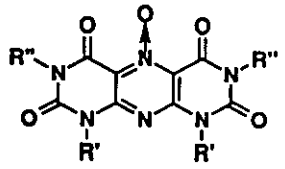
SYNTHESIS AND DNA PHOTO-CLEAVING ACTIVITY OF NOVEL HETEROCYCLIC *N*-OXIDE - ACRIDINE HYBRID MOLECULES †

Magoichi Sako*, Yoshifumi Takeda, Kosaku Hirota, and Yoshifumi Maki*

Gifu Pharmaceutical University, 5-6-1, Mitahora-higashi, Gifu 502, Japan

Abstract —— The novel DNA photo-cleaver consisting of the heterocyclic *N*-oxide, which is an efficient photochemical generator of hydroxyl radicals, an acridine intercalator, and an amide-type methylene linker was designed and synthesized. The preliminary DNA strand-breakage study of the hybrid compounds demonstrated that the DNA photo-cleaving activity of the parent heterocyclic *N*-oxide increased by linking with the acridine intercalator.

Our previous works have documented that pyrimido[5,4-*g*]pteridinetetrone *N*-oxides, (**1**) and (**2**), function as an electron acceptor to cause efficiently photochemical oxygenation or dehydrogenation of electron-rich substrates and photochemical generation of hydroxyl (OH) radicals, depending on the nature of the substrates and solvents employed. [1,2] The most intriguing observation is that water-soluble *N*-oxides (*e.g.*, **2**) generate efficiently OH radicals in a bimolecular fashion from a water-solvated excited form upon irradiation with uv-visible light in water. [3]



1: R'= R"= ^{n}Bu
2: R'= MOM, R"= Me

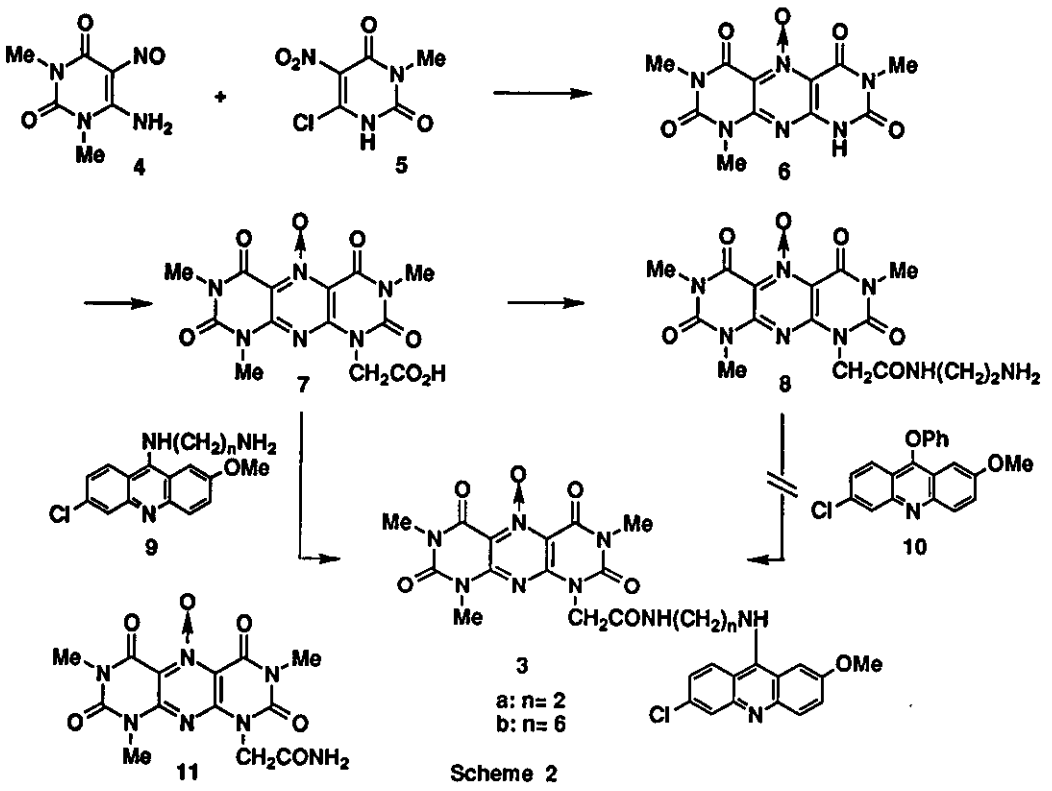
Scheme 1

The usefulness of **2** as an OH radical generator was demonstrated by the application to a DNA photo-cleaving agent: the ability of **2** to induce photochemical breakage of DNA strand was estimated by using supercoiled circular ΦX 174 RF-1 (Form I) DNA. Essentially complete conversion of Form I DNA to relaxed circular (Form II) DNA was achieved at a 2.0 μM concentration of the *N*-oxide (**2**) after irradiation in a pH 7.5 buffer solution for 10 min. [3] No intercalation of **2** with the DNA under the conditions employed was proved by the fluorescence quenching experiments. [4]

In above context, we designed the hybrid molecules (**3a,b**) in which the heterocyclic *N*-oxide moiety is linked with an acridine intercalator by methylene chains through an amide group, because intercalators have high affinity toward DNA and bring the attached strand-breakage group close to DNA to enhance the cleavage ability. [5]

In this paper, we describe the synthesis and a result in the preliminary DNA strand-breakage study of the novel hybrid compounds (**3a,b**).

† This paper is dedicated to the memory of the late Dr. Yoshio Ban.



Scheme 2

There have been two methods for the construction of the pyrimido[5,4-*g*]pteridinetetrone ring system involving oxidative dimerization of 1,3-disubstituted 6-amino-5-nitrosouracils by using lead tetraacetate in acetic acid [6] and thermal condensation of 6-amino-5-nitrosouracils with 6-chloro-5-nitrouracils in dimethylformamide (DMF). [7] The former method is versatile and efficient for the synthesis of symmetrically *N*-substituted *N*-oxides such as **1** and **2**. The latter method is advantageously used for the preparation of unsymmetrically *N*-substituted *N*-oxides. Thus, the preparation of the requisite 1,3,7-trimethylpyrimido[5,4-*g*]pteridine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)-tetrone 5-oxide (**6**), mp >300°C, [8] was achieved by refluxing a mixture of 6-amino-1,3-dimethyl-5-nitrosouracil (**4**) and 6-chloro-3-methyl-5-nitrouracil (**5**) [9] in DMF for 3 min (yield: 35%). The reaction of **6** with ethyl bromoacetate in the presence of sodium hydride in dry DMF followed by hydrolysis with 6N-hydrochloric acid gave 9-carboxymethyl derivative (**7**), mp >300°C, in 84% yield. Photolysis of **7** in water (>355 nm, for 1 h) gave a complicated mixture of products resulting from decarboxylation [10] together with deoxygenation. In sharp contrast, the amide derivative of **7**, (**11**), mp >300°C, [11] was smoothly converted to give the corresponding deoxygenated product upon irradiation under analogous conditions, indicating efficient generation of OH radicals. [3]

On the basis of this fact, an amide group was chosen for the linkage between the photo-reactive *N*-oxide moiety and an acridine intercalating moiety.

When **7** was allowed to react with *N*-Boc ethylenediamine in the presence of *N*-hydroxysuccinimide and DCC in dry DMF at room temperature and subsequently was deprotected by acid-treatment, 9-(*N*-aminoethyl)carbamoyl-

methyl derivative (**8**), mp 268°C, was obtained in 60% yield. The reaction of **8** with excess 9-phenoxyacridine derivative (**10**) in phenol under reflux, according to the Buchardt's procedure, [12] resulted in the formation of the deoxygenated hybrid compound which was identified by a sample prepared by reduction of **3a** with sodium hydrosulfite. Analogous thermal deoxygenation of the *N*-oxide group has already been observed in the reaction of **1** with aromatic hydrocarbons. [13]

Thus, an alternative route was examined and the preparation of the hybrid compounds (**3a,b**) was achieved as follows: the condensation of **7** with 9-(2-aminoethyl- or 6-aminohexyl)aminoacridine (**9a** or **9b**) obtained by the Buchardt's procedure,[12] was carried out in dry DMF in the presence of *N*-hydroxysuccinimide and DCC. The desired hybrid compounds (**3a,b**) were obtained in 36% and 69% yields, respectively. The uv spectra of **3a** and **3b** strongly support the hybrid structures: Uv (H_2O) λ_{max} 422, 368, 265, and 229 nm for **3a**; 422, 368, 265, and 230 nm for **3b**. Fluorescence quenching experiments showed that the fluorescence of the acridine moiety (490 nm) in **3a,b** markedly decreases with concentration dependence by the addition of supercoiled circular DNA as expected, indicating that the acridine moiety of **3a,b** strongly intercalates with the DNA. The smooth conversion of **3a** to the deoxygenated hybrid compound was observed in the photolysis of **3a** in water and the generated OH radicals were detected by ESR spin-trapping method using 5,5-dimethylpyrroline *N*-oxide (DMPO). [14]

Table 1. Cleavage of Supercoiled Circular ΦX 174 RF 1 (Form I) DNA into Relaxed Circular (Form II) DNA by Photo-irradiation of Hybrid Compounds(**3a,b**)and Component Compounds (**9a** and **8**), and its Inhibition with Dimethyl Sulfoxide (DMSO). a)

Compound	Concentration (μM)	DMSO (%)	Form I (%) b)	Form II (%) b)
9a	5.0	-	86	14
8	1.0	-	65	35
3a	0.1	-	74	26
3a	0.5	-	29	71
3a	1.0	-	N.D.	100
3a	1.0	0.1	4	96
3a	1.0	1.0	27	73
3b	1.0	-	16	84
Blank	-	-	86	14

a) A solution (30 μl total volume) of Form I DNA (200 ng) in 50 mmol sodium cacodylate buffer (pH 7.5) containing the hybrid molecules (**3a,b**) or component compounds (**9a** and **8**) at varying concentrations was irradiated in the absence or presence of DMSO at a distance of 5 cm from a 400 W high-pressure mercury arc lamp through a $BiCl_3$ solution filter (>355 nm) at ambient temperature for 10 min and then analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The employed DNA contains a small amount of Form II DNA (*ca.* 10%).

b) Yields were estimated by densitometric analyses of a photographic negative of the agarose gel after ethidium bromide staining.

The ability of **3a,b** to induce photochemical cleavage of DNA strand was estimated by using Form I DNA (see Table 1). For comparison, the DNA photo-cleaving activity of the 9-aminoacridine derivative (**9a**) or the 9-carbamoylmethyl *N*-oxide derivative (**8**) also was measured under the same conditions.

An efficient single-strand breakage of Form I DNA was observed as evidenced by the production of Form II DNA with concentration dependence of **3a**. Essentially complete conversion of Form I DNA to Form II DNA was achieved at a 1.0 μM concentration of **3a** after irradiation for 10 min. The photochemical DNA strand-breakage with **3a** was effectively inhibited by the addition of an OH radical scavenger, dimethyl sulfoxide, with concentration dependence. Practically, no photo-cleaving activity of **9a** was observed. The *N*-oxide (**11**) exhibited the cleaving activity at a 1.0 μM concentration to cause 35% conversion of Form I DNA to Form II DNA. Analogous conversion to Form II DNA in the case of **3a** was attained at *ca.* 0.1 μM concentration. Thus, it is roughly evaluated that the hybridization of the *N*-oxide with the intercalator increases approximately 10 times the photo-cleaving activity of the *N*-oxide itself. The hybrid compound with a long-chain linker (**3b**) showed the cleaving activity at the almost same level comparing with **3a**.

Contrary to our expectation, the activity of the hybrid compounds as a DNA photochemical cleaver is not prominent in comparison with that of the component *N*-oxide. Further studies on the application of the novel heterocyclic *N*-oxide to the construction of more efficient and site-specific DNA photo-cleaver and on the mechanism for the DNA photo-cleavage are now in progress.

REFERENCES AND NOTES

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