COMMUNICATION

Development of arylhydrazines and diphenyl arylhydrazidophosphates as photo-induced DNA-cleaving agents

Jih Ru Hwu,*[,a,b] Chii Shyang Yau,[a] Fu Yuan Tsai[a,b] and Shwu-Chen Tsay[b]

[a] *Organosilicon and Synthesis Laboratory, Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan 30043, Republic of China*
[b] *Institute of Chemistry, Academia Sinica, Nankang, Taipei, Taiwan 11529, Republic of China*

Single-strand cleavage of DNA in a sodium phosphate buffer at pH 7.4 has been accomplished by photolysis of various arylhydrazines or diphenyl arylhydrazidophosphates with 312 nm UV light; the new processes involve the generation of HO• and singlet oxygen, respectively.

Hydrazine cleaves DNA at the thymine and cytosine residues;[1] however, its derivative phenylhydrazine is not in itself a DNA-damaging agent.[2,3] Oxidation of phenylhydrazine initiated by Cu^{II}, oxyhemoglobin, or Fe^{III}–EDTA in buffer solution produces H_2O_2, O_2^-, phenyl or hydroxyl radicals.[2] The hydroxyl radical, being more potent than the phenyl radical, was reported as being an efficient DNA-cleaving species.[2] Thus the oxidation of phenylhydrazine may cause DNA damage.

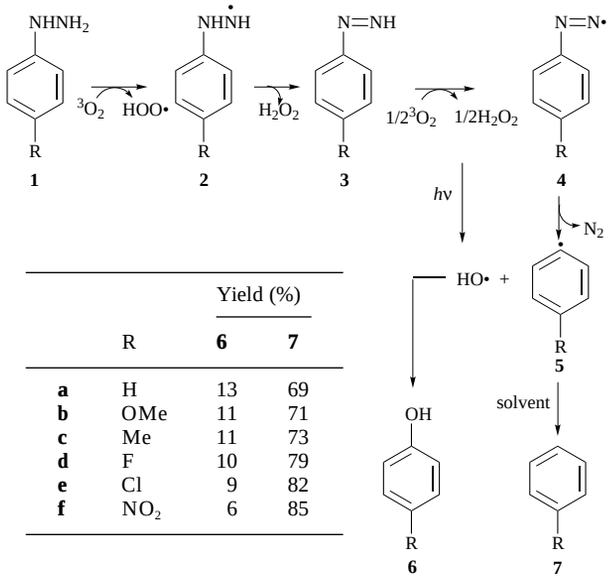
In the development of new DNA-cleaving agents, we investigated the photochemical behavior of various arylhydrazines and arylhydrazidophosphates. Herein we report our new findings that these compounds function as photo-induced DNA-cleaving agents.

To establish the photochemical properties of arylhydrazines, we irradiated a sodium phosphate buffer (pH 7.4) of **1a**–**f** (10 mmol dm^{-3}) with a 450 W medium-pressure mercury UV lamp through a Pyrex filter ($\lambda \geqslant 300$ nm) under aerobic conditions at 37 °C for 2.0 h (Scheme 1). Phenols **6a**–**f** were obtained in 6–13% yields and arenes **7a**–**f** in 69–85% yields.

To investigate the reaction mechanism, we performed an EPR experiment by irradiating with UV light ($\lambda \geqslant 300$ nm, 400 W) a phosphate buffer (pH 7.4) containing **1a**, molecular oxygen, and the spin trapping agent 5,5-dimethyl-4,5-dihydro-3*H*-pyrrole *N*-oxide (DMPO). The EPR spectrum obtained was superimposable with a simulated one resulting from a mixture of phenyl and hydroxyl radical species (9:1). The *g* value and hyperfine splitting constants (*a*) for the DMPO–phenyl radical adduct ($g = 2.0050$, $a_N = 15.98$ G, $a_H = 23.01$ G) and DMPO–HO• adduct ($g = 2.0058$, $a_N = a_H = 14.90$ G) agreed well with the reported data.[2,4]

Given the established chemistry on oxidation of arylhydrazines[5] and our new results from photolysis, we illustrate a plausible mechanism as shown in Scheme 1 for the photo-oxidation of arylhydrazines. Arylhydrazines **1** are oxidized with molecular oxygen to give aryldiazenes **3** through arylhydrazino radicals **2**.[6] Further oxidation of **3** with molecular oxygen generates arylazo radicals **4** and H_2O_2.[7] Homolytic fission of H_2O_2 *in situ* by UV light produces HO•.[8] Meanwhile, nitrogen gas is extruded from arylazo radicals **4** to give aryl radicals **5**. Coupling of aryl radicals with HO• gives phenols **6**; while hydrogen abstraction from the solvent by **5** affords arenes **7**.[9] Because HO• is produced during the photo-oxidation process, an arylhydrazine can be regarded as a 'photo-Fenton reagent'.[10]

Consequently we planned to develop arylhydrazines as photo-induced DNA-cleaving agents. We irradiated various substituted arylhydrazines (50 μM) in a sodium phosphate buffer (pH 7.4) containing the supercoiled circular *φX174* RFI DNA (form I; 50 μM/base pair) with UV light (312 nm, 16 W) under



	R	Yield (%) 6	7
a	H	13	69
b	OMe	11	71
c	Me	11	73
d	F	10	79
e	Cl	9	82
f	NO_2	6	85

Scheme 1

aerobic conditions at 37 °C for 2.0 h. Analytical results from gel electrophoresis on 1% agarose with ethidium bromide staining showed that **1a**–**f** exhibited appealing DNA-cleaving activity and gave the relaxed circular DNA (form II). The ratios of (form II)/(form I) ranged from 1.5–2.6 (Table 1). Among **1a**–**f**, 4-fluorophenylhydrazine (**1d**) exhibited the greatest potency. At pH 7.4, it was able to cleave DNA with (form II)/(form I) = 1.1 at the concentration even as low as 10 μM (Fig. 1).[11] Furthermore, we found that DNA cleavage did not occur with **1a** in the dark (entry 7 in Table 1). Thus the UV light functioned as a 'trigger'[12] for arylhydrazines to initiate the single-strand scission of DNA.

The pH value of the media over the range 5.0–8.0 did not affect the cleaving efficiency of **1a** significantly (entries 1 and 14–17 of Table 1). In control experiments, we removed molecular oxygen from the buffer by bubbling argon gas through (entry 2). Moreover, we reduced the concentration of HO• by adding hydroxyl radical scavengers (*e.g.* ethanol,[13,14] DMSO[14,15] and KI[16]) or a general radical scavenger (*e.g.* cysteine[13]). The cleaving potency of **1a** decreased significantly (entries 3–6 of Table 1). These results provide extra evidence of the generation of HO•, which is responsible for the DNA cleavage.

Furthermore, we investigated the ability of diphenyl arylhydrazidophosphates as photo-induced DNA-cleaving agents. The diphenyl arylhydrazidophosphates **8a**–**f** were prepared in 73–93% yields by reaction of the corresponding arylhydrazines (**1a**–**f**) with diphenyl chlorophosphate (1.0 equiv.) and sodium acetate (1.2 equiv.) in THF at 25 °C (Scheme 2).

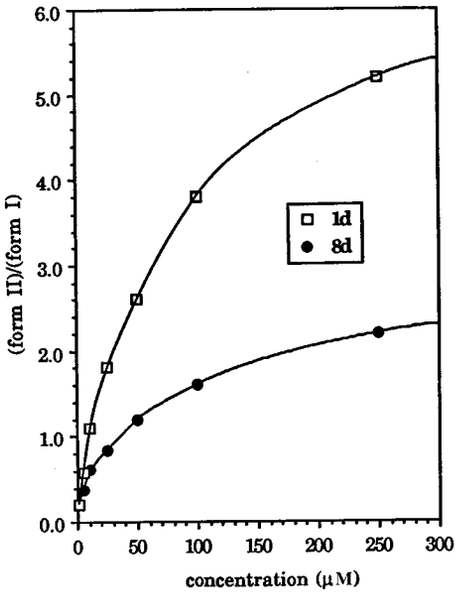


Fig. 1 Dose measurements of 4-fluorophenylhydrazine **1d** (□) and 4-fluorophenylhydrazidophosphate **8d** (●) for their DNA cleaving ability in a sodium phosphate buffer (pH 7.4) upon irradiation with 312 nm UV light

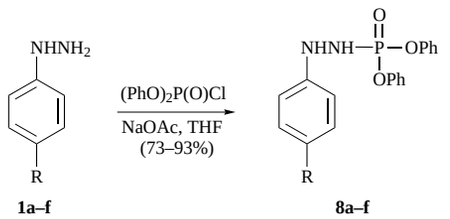
Table 1 Single-strand cleavage of supercoiled circular *φX174* RFI DNA (form I) to relaxed circular DNA (form II) by photolysis of **1a–f** with 312 nm UV light at 37 °C for 2.0 h

Entry	Aryl-hydrazines[a]	pH	% Form I[b]	% Form II[b]	Form II/ form I
1	**1a**	7.4	35	65	1.9
2	**1a**[c]	7.4	78	22	0.28
3	**1a**[d]	7.4	80	20	0.25
4	**1a**[e]	7.4	78	22	0.28
5	**1a**[f]	7.4	82	18	0.22
6	**1a**[g]	7.4	85	15	0.18
7	**1a**[h]	7.4	88	12	0.14
8	**1b**	7.4	40	60	1.5
9	**1c**	7.4	37	63	1.7
10	**1d**	7.4	28	72	2.6
11	**1e**	7.4	35	65	1.9
12	**1f**	7.4	32	68	2.1
13	None	7.4	91	9.0	0.10
14	**1a**	5.0	32	68	2.1
15	**1a**	6.0	33	67	2.0
16	**1a**	7.0	35	65	1.9
17	**1a**	8.0	37	63	1.7

[a] A sodium phosphate buffer (Na_2HPO_4 and NaH_2PO_4; 0.10 M) containing an arylhydrazine (50 μM), 50 μM/base pair of form I DNA (molecular weight 3.50×10^6, 5386 base pairs in length). [b] Analyzed by gel electrophoresis with 1% agarose and ethidium bromide staining. [c] Molecular oxygen was removed from the buffer by argon gas. [d] Ethanol (2.0 M) was added. [e] Dimethyl sulfoxide (2.0 M) was added. [f] Potassium iodide (50 mM) was added. [g] Cysteine (50 mM) was added. [h] In the dark.

Then we performed a series of DNA-cleaving experiments as described above by using arylhydrazidophosphates **8a–f** (500 μM) in a sodium phosphate buffer (pH 7.4) containing 10% THF. Among those hydrazidophosphates, fluoro-containing derivative **8d** exhibited the greatest potency of single-strand scission. At pH 7.4, it was able to cleave DNA with (form II)/(form I) = 1.2 at a concentration as low as 50 μM (see Fig. 1).

In addition, we found that addition of radical scavengers, including EtOH, DMSO, KI and cysteine, did not inhibit their cleaving potency (entries 3–6 of Table 2). Thus radical species were not responsible for the DNA cleavage by phenylhydrazidophosphates. However, when we replaced H_2O by D_2O to increase the lifetime of singlet oxygen,[17] the potency of **8a** increased significantly (entry 7 in Table 2). Furthermore, when we removed molecular oxygen from the buffer by bubbling argon gas through, or removed singlet oxygen by adding sodium azide as a scavenger,[15,18] the cleaving potency of the phenylhydrazidophosphates was inhibited. In the dark, those compounds did not exhibit DNA-cleaving ability. Accordingly we conclude that the arylhydrazidophosphates can function as a photo-sensitizer to generate singlet oxygen;[19] nevertheless, the possibility of the generation of other species that cause DNA-cleavage cannot be excluded.



1a–f **8a–f**

a: R = H; b: R = OMe; c: R = Me; d: R = F;
e: R = Cl; f: R = NO_2

Scheme 2

Table 2 Single-strand cleavage of supercoiled circular *φX174* RFI DNA (form I) to relaxed circular DNA (form II) by irradiation of **8a–f** with 312 nm UV light at 37 °C for 2.0 h

Entry	Arylhydrazido-phosphates[a]	pH	% Form I[b]	% Form II[b]	Form II/ form I
1	**8a**	7.4	36	64	1.8
2	**8a**[c]	7.4	84	16	0.19
3	**8a**[d]	7.4	36	64	1.8
4	**8a**[e]	7.4	38	62	1.6
5	**8a**[f]	7.4	36	64	1.8
6	**8a**[g]	7.4	36	64	1.8
7	**8a**[h]	7.4	24	76	3.2
8	**8a**[i]	7.4	82	18	0.22
9	**8a**[j]	7.4	88	12	0.14
10	**8b**	7.4	42	58	1.4
11	**8c**	7.4	38	62	1.6
12	**8d**	7.4	26	74	2.8
13	**8e**	7.4	32	68	2.1
14	**8f**	7.4	30	70	2.3
15	None	7.4	91	9.0	0.10
16	**8a**	5.0	32	68	2.1
17	**8a**	6.0	34	66	1.9
18	**8a**	7.0	36	64	1.8
19	**8a**	8.0	38	62	1.6

[a] A sodium phosphate buffer (Na_2HPO_4 and NaH_2PO_4; 0.10 M) containing an arylhydrazidophosphate (500 μM), 50 μM/base pair of form I DNA (molecular weight 3.50×10^6, 5386 base pairs in length) and 10% THF. [b] Analyzed by gel electrophoresis with 1% agarose and ethidium bromide staining. [c] Molecular oxygen was removed from the buffer by argon gas. [d] Ethanol (2.0 M) was added. [e] Dimethyl sulfoxide (2.0 M) was added. [f] Potassium iodide (50 mM) was added. [g] Cysteine (50 mM) was added. [h] H_2O was replaced by D_2O. [i] Sodium azide (50 mM) was added. [j] In the dark.

Furthermore, we pre-incubated a mixture of ^{32}P end-labeled dsDNA fragments and a cleaving agent arylhydrazine **1d** or hydrazidophosphate **8d** (2000 μM) in a sodium phosphate buffer (pH 7.4) containing 10% THF at 37 °C for 30 min. The double stranded 16-mer d(5′-^{32}P-GGACGAAGGATTACGT)·d(ACGTAATCCTTCGTCC) was prepared on a DNA synthesizer and labeled by use of γ-^{32}P-ATP and T4 polynucleotide kinase. Photo-irradiation (312 nm) of the mixtures followed by piperidine treatment at 95 °C for 30 min generated a sequence independent pattern on an autoradiogram of a 20% polyacrylamide–8 M urea gel. The results indicate that arylhydrazines and arylhydrazidophosphates could be useful for DNA footprinting.[20]

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