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C−H to N Substitution Dramatically Alters the Sequence-Specific DNA Alkylation, Cytotoxicity, and Expression of Human Cancer Cell Lines

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As a result of recent research, many diseases, including malignant lymphoma and cancer, are better understood at a sequence level, and thus many areas of medical science such as diagnosis, treatment, and prevention are changing dramatically.[1] For instance, new types of anticancer agents such as STI-571 (Glivec) that target mutated gene products are showing great promise. This targets the ABL (Abelson leukemia viral oncogene) kinase in patients with chronic myelogenous leukemia.[2]

DNA alkylating agents, which include well-known anticancer agents such as cisplatin and mitomycin C, are routinely used in antitumor treatments. However, these drugs are extremely toxic. One important question to consider is whether the introduction of sequence selectivity to an alkylating agent can improve its efficacy as an anticancer agent. To address this question, we have designed and synthesized a series of sequence-specific alkylating agents. We demonstrated that hybrids between segment A of DU-86[3] and Py-Im hairpin polyamides[4] selectively alkylate matched sequences according to the recognition rule of Py-Im polyamides. For example, conjugate **1** (Figure 1) alkylates DNA at the purine of sequence 5′-(A/T)G(A/T)C*Pu*-3′ at nanomolar concentrations.[5] Recently, we demonstrated that conjugate **1** effectively inhibits transcription at the alkylation site in the GFP-coding region.[6] These results suggest that sequence-specific DNA-alkylating Py-Im polyamides[7] could be used as novel antitumor drugs that target specific gene expression. We describe here a comparative study of sequence-specific DNA alkylation, cytotoxicity in 39 human cancer cell lines, and the effect on expression levels in cancer cell lines by Py-Im conjugates **1** and **2**. They differ only in that the C−H is substituted by an N in the second ring.

Sequence-selective alkylation by compounds **1** and **2** was examined on 5′-Texas Red-labeled 993-bp DNA fragments using an automated DNA sequencer, as described previously.[5−7] Alkylation was carried out at 23 °C for 1 h and quenched by the addition of calf thymus DNA. The sites of alkylation were visualized by thermal cleavage of the DNA fragment at the alkylated sites (Figure 2). Conjugate **1** mainly alkylated at the G of 5′-AGTC*G*-3′ (lanes 4−6) at the lower concentration of **1** as reported previously.[6] With increasing concentration of **1**, alkylation occurred at all match sequences, 5′-(A/T)G(A/T)C*Pu*-3′, in a 993-bp DNA fragment (lane 2). Similarly, conjugate **2** selectively alkylated at the A of 5′-TGCC*A*-3′ (lanes 10 and 11) at the lower concentration of **2** and alkylated at all of the eight matched sequences, 5′-(A/T)-GCC*Pu*-3′, at higher concentrations of **2** (lanes 7 and 8). These results indicate that conjugates **1** and **2** precisely recognize and effectively alkylate DNA according to the pairing rule of Py-Im polyamides.[4] Alkylation experiments using two sets of deoxydecanucleotides, their sequences copied from the major alkylation sites, demonstrated that the rates of guanine alkylation by **1** and

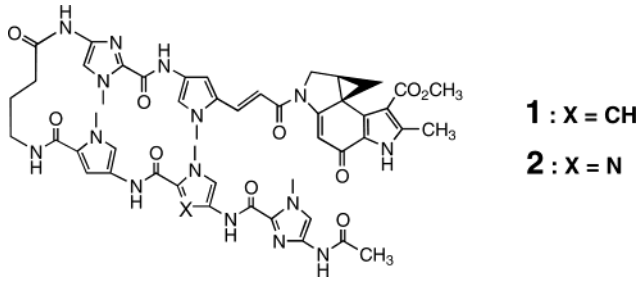

Figure 1. Structures of Py-Im conjugates **1** and **2**.

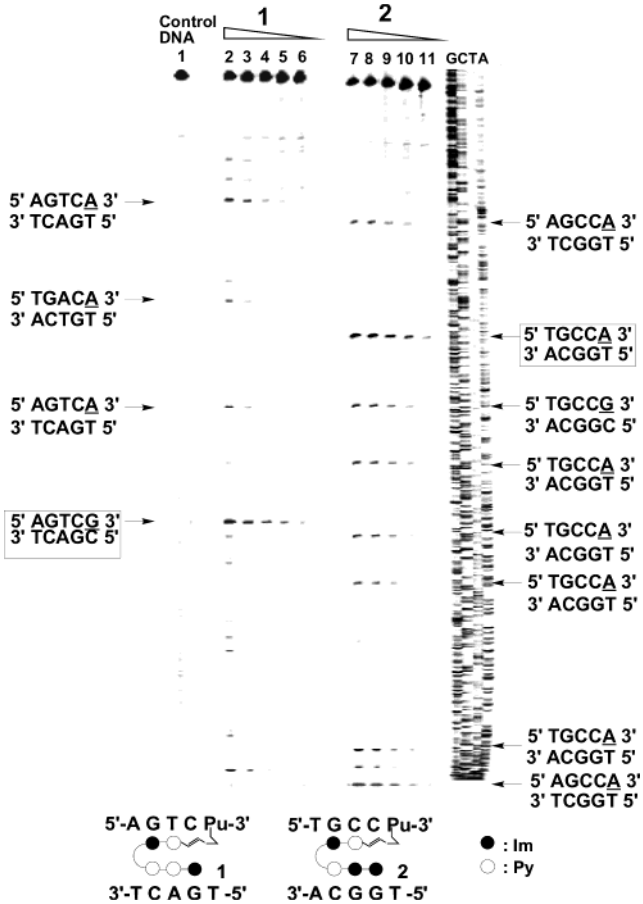

Figure 2. Thermally induced strand cleavage of 5′-Texas Red-labeled 993-bp DNA fragments alkylated by conjugates **1** and **2**. Lane 1: DNA control. Lanes 2−6: 12.5, 5, 2.5, 1.2, 0.6 nM of **1**, respectively. Lanes 7−11: 12.5, 5, 2.5, 1.2, 0.6 nM of **2**, respectively. Sequences containing alkylation sites are represented.

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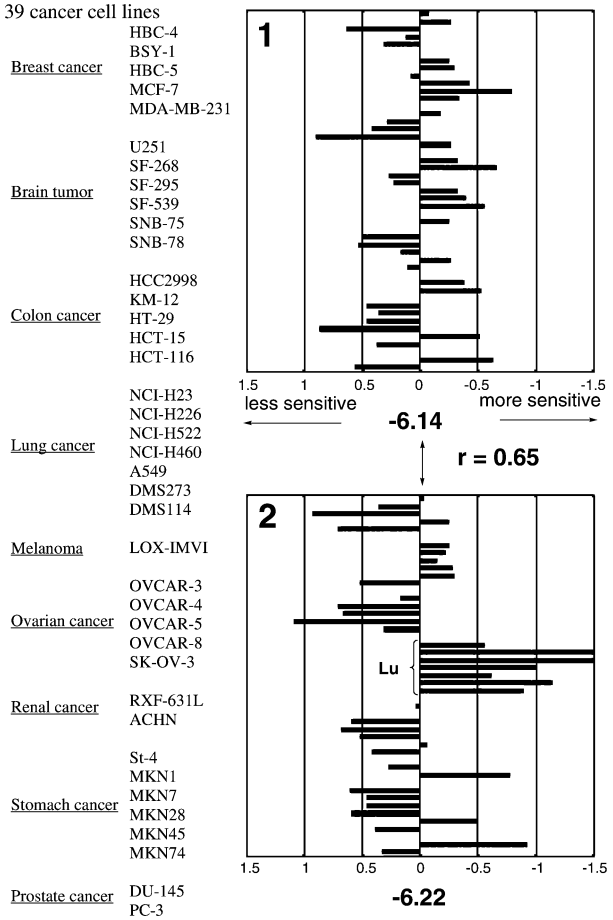

Figure 3. Mean graphs of 50% growth inhibition against a panel of 39 human cancer cell lines recorded for conjugates **1** and **2**. The log IC_{50} for each cell line is indicated. Columns extending to the right are more sensitive to agents; columns extending to the left are less sensitive to agents. One unit represents one log difference.

adenine alkylation by **2** are almost the same and that both adducts are relatively stable at 37 °C in pH 7.0 ($\tau_{1/2}$ > 1 week) (Figures 1S and 2S in Supporting Information).

To evaluate the potency of these conjugates, we investigated the 50% cell growth inhibition (IC_{50}) values of conjugates **1** and **2** using 39 human cancer cell lines as shown in Figure 3. The mean log IC_{50} values of **1** and **2** were −6.14 (0.72 μM) and −6.22 (0.60 μM), respectively, which are comparable with mitomycin C (−6.0) and cisplatin (−5.2). The results indicate that the sequence-specific nature of the alkylation by these agents does not impart any outstanding potency to the compounds.

Importantly, we observed a substantial difference in the cytotoxicities between conjugates **1** and **2**. Conjugate **2** in particular has a relatively strong cytotoxicity against seven lung cancer cell lines (IC_{50} = 18−170 nM). The COMPARE analysis of the mean graphs showed that the Py-Im conjugates **1** and **2** did not correlate well with each other (r = 0.65) despite having a common DNA-alkylating mechanism (purine N3 alkylation).[7] It is generally accepted in a COMPARE analysis that higher correlation coefficients (r > 0.75) are observed for anticancer agents possessing the same reaction mechanism.[8] For example, the correlation coefficient between the DNA intercalators doxorubicin and daunorubicin is 0.93. The correlation coefficient between ecteinascidin Et 743 and phthalascidin Pt 650 (guanine N2 alkylation) is 0.90.[9] These results suggest that differences in sequence specificity might affect the pattern of cytotoxicities.

Table 1. List of Genes that Were Oppositely Regulated by **1** and **2** in Human Bladder Cancer RT4 Cells[a]

fold change by **1**	fold change by **2**	control gene	name	NCBI RefSeq
163(**2.5**)	15(−**4.3**)	64	ARPC5	NM 005717
182(**2.7**)	13(−**5.1**)	66	ARPP-19	NM 006628
233(**2.6**)	30(−**3.1**)	92	EMR1	NM 001974
369(**2.6**)	41(−**3.5**)	143	ETFB	NM 001985
239(**2.8**)	27(−**3.1**)	85	DNCLI2	NM 006141
202(**2.6**)	26(−**3.0**)	79	CAV1	NM 001753
58(−**3.2**)	699(**3.7**)	187	FPGS	NM 004957
44(−**3.1**)	907(**6.5**)	139	CENPF	NM 005196
3(−**4.3**)	42(**3.2**)	13	SOX12	NM 006943
1(−**13.0**)	40(**3.1**)	13	TANK	NM 004180

[a] Expression analysis was carried out on an Atlas Glass Human 7.6 Microarray apparatus (Clontech, USA). Human bladder cancer RT4 cells were treated with 10 nM conjugate for 3 h, and then the expressions were analyzed using the manufacturer's recommended protocols.

To further examine this point, array-based gene expression analyses were performed using human bladder cancer RT4 cells. If their sequence specificity causes different biological effects against cancer cells, it is possible that two agents induce different expression levels.[10] It was found that incubation with **1** or **2** (10 nM) induced up-regulation of 58 and 99 genes (>4-fold) and down-regulation of 221 and 208 genes, respectively, among 7548 genes in human bladder cancer RT4 cells. Interestingly, there are 10 oppositely regulated genes (Table 1). Although a direct relationship between the number of possible alkylation sites and the expression level of these genes was not found, the results confirm that the differences in sequence specificity lead to marked differences in biological activity.

The present results clearly demonstrate that C−H to N substitution dramatically alters the sequence specificity of the agents as well as the biological effects upon the cells. The results suggest the intriguing possibility that DNA-alkylating agents recognizing longer base-pair sequences may provide a promising approach for developing new types of antigene agents.

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Supporting Information Available: Synthesis of conjugate **1** and **2** and their alkylation of decanucleotide. This material is available free of charge via the Internet at http://pubs.acs.org.

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