Photoactivated thiazole-based DNA-cleaving agents: dramatic change of recognition sequence depending on the number of thiazole units

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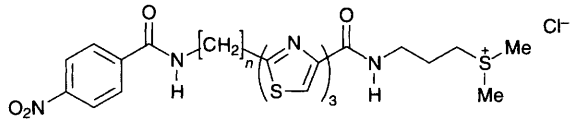
By increasing the number of thiazole units in the photoactive DNA cleaving compounds, 4-(3-dimethylsulfoniopropyl- aminocarbonyl)-2-[2-(4-nitrobenzoylamino)ethyl]oligo- thiazole, from one or two to three, the specificity of their DNA cleavage was dramatically altered from 5′-AAATN-3′ (N ≠ G) to 5′-GG-3′.

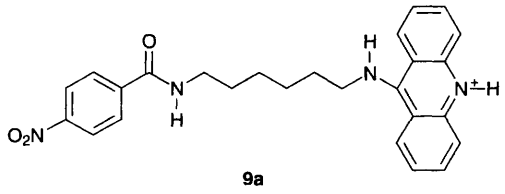
In an attempt to understand the mechanism of sequence recognition by DNA ligands, we have been studying a series of compounds consisting of the *p*-nitrobenzoyl group linked to a sequence recognizing moiety. The *p*-nitrobenzoyl group acts as a non-diffusible DNA breaking agent on irradiation with UV light (*ca.* 310 nm) by forming an excited triplet state and eventually leading to DNA strand scission.[1,2] The sequence recognizing moiety we used is either the *N*-methylpyrrolecarboxamide group,[3,4] constituting part of the DNA-binding natural antibiotics netropsin or distamycin, or the thiazole group, found in the antitumour antibiotic bleomycin A_2.[5] We have shown that these synthetic compounds, particularly those containing the thiazole group, are highly sequence specific.[5] The sequence recognized by the unithiazole analogue is the degenerate motif 5′-(A/T)(AA/TT)Tpu(A/T)-3′, whereas the bithiazole derivative acts at the more stringent sequence subset 5′-AAATN-3′ (N ≠ G).

We have now extended this work to compounds which contain three thiazole units as the sequence recognizing moiety. Both compounds **1a** and **1b** contain a terthiazole, but differ in the length of the methylene chain which links the terthiazole to the photoactivatable *p*-nitrobenzoyl group: compound **1a** contains dimethylene while **1b** has a tetramethylene chain. The compound **9a**, lacking the sequence recognizing terthiazole group, was included in this study for comparison. Attachment of the intercalator 9-aminoacridine was necessary to increase the otherwise weak affinity of the *p*-nitrobenzoyl group for DNA.

Compound **1a** was synthesized following the method published by us previously.[6] Compound **1b** was newly synthesized according to Scheme 1. After protecting the amino group of **2** with phthalic anhydride, it was oxidized to give the aldehyde **3**. L-Cysteine methyl ester and **3** were condensed and oxidized to form a thiazole ring **4**. After changing the protecting group **5**, two more thiazole rings were added using the method of Shioiri *et al.*[7] The methyl ester of **6** was hydrolysed with 1 M NaOH, and 3-methylsulfanylpropylamine was introduced by the 2-chloro-1,3-dimethylimidazolinium chloride (DMC) method. The protecting group (Boc) was removed by treatment with 1 M HCl and the *p*-nitrobenzoyl group was successfully introduced by condensation with *p*-nitrobenzoic acid. The terminal methyl thioether was converted to the dimethylsulfonium ion by treatment with methyl iodide, followed by a column chromatography on Diaion® SA10A. Recrystallization from methanol afforded **1b** (yellow powder) in 46%. The products were verified by elemental analysis, ^{1}H NMR and high resolution mass spectroscopy.



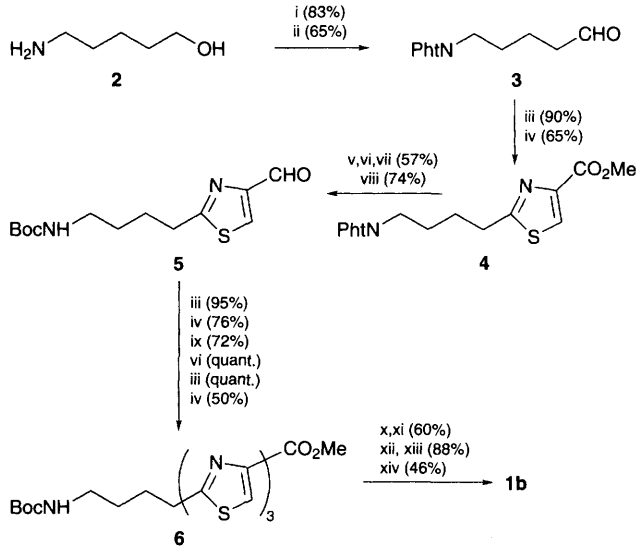
1a $n = 2$
b $n = 4$



9a

The terthiazole compounds cleaved DNA on UV irradiation in a reaction which depended on the compound : DNA base-pair ratio (data not shown). In the absence of UV irradiation no cleavage of DNA was observed. The cleavage efficiencies of **1a** and **1b** were similar, in contrast to the bithiazole analogues where the tetramethylene compound exhibited greatly reduced cleavage efficiency compared to its dimethylene counterpart.

DNA cleavage sites induced by the compounds were determined by high-resolution sequencing gel electrophoresis using as substrates 5′ ^{32}P-end labelled 189 bp *Eco*RI*-*Eco*RV as well as 3′ ^{32}P-end labelled 154 bp *Hin*dIII*-*Eco*RV and 220 bp *Hin*dIII*-*Ssp*I restriction fragments from plasmid pBR322 DNA (Fig. 1). The nucleotide positions of cleavage sites were read[8] from the Maxam–Gilbert sequencing reactions[9] run on the same fragments. Compared with **9a**, which lacks the thiazole



Scheme 1 *Reagents and conditions*: i, phthalic anhydride, $MgSO_4$, toluene, reflux; ii, PCC, CH_2Cl_2; room temp.; iii, L-Cys-OMe·Cl, NEt_3, $MgSO_4$, CH_2Cl_2 room temp.; iv, MnO_2, pyridine, benzene, 50 °C; v, $NaBH_4$, Pr^iOH–H_2O, room temp.; vi, AcOH, 80 °C; vii, $(Boc)_2O$, NEt_3, dioxane–H_2O, room temp.; viii, MnO_2, dioxane, 50 °C; ix, $NaBH_4$, LiCl, THF, room temp.; x, 1 M NaOH, THF, room temp.; xi, DMC, NEt_3, CH_2Cl_2, room temp.; xii, 1 M HCl, dioxane, room temp.; xiii, *p*-$NO_2C_6H_4CO_2H$, DMC, NEt_3, DMF, room temp.; xiv, MeI, MeOH, room temp. DMC = 2-chloro-1,3-dimethylimidazolinium chloride, PCC = pyridinium chlorochromate.

group (lane 11), DNA cleavage by **1a** (lanes 3–5) and **1b** (lanes 6–8) was highly sequence selective. Compounds **1a** and **1b** cleaved DNA at exactly the same sites, an observation that differs from that of the corresponding bithiazole analogues. For the bithiazole compounds, cleavage induced by the tetramethylene compound was shifted towards the 3′ direction by one base compared with DNA breakage by the dimethylene derivative,[5] clearly indicating that the bithiazole group recognizes the DNA base sequence and the *p*-nitrobenzoyl group cleaves the DNA strand. The sites of cleavage were dramatically altered on increasing the number of thiazole rings. Both of the terthiazole compounds cleaved DNA strongly at the first G of 5′-GG-3′, less strongly at G of 5′-GA-3′ and weakly at all single G residues. These cleavage sites are completely different from the AT-containing consensus cleavage sequence observed for the uni- and bi-thiazole analogues. For these compounds, increasing the number of thiazole units in the DNA ligand

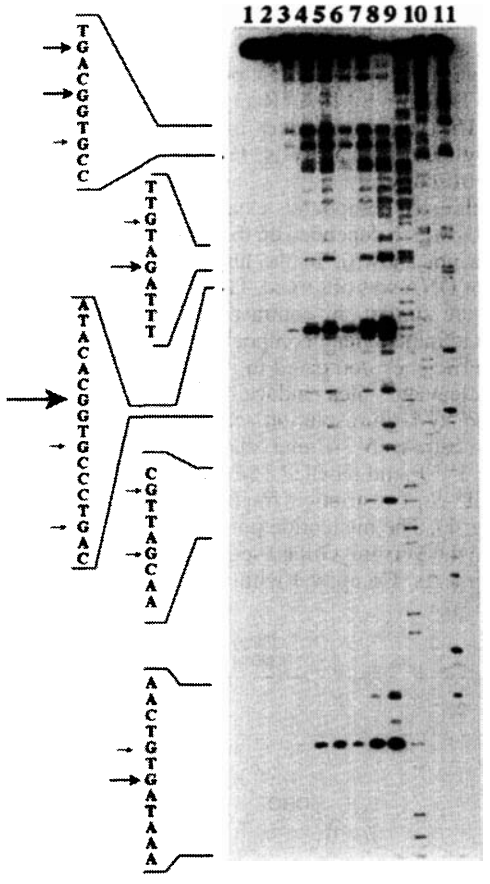


Fig. 1 Photocleavage of the 3′ ^{32}P-end labelled 154 bp *Hin*dIII*-*Eco*RV fragment from pBR322 DNA. Lane 1 is the starting DNA fragment. Lane 2 contained no drug; lanes 3–5, **1a**; lanes 6–8 **1b**; lane 11, **9a**. The DNA concentration was 25 μM and the concentration of the drug was 12.5 (lane 11), 25 (lanes 3 and 6), 50 (lanes 4 and 7) and 100 μM (lanes 5 and 8). Lanes 9 and 10 are the Maxam–Gilbert A+G and C+T sequencing reactions, respectively.

appeared to confer greater sequence selectivity within the AT consensus. However, instead of further increasing the AT selectivity, compounds with three thiazole units recognized a completely different base sequence. No cleavage was observed on the opposite strand to the GG step. In the uni- and bi-thiazole analogues, the opposite strand to the 5′-AAATN-3′ (N ≠ G) was weakly and more diffusively cleaved with a shift towards the 3′ side, suggesting the compound binds more closely to one of the strands of DNA in the minor groove. In the case of uni- and bi-thiazole compounds, photo-irradiation of DNA in the presence of the compounds induced DNA cleavage, and the subsequent piperidine treatment (90 °C, 15 min) increased the cleavage efficiency without altering the cleavage pattern or band mobilities.[5] However, for the current terthiazole compounds, cleavage was observed only after piperidine treatment.

Together, these results clearly indicate that the mechanism of the sequence recognition and/or DNA cleavage is different for the terthiazole compounds compared to their uni- and bi-thiazole analogues. It is known that the most readily oxidizable sites in duplex DNA are the G residues located 5′ to G, due to the π-stacking interaction of the two guanine bases.[10] Thus, the strongest cleavage at the GG and weaker cleavage at the GA step by the terthiazole compounds may be due to the electron transfer from guanine to the ligand, rather than *via* hydrogen abstraction from the sugar backbone by the photo-excited nitro group. Thus, the nature of drug–DNA interactions in the oligothiazole compounds depends critically on whether the number of the thiazole unit is one/two or three. Further work is underway to clarify the involvement of the *p*-nitrobenzoyl and the terthiazole moieties in the cleavage reaction, and to elucidate the determinants governing DNA–ligand interactions, *i.e.* DNA cleavage by the photo-excited nitro group, and DNA damage presumably by electron transfer from guanine bases.

This work was supported by a Grant-in-Aid for priority research from the Ministry of Education Japan, Nissan Science Foundation and Sankyo Co., Ltd. to R. K.

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Received, 29th April 1996; Com. 6/03012B