxamide] (22). 2-Cyanoaziridine-1-[*N*-(4-chlorosulfonylphenyl)carboxamide] (**24**) was prepared from 2-cyanoaziridine (**29**) and 4-chlorosulfonylphenyl isocynate (**28**) by the general procedure. It had mp 142−144 °C. Without further purification, it was converted by treatment with excess liquid ammonia into **22** in an overall yield of 39%. The properties of **22** are given in Table 1.

Preparation of 2-Cyanoaziridine-1-[*N*-(3-pyridyl)carboxamide] (24). 3-Pyridyl isocyanate (**32**) was prepared from nicotinic acid hydrazide (**30**) by way of nicotinic acid azide (**31**)

Table 4. Activity of **13** and Imexon against Cloned Fresh Human Tumors[a]

		no. sensitive					
		imexon (μM)			**13** (μM)		
tumor type	no. tested	0.1	1.0	10	0.2	2.0	2.0
breast	4	0	2	3	2	2	3
lung	2	0	0	2	0	1	2
melanoma	10	3/5	4	8	3/5	8	10
myeloma	3	3	3	3	2/2	2/2	2/2
ovary	12	4	3/11	8	3/10	10/11	10
other gynecologic	4	0	0	1	0	2	3
stomach	1	0	0	1	1	1	1
sarcoma	8	0/2	2	5	2/2	4	5

[a] Sensitivity is defined as a decrease of 50% or greater in the amount of [^{3}H]thymine taken up by treated tumor-colony-forming cells compared with control tumor-colony-forming cells. The assay followed a standard protocol (Salmon, S. E.; Hersh, E. M. Sensitivity of Multiple Myeloma to Imexon in the Human Tumor Cloning Assay. *J. Natl. Cancer Inst.* **1994**, *86*, 228−230).

according to the published procedure.[19] This intermediate was converted into **24** by the general method, except that the solvent was benzene. The properties of **24** are given in Table 1.

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