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Molecular design and evaluation of quinoxaline-carbohydrate hybrids as novel and efficient photo-induced GG-selective DNA cleaving agents

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Quinoxaline, found in antitumor quinoxaline antibiotics, was found to cleave double stranded DNA at the 5′ side guanine of 5′-GG-3′ site on irradiation with long wavelength UV light without any additive; furthermore, a bis(quinoxaline–carbohydrate) hybrid system was very effective for DNA cleavage.

The development of photochemical DNA cleaving agents, which selectively cleave DNA by irradiation with a specific wavelength light under mild conditions and without any additives such as metals and reducing agents, is very interesting from a chemical and biological standpoint and offers considerable potential in medicine especially in the post-genome era.[1] Furthermore, photodynamic therapy using a photosensitizing drug has recently emerged as a promising modality against cancer and allied diseases.[2] In this communication, we discuss the molecular design and DNA photocleaving properties of such novel and artificial light activatable DNA cleaving agents, that include the quinoxaline–carbohydrate hybrids **5**–**9**.

In our first approach to create such novel DNA cleaving molecules, we noted the quinoxaline structure, because quinoxaline was found in DNA interactive and antitumor quinoxaline antibiotics[3] such as echinomycin and triostin A. Although quinoxaline is known only as a DNA intercalator,[4] we expected that quinoxaline would be a novel DNA photocleaving agent, because the conjugated C=N bond in quinoxaline was expected to generate the photo-excited $^3(n–\pi^*)$ and/or $^3(\pi–\pi^*)$ state(s) by photoirradiation which could be capable of cleaving DNA by H-abstraction and/or electron-transfer pathway(s).[1] Therefore, we first examined the photo-induced DNA cleaving activities of the quinoxaline derivatives and the related compounds **1**–**4** (Fig. 1) using supercoiled ΦX174 DNA (form I). As is obvious from Fig. 2, **1** (500 μM) and **2** (500 μM) caused significant single-strand scission of DNA by photoirradiation using a long wavelength UV light (365 nm) without any further additive, leading to the nicked open circular DNA (form II). On the other hand, **3** and **4** showed little DNA cleaving activity under the same conditions. These result clearly demonstrate, for the first time, that the quinoxaline moiety present in many antitumor antibiotics is able to cleave DNA on irradiation with UV light with a long wavelength without any additive and the two nitrogen atoms in quinoxaline are essential for the DNA cleavage. Furthermore, it was found that the ester function at the C-2 position of quinoxaline improved the DNA cleaving activity. Because, in our previous studies, we had found that a suitably substituted amino sugar showed a high affinity to DNA, and significantly enhanced the intercalating ability of certain intercalators,[5] in order to further improve the DNA cleaving ability of the quinoxaline derivatives, we designed and synthesized the quinoxaline–carbohydrate hybrids **5**–**9** (Fig. 1).† The photo-induced DNA cleaving activities of the hybrids **5**–**9** along with the reference compound **1** were assayed using supercoiled ΦX174 DNA in concentrations of 500–3 μM. Based on the results shown in Fig. 3, the quinoxaline–carbohydrate hybrids **5**–**9** caused effective DNA cleavage during irradiation with a long wavelength UV light (365 nm). It was confirmed that no DNA cleavage by **1** and **5**–**9** was observed in the absence of light. Thus the UV light functioned as a trigger to initiate these quinoxaline derivatives for the DNA strand scission. Furthermore, the DNA cleaving abilities of the quinoxaline–carbohydrate hybrids **5** and **6** were found to be stronger than that of **1**. These results strongly suggest that the suitably substituted amino sugar in these hybrids works as the DNA groove binder and significantly enhances the intercalating ability of the quinoxaline. Moreover, the bis(quinoxaline–carbohydrate) hybrids **7**–**9** were the most effective photo-induced DNA cleaving agents among them. Thus, the strongest DNA cleaving hybrids **7**–**9** cleaved DNA in concentrations over 3 μM, and caused 100% DNA breakage at concentrations over 30 μM. These results clearly show that the dimerization of the quinoxaline–carbohydrate hybrids **5** and **6** *via* the *p*-xylene

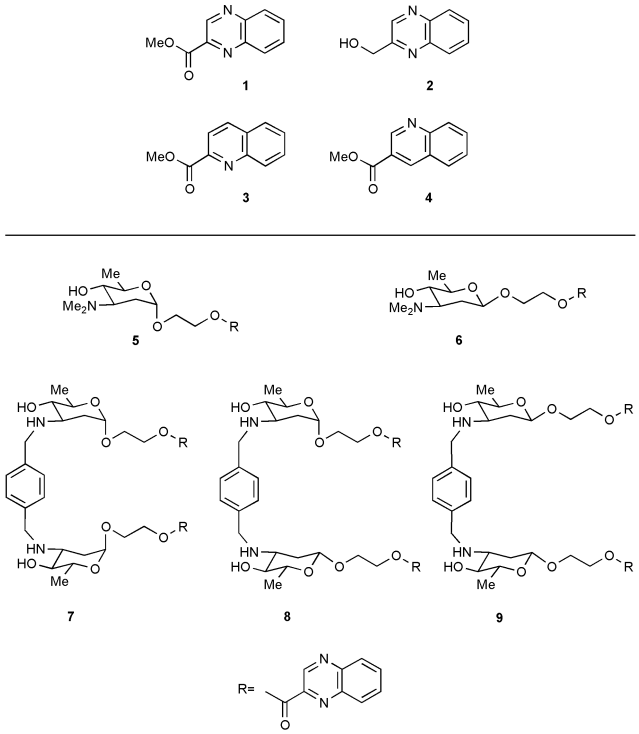

Fig. 1

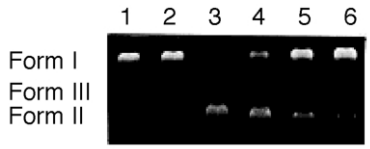

Fig. 2 Photocleavage of supercoiled ΦX174 DNA. ΦX174 DNA (50 μM per base pair) was incubated with various compounds in 20% acetonitrile in Tris–HCl buffer (pH 7.5, 50 mM) at 25 °C for 1 h under irradiation of a UV lamp (365 nm, 15 W) placed at 10 cm from the mixture, and analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain): lane 1, DNA alone; lane 2, DNA with UV; lanes 3–6, compounds **1**, **2**, **3** and **4** (500 μM), respectively, following UV irradiation. Form I: covalently closed supercoiled DNA, Form II: open circular DNA, and Form III: linear DNA.

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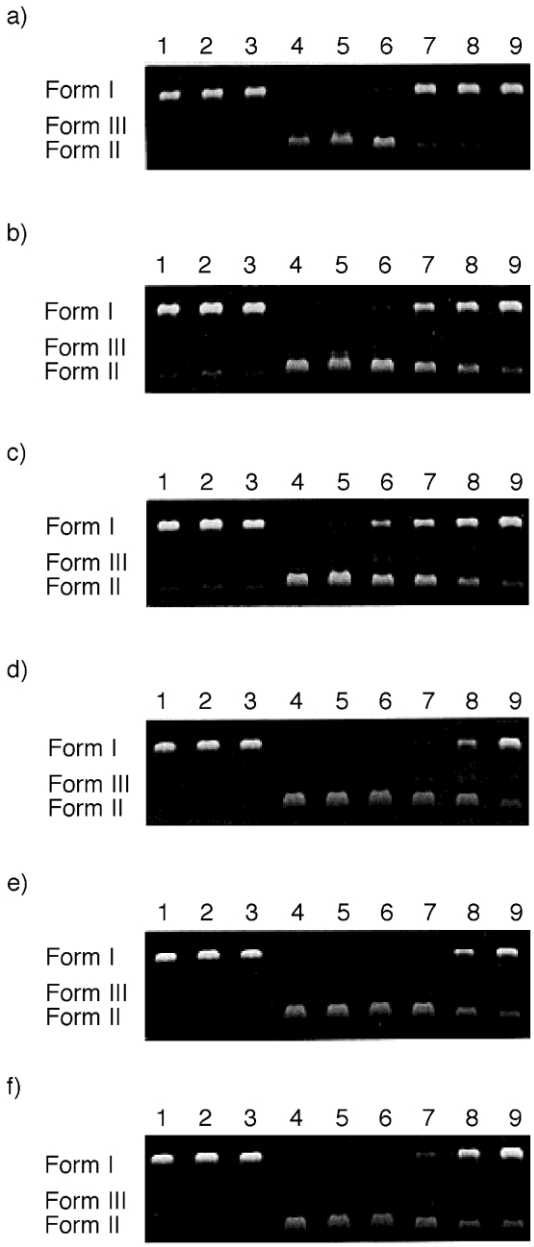

Fig. 3 Photocleavage of supercoiled ΦX174 DNA. The same protocol as that mentioned in Fig. 2 was carried out: (a),(b), (c), (d), (e) and (f) for the compounds **1**, **5**, **6**, **7**, **8** and **9**, respectively: lane 1, DNA alone; lane 2, DNA with UV; lane 3, DNA + compound (500 μM) without UV; lanes 4–9, compound (500), compound (300), compound (100), compound (30), compound (10) and compound (3 μM), respectively, following UV irradiation.

linker is very effective for the DNA cleavage.[6] The DNA cleaving site specificity of the representative quinoxaline derivatives **1**, **5**, **7** and **9** was analyzed according to the Sanger protocol.[7] Since the Sanger sequencing reactions result in base incorporation, cleavage at nucleotide *N* (sequencing) represents a cleaving site by the agent or the Maxam–Gilbert reaction at *N* + 1.[8] The results shown in Fig. 4 clearly demonstrated that all the quinoxaline derivatives selectively cleaved DNA at the 5′ side guanine of the 5′-GG-3′ sites and the site-selective DNA cleavage was drastically enhanced upon treatment with hot piperidine (data without hot piperidine not shown). Since both a free radical and a singlet oxgen scavengers, dimethyl sulfoxide and 2,2,6,6-tetramethylpiperidine, did not inhibit the DNA cleavage, it is very likely that an electron transfer from the electron-rich 5′-GG-3′ site to the photo-excited quinoxaline is the initial step for the photo-induced destruction of the guanine base at the 5′ side of 5′-GG-3′ sites.[1,9]

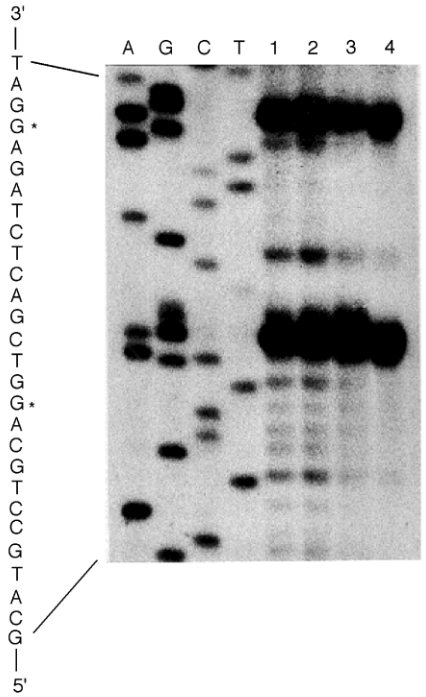

Fig. 4 Autoradiogram of 12% polyacrylamide–8 M urea slab gel electrophoresis for sequence analysis. The 5′-end-labeled M13mp 18 DNA at the primer site was cleaved by the compound at pH 7.5 and 25 °C for 1 h under irradiation of the UV lamp (365 nm, 15 W) placed at 10 cm from the mixture (bases 50–77 are shown): lanes A, G, C and T; Sanger A, G , C and T reactions, respectively; lanes 1, 2, 3 and 4; the compounds **7**, **9**, **5**and **1** (500 μM), respectively, following UV irradiation: DNAs for lanes 1–4 were treated with hot piperidine prior to gel electrophoresis.

In summary, the present work demonstrates not only the determining of a novel property of quinoxaline as a DNA photocleaver, but also the molecular design and DNA photocleavage profile of novel quinoxaline–carbohydrate hybrids. The described chemistry and evaluation also provide significant information about the molecular design of novel and artificial DNA photocleaving agents.

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Notes and references

† The synthesis of these compounds will be reported in detail elsewhere.

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