SYNTHESIS OF HALOGENATED OLIGO-N-METHYLPYRROLE-CARBOXAMIDE DERIVATIVES AND THEIR PHOTOCHEMICAL DNA CLEAVING ACTIVITIES

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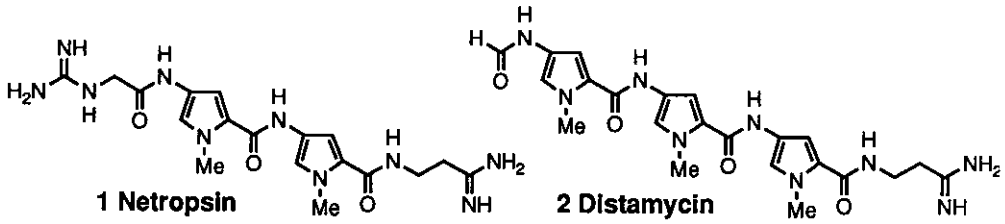
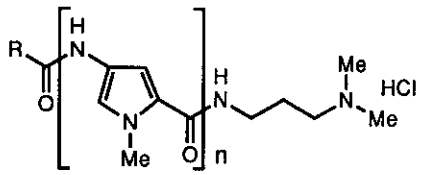
Abstract – Synthesis of various halogenated oligo-N-methylpyrrole-carboxamide derivatives and their DNA cleaving activities under UV-A irradiation were described.

Low molecular weight substances which possess both sequence specific DNA binding and cleaving activities are of great current importance in the field of fundamental molecular biology.[1] There are a number of substances that bind to DNA in the minor groove.[2] Among them, oligo-N-methylpyrrolecarboxamides such as netropsin (**1**) and distamycin (**2**) and their analogues have attracted attention because of their strong minor groove nonintercalative binding ability to double-stranded B-DNA at specific AT rich region.[3] On the other hand, rational design of compounds which cleave DNA under photo-irradiation is of great importance not only from a fundamental biological point of view but also in a photodynamic therapeutic approach as antitumor agents.[4] We have reported the synthesis of several oligo-N-methylpyrrolecarboxamide derivatives which possess photo induced DNA cleaving activities for this pourpose.[5] As it has been reported for the UV-induced DNA cleavage by chlorpromazine,[6] halogenated heteroaromatic compounds are generally expected to cleave DNA under photo-irradiation *via* carbon radical formation. Here we report the synthesis of various oligo-N-methylpyrrolecarboxamides which possess halogenated heteroaromatic groups and their DNA cleaving activities under UV-A irradiation.



Various oligo-N-methylpyrrolecarboxamides having halogenated heteroaromatic rings as well as the monoamides listed in Table 1 were synthesized by the way summarized in Scheme 1.

Table 1



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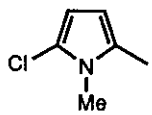
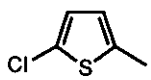
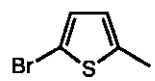


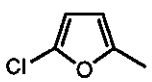
3a~c (n = 0~2)



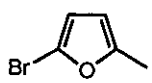
4a~c (n = 0~2)



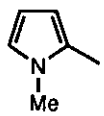
5a~c (n = 0~2)



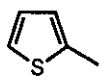
6a~c (n = 0~2)



7a~c (n = 0~2)

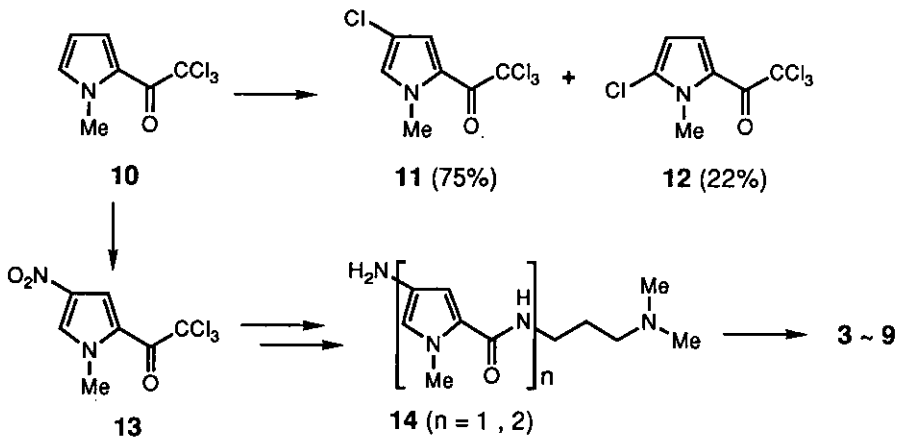


8a~c (n = 0~2)



9a~c (n = 0~2)

Scheme 1



The trichloroacetylpyrrole (**10**),[5a] which is readily available in large scale from N-methylpyrrole, was chlorinated with N-chlorosuccinimide (NCS) to afford a mixture of the 4-chloropyrrole (**11**) and 5-chloropyrrole (**12**), which was separated by column chromatography. The trichloroacetylpyrroles (**10**) and (**12**) were condensed respectively with 3,3-dimethylaminopropylamine to afford the amides (**8**a; n=0) and (**3**a; n=0), respectively. Similarly, the oligopeptides (**8**b,c; n=1,2) and (**3**b,c; n=1,2) were synthesized by condensation of **10** and **12** with the aminopyrroles (**14**) prepared from **10** *via* the nitropyrrole (**13**) according to the method which we previously reported.[5a,c] The halogenated thiophene and furane derivatives (**4**a~c, **5**a~c, **6**a~c, **7**a~c, and **9**a~c) were synthesized by the acylation of 3,3-dimethylaminopropylamine or **14** with appropriate acyl chlorides in the presence of triethylamine in DMF.

DNA cleaving activities of the peptides (**3** ~ **9**) were assayed with supercoiled plasmid Col E1 (*ca.* 40 μg/ml) under UV-A light (365 nm maximum, 13 $J \cdot m^{-2} \cdot sec^{-1}$) irradiation at 20°C. A single-strand break converted covalently closed circular DNA (form I) into the open circular DNA (form II). A double-strand break of close-spaced single-strand breaks changed form I DNA into linear DNA (form III). After electrophoresis each DNA was quantitated by ethidium bromide staining and densitometry. The monopeptides (**3a** ~ **9a**) exhibited very low activities. All halogenated compounds tested exhibited activities, depending on the drug concentrations (0.1, 1, 10, and 100 μM final concentrations) whereas compounds (**8**) and (**9**) which have no halogene atom were much less active.

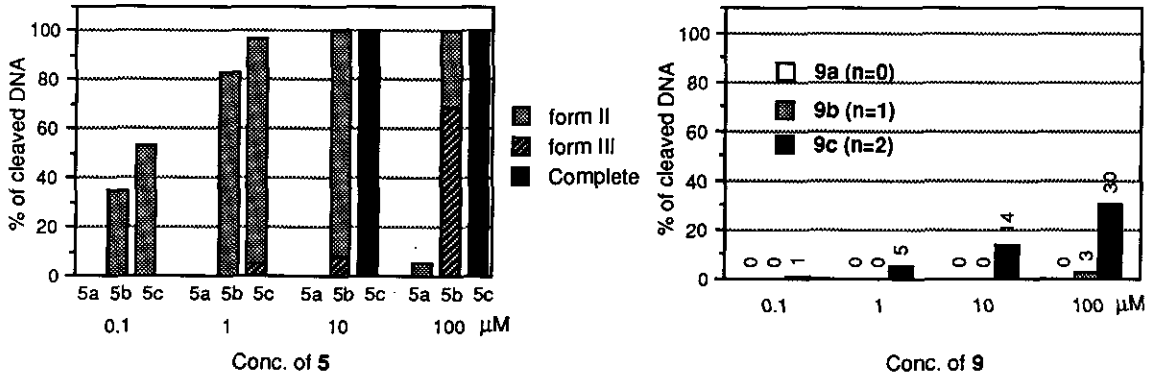


Figure 1. Photo-induced DNA-cleavage by compounds (**5**) and (**9**). Col E1 was incubated in 20 ml of Tris-acetate (TAE) buffer (pH 7.8) with various amount of compounds and irradiated for 2 h. Results presented are mean value of three runs. A control reaction mixture without the addition of drug was irradiated and used as the background to be subtracted from the obtained values. Complete means complete fragmentation of DNA.

Activities of compounds (**5a**; n=0)~ (**5c**; n=2) are typically shown in Figure 1, comparing with that of the corresponding non-halogenated compounds (**9a** ~ **9c**). Table 2 summarizes the relative cleavage efficiency of compounds (**3** ~ **9**) by comparing the activities at 1 μM drug concentrations. Single strand cleavage was predominant in the experiments at 1 μM drug concentrations, and a remarkable correlation between the peptide chain lengths and the activities was observed in each series of the compounds.

compound	3	4	5	6	7	8	9
	% of form I DNA converted into form II DNA						
a (n = 0)	4	0	0	1	1	1	0
b (n = 1)	32	4	83	4	2	2	0
c (n = 2)	98	42	97	88	100	10	5

Table 2. Photoinduced DNA-cleavage at 1 μM drug concentrations. The reaction mixture containing 1 μM drug was irradiated for 2 h. Values were obtained from mean values of three runs.

In order to investigate the participation of hydroxyl radical (OH•) in the reaction, irradiation of Col E1 sensitized by compounds (**3** ~ **9**) were carried out in the presence of OH• scavengers such as phenol, potassium iodide, sodium formate, and sodium benzoate. Tested at two different concentrations, these four scavengers partially inhibited the nicking reactions by **8** and **9** whereas exhibited no effect on the reaction by compounds (**3** ~ **7**). The results of scavenge by sodium benzoate for compounds (**9c**; n=2) and (**5c**; n=2) are typically shown in Figure 2. In the experiments at two different drug concentrations, SOD (O_2^- scavenger) and catalase (H_2O_2 scavenger) had little effect on the extent of photo-nicking activities by compounds (**3** ~ **9**) (data not shown).

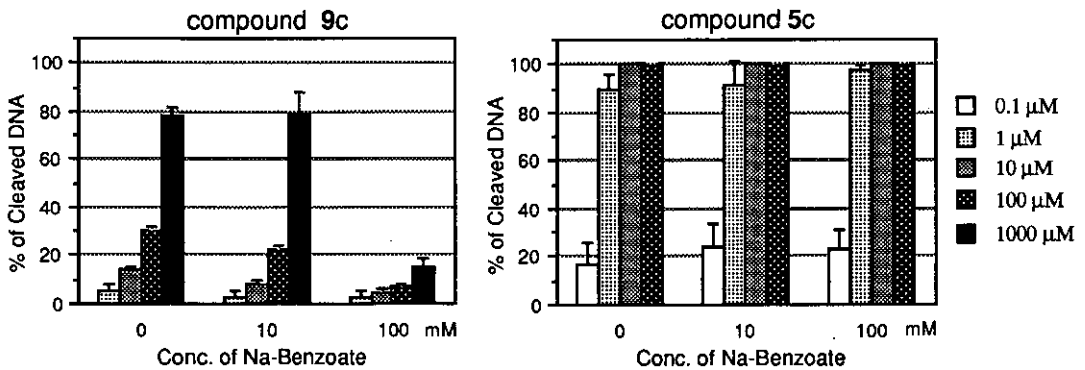


Figure 2. Effect of sodium benzoate as hydroxyl radical scavenger on photo-cleavage of Col E1 DNA by compounds **9c** and **5c**. Results presented are mean values ±SD of three runs.

We previously reported the synthesis of various oligo-N-methylpyrrolecarboxamides which do not possess special side groups sensitive to uv light.[5c] Their DNA cleaving activities under uv-A irradiation were demonstrated and the mechanism in which both hydroxyl radical and molecular dioxygen participate were proposed. The mechanism of action of compounds (**8**) and (**9**) must be similar as that we reported previously.[5c] On the other hand, the possibility of major participations of active oxygens for the photo-DNA-cleaving activities of compounds (**3** ~ **7**) could be excluded on the basis of above experiments using several active oxygen scavengers. Motten *et al.*[6b] reported the photo-production of promazine radical (P•) from chloropromazine and found that the reactivity of P• is similar to that of hydroxyl or phenyl radical in its capability to abstract hydrogen atoms from a variety of substrates. Similar aryl radical production by a photo-homolysis of carbon-halogen bond, is probable for the action of compounds (**3** ~ **7**). Further investigations will be necessary in establishing the mechanisms of DNA cleavage by our compounds, which are now in progress in our laboratory.

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Received, 24th April, 1992