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

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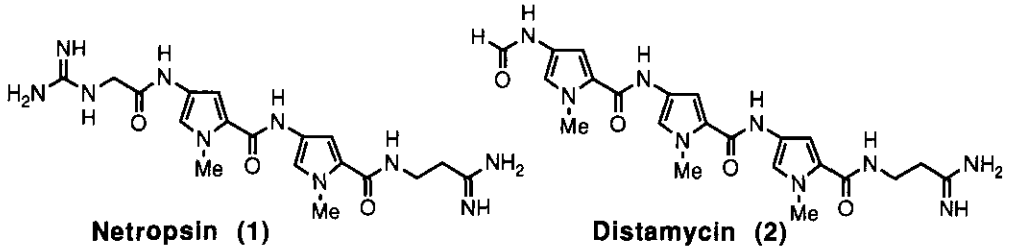
Abstract – Synthesis of various sulfonamido oligo-N-methylpyrrolecarboxamide derivatives and their DNA cleaving activities under uv-A irradiation were described.

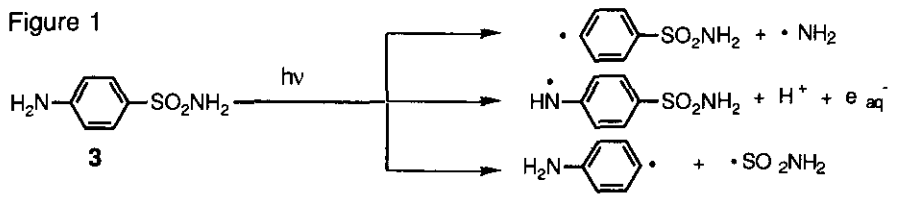
Synthetic reagents which cleave DNA are of considerable interest as tools in molecular biology. This has led to the development of substances possessing sequence specific DNA binding and cleaving activities.[1] Among them, oligo-N-methylpyrrolecarboxamides such as netropsin (**1**) and distamycin (**2**) 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.[2] 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. Sulfanilamide (**3**) and its derivatives are historically important therapeutic agents, and they are reported to produce several radical species under photo-irradiation.[3] (Figure 1) Here we report the synthesis of various oligo-N-methylpyrrolecarboxamides which possess sulfonamido terminal group and their DNA cleaving activities under uv-A irradiation.



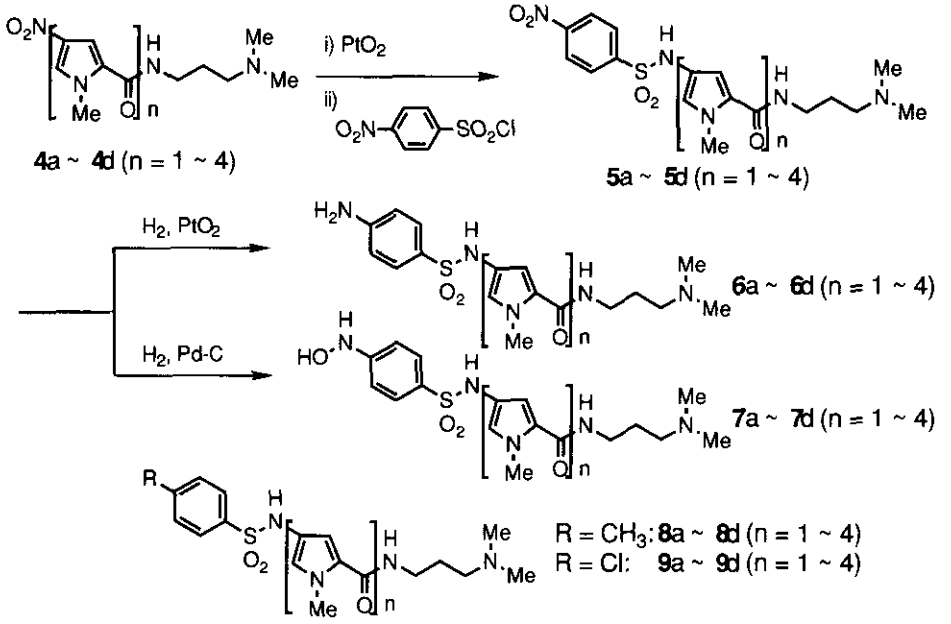
Netropsin (1) **Distamycin (2)**

†Dedicated to Dr. Masatomo Hamana on the occasion of his 75th birthday.

Figure 1



Starting oligopeptides (**4**a ~ **4**d) (n = 1 ~ 4) were synthesized by the similar way we reported previously.[4] The aminopyrrole derivatives obtained by the reduction of **4**a ~ **4**d (n = 1 ~ 4) were condensed with 4-nitrobenzenesulfonyl chloride in the presence of triethylamine to give the appropriate sulfonamides (**5**a ~ **5**d) (n = 1 ~ 4) in good yields, respectively . Hydrogenation of **5**a ~ **5**d (n = 1 ~ 4) with PtO_2 as a catalyst gave the aniline derivatives (**6**a ~ **6**d) (n = 1 ~ 4), respectively. On the other hand, catalytic hydrogenation with 10% Pd-C afforded selectively the hydroxyamino derivatives (**7**a ~ **7**d) (n = 1 ~ 4). Compounds (**8**a ~ **8**d) (n = 1 ~ 4) and (**9**a ~ **9**d) (n = 1 ~ 4) were also synthesized by the similar way as that for **5**, respectively, in order to compare their DNA cleaving activities with those of **6** and **7**



DNA cleaving activities of HCl-salts of the peptides (**6** ~ **9**) were assayed with supercoiled plasmid Col E1 (*ca.* 40 μg/ml) under uv-A light (360 nm maximum, 13 $J \cdot m^{-2} \cdot sec^{-1}$) irradiation at 20°C. DNA strand cleavage was estimated on agarose gels by conversion of the covalently closed circular (ccc) DNA to the open circular (oc) form. After electrophoresis each DNA was quantitated

by ethidium bromide staining and densitometry. All compounds tested exhibited activities, depending on the drug concentrations (1, 10, and 100 μM final concentrations). Single strand cleavage was predominant in all experiments, and a remarkable correlation between the peptide chain lengths and the activities was observed in each series of the compound.(Figure 2 ~ 5) Unexpectedly, compounds (**6**) showed almost the same activity as that of the *p*-toluenesulfonamide derivatives (**8**)(Figure 2 and 4), while the *p*-hydroxyaminosulfonamide derivatives (**7**) showed slightly higher activity.(Figure 3) We previously reported the synthesis of various oligo-N-methylpyrrolecarboxamides which do not possess special side groups sensitive to uv light.[5] We demonstrated their DNA cleaving activities under uv-A irradiation and proposed the mechanism in which both hydroxyl radical and molecular dioxygen participate.[5] The mechanism of action of compounds (**6** ~ **8**) presumably be similar as that we reported previously.[5] On the other hand, the *p*-chlorobenzenesulfonamide derivatives (**9**) showed relatively higher activity.(Figure 5) The mechanism in which phenyl radical participates is probable for the action of **9**. Further investigations on the mechanisms of DNA cleavage by our compounds are now in progress.

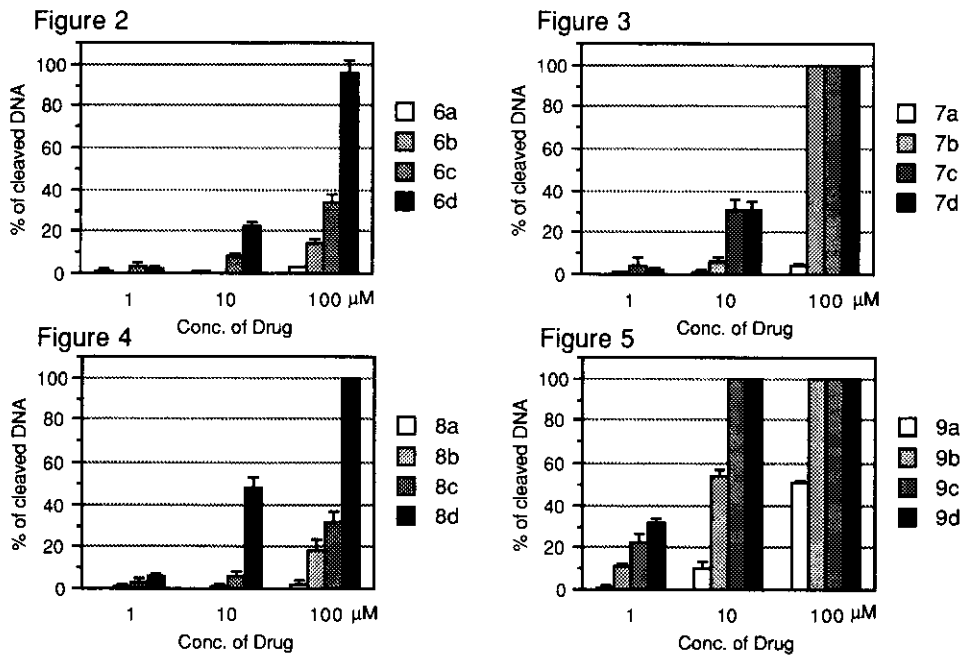


Figure 2 ~ 5: Photoinduced DNA-cleavage. Col E1 was incubated in 20 μl of Tris-acetate (TAE) buffer (pH 7.8) with various amount of compounds (**6** ~ **9**) (n = 0 ~ 4) and irradiated for 2 h. Results presented are mean value±SD of three runs. A control reaction mixture without the addition of drug was irradiated and used as the background to be subtracted from the obtained values.

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