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Bioorganic & Medicinal Chemistry 12 (2004) 2509–2515
Bioorganic & Medicinal Chemistry

Aminyl and iminyl radicals from arylhydrazones in the photo-induced DNA cleavage

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Received 3 February 2004; revised 17 March 2004; accepted 18 March 2004

Available online 10 April 2004

Abstract—Photolytic cleavage of the nitrogen–nitrogen single bond in benzaldehyde phenylhydrazones produced aminyl ($R_2N\cdot$) and iminyl ($R_2C{=}N\cdot$) radicals. This photochemical property was utilized in the development of hydrazones as photo-induced DNA-cleaving agents. Irradiation with 350 nm UV light of arylhydrazones bearing substituents of various types in a phosphate buffer solution containing the supercoiled circular $\phi X174$ RFI DNA at pH 6.0 resulted in single-strand cleavage of DNA. Attachment of the electron-donating OMe group to arylhydrazones increased their DNA-cleaving activity. Results from systematic studies indicate that both the aminyl and the iminyl radicals possessed DNA-cleaving ability.
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1. Introduction

Cleavage of DNA by chemical methods often involves carboradicals and the hydroxyl radical HO·.[1–13] Recently 1,5-azulenequinones have also been developed as photo-induced DNA cleaving agents.[14,15] In a systematic study of DNA cleavage, we made efforts to understand the cleaving ability of different species.

Utility of nitrogen-centered radical species as 'DNA nucleases' has recently been documented.[16,17] Binkley[18] reported the photolytic cleavage of the nitrogen–nitrogen single bond in benzaldehyde phenylhydrazone. An aminyl radical ($R_2N\cdot$) and an iminyl radical ($R_2C{=}N\cdot$) are generated, which can escape from solvent cage and perform hydrogen abstraction.

Hydrazones are an important class of organic compounds, some of which show significant biological activities. For example, Heinisch and co-workers[19] developed a series of *N*-heteroaryl hydrazones as potential novel antitumor agents.

Alvarez-Builla and co-workers[20] reported that 2,6-di-*tert*-butyl-4-(2-azinylhydrazonomethyl)phenol derivatives possess potential as 5-lipoxygenase inhibitors. 5-Lipoxygenase metabolites of arachidonic acid are implicated as mediators in a diversity of diseases, including asthma and many other inflammatory pathologies. Moreover, Barreiro and co-workers[21] utilized arylcarbaldehyde 4-(1-phenyl-3-methylpyrazolo-[3,4-*b*]pyridine) hydrazone derivatives as a new pharmacophoric tool for the development of more efficacious analgesics.

Two factors associated with phenylhydrazones must be taken into account for their development as DNA-cleaving agents. First, the size and shape of these 'guests' should allow themselves to fit well into the host (i.e., DNA). Performance of graphic molecular modeling of oligodeoxyribonucleotides and hydrazones provided us valuable information on the molecular design of

Keywords: Aminyl radicals; Iminyl radicals; Photo-induced; DNA cleavage.
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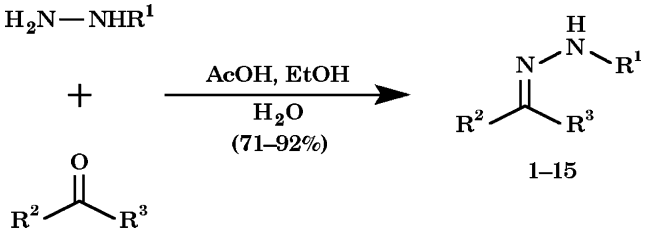
doi:10.1016/j.bmc.2004.03.037



1. $R^1 = R^2 = Ph$, $R^3 = H$
2. $R^1 = Ph$, $R^2 = Ph\text{-}p\text{-}OMe$, $R^3 = H$
3. $R^1 = Ph$, $R^2 = Ph\text{-}p\text{-}NHCOMe$, $R^3 = H$
4. $R^1 = Ph$, $R^2 = Ph\text{-}p\text{-}NO_2$, $R^3 = H$
5. $R^1 = Ph\text{-}p\text{-}F$, $R^2 = Ph$, $R^3 = H$
6. $R^1 = Ph\text{-}p\text{-}OMe$, $R^2 = Ph$, $R^3 = H$
7. $R^1 = Ph\text{-}p\text{-}NO_2$, $R^2 = Ph$, $R^3 = H$
8. $R^1 = Ph\text{-}p\text{-}OMe$, $R^2 = Ph\text{-}p\text{-}NO_2$, $R^3 = H$
9. $R^1 = Ph\text{-}p\text{-}NO_2$, $R^2 = Ph\text{-}p\text{-}OMe$, $R^3 = H$
10. $R^1 = R^2 = Ph\text{-}p\text{-}OMe$, $R^3 = H$
11. $R^1 = Ph$, $R^2 = 1\text{-naphthyl}$, $R^3 = H$
12. $R^1 = Ph$, $R^2 = 3\text{-indolyl}$, $R^3 = H$
13. $R^1 = R^2 = R^3 = Ph$
14. $R^1 = \text{purin-6-yl}$, $R^2 = Ph$, $R^3 = H$
15. $R^1 = \text{2-chloropyrimidin-4-yl}$, $R^2 = Ph$, $R^3 = H$

Scheme 1. Synthesis of arylhydrazones.

the cleaving agents **1**–**15** shown in Scheme 1. Second, stability and lifetime of the radical intermediates generated photochemically are essential to their cleaving ability.

Ingold and co-workers[22] found that the second-order decay of dimethylaminyl and diethylaminyl radicals occurs with the range $(2 \pm 1) \times 10^9\ M^{-1}\ s^{-1}$ by the ESR technique during photolysis. The rate constants for the bimolecular self-reactions are 4×10^7 and $4 \times 10^9\ M^{-1}\ s^{-1}$ for diisopropyliminyl and ditrifluoromethyliminyl radicals, respectively.[23] Lusztyk and co-workers[24] reported that the lifetimes of diethylaminyl and piperidinyl radicals are 1.6 and 2.5 μs in 0.05 M malonic acid/acetonitrile solution. The corresponding phenyl substituted aminyl radicals are more stable than the dialkylaminyl radicals; lifetimes of $PhMeN^{\cdot}$ and $Ph_2N^{\cdot}$ are 50 and 80 μs, respectively. Consequently, we planned to synthesize arylhydrazones bearing various substituents on both aryl rings. Their influence on DNA cleavage could result from the inductive and the field effects.[25]

Herein we report our new findings that cleavage of DNA took place upon irradiation of arylhydrazones with UV light. Our results indicate that both the nitrogen-centered radicals $R_2N^{\cdot}$ and $R_2C{=}N^{\cdot}$ can function as the DNA-cleaving species.

2. Results

2.1. Synthesis of arylhydrazones

We prepared various substituted arylhydrazones **1**–**15** in 71–92% yields by condensation of the corresponding carbonyl compounds with different arylhydrazines in a mixture of glacial acetic acid and ethanol (Scheme 1).[26]

The electrical effects[27] resulting from substituents attached to the aromatic rings of arylhydrazones could play vital roles in the DNA-cleaving process. Consequently, we used arylhydrazines containing an electron-donating (+M) group, such as OMe, and electron-withdrawing (−M) group, such as NO_2, in investigation. Given the same reason, carbonyl compounds containing an OMe, NHCOMe, or NO_2 substituted aryl group were allowed to react with hydrazines. Furthermore, we introduced special moieties, including naphthyl, 3-indolyl, purin-6-yl, and 2-chloropyrimidin-4-yl groups, into the arylhydrazones (e.g., **11**, **12**, **14**, and **15**).

We listed the UV spectroscopic data of arylhydrazones **1**–**15** in Table 1. Most of the compounds possessed a strong UV absorption between 320 and 360 nm, which fits into an ideal range of wavelengths in the photochemical activation of chemical agents for DNA scission.

2.2. Photo-induced DNA cleavage by hydrazones

To investigate their DNA-cleaving potency, we dissolved hydrazones **1**–**15** (250 μM) individually in a sodium phosphate buffer (pH 6.0) and 10% ethanol containing the supercoiled circular $\phi X174$ RFI DNA (form I; 50 μM/base pair). These solutions were irradiated with UV light (350 nm, 16 W) under aerobic conditions at room temperature for 2.0 h. By analyzing the

Table 1. UV spectroscopic data of arylhydrazones **1**–**15**

Arylhydrazone	UV–vis λ_{max} (EtOH)/nm[a]
1	236 (18,057), 296 (16,129), **344** (29,187)
2	243 (22,344), 302 (30,679), **345** (47,771)
3	250 (11,787), 304 (9930), **354** (25,694)
4	264 (26,607), 424 (42,350)
5	236 (11,672), 312 (14,530), **346** (20,189)
6	236 (13,662), 312 (14,924), **346** (20,235)
7	299 (3061), **328** (2493), 404 (17,942)
8	275 (11,392), 444 (22,394)
9	235 (10,471), 292 (9014), **329** (5133), 416 (29,035)
10	246 (11,995), 314 (17,733), **354** (19,260)
11	233 (24,477), 293 (7862), **354** (23,791)
12	**339** (22,537)
13	236 (16,733), 298 (10,025), **340** (19,148)
14	211 (25,000), 268 (18,173)
15	224 (12,309), **326** (23,183)

[a] Numbers marked with bold face represent the absorption between 320 and 360 nm.

results from gel electrophoresis on 1% agarose gel with ethidium bromide staining, we found that arylhydrazones **1**–**15** caused the single-strand cleavage of DNA to give the relaxed circular DNA (form II). The ratios of (form II)/(form I) ranged from 0.19 to 24 (see Fig. 1 and Table 2). The cleaving efficacy of **1** in phosphate buffers over the pH range between 5.0 and 8.0 was found to be slightly pH dependent.

We found that the UV light was essential for arylhydrazones to cleave DNA. Under aerobic conditions with 250 μM of **1**, the ratios of (form II)/(form I) were 0.14 in the dark (the third row in Table 2) and 1.3 with UV light (the second row). Under photolytic conditions for 2.0 h without addition of **1**, the ratio was 0.091 (the last row). Thus the UV light functioned as a 'trigger' to initiate an arylhydrazone for the DNA strand scission.

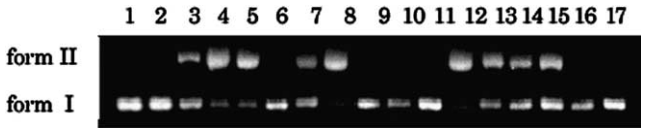


Figure 1. Single-strand cleavage of supercoiled circular $\phi X174$ RFI DNA (form I, 50 μM/base pair, molecular weight 3.50×10^6, 5386 base pairs in length) to relaxed circular DNA (form II) was carried out by irradiation of a hydrazone (250 μM) with 350 nm UV light in a sodium phosphate buffer (pH 6.0) under aerobic conditions at room temperature for 2.0 h. The resultant products were subjected to electrophoresis on 1% agarose gel followed by ethidium bromide staining: lane 1, DNA alone; lane 2, **1** in the dark; lane 3, **1**; lane 4, **2**; lane 5, **3**; lane 6, **4**; lane 7, **5**; lane 8, **6**; lane 9, **7**; lane 10, **8**; lane 11, **9**; lane 12, **10**; lane 13, **11**; lane 14, **12**; lane 15, **13**; lane 16, **14**; lane 17, **15**.

Table 2. Single-strand cleavage of supercoiled circular $\phi X174$ RFI DNA (form I) to relaxed circular DNA (form II) by photolysis of hydrazones **1**–**15** under aerobic conditions at room temperature with 350 nm UV light for 2.0 h

Hydrazone[a]	pH	% Form I[b]	% Form II[b]	(Form II)/(Form I)
1	5.0	37	63	1.7
1	6.0	43	57	1.3
1[c]	6.0	88	12	0.14
1	7.0	45	55	1.2
1	8.0	50	50	1.0
2	6.0	16	84	5.4
3	6.0	13	87	6.7
4	6.0	70	30	0.42
5	6.0	45	55	1.2
6	6.0	6.0	94	16
7	6.0	76	24	0.31
8	6.0	75	25	0.33
9	6.0	76	24	0.31
10	6.0	4.0	96	24
11	6.0	33	67	2.0
12	6.0	45	55	1.2
13	6.0	45	55	1.2
14	6.0	84	16	0.19
15	6.0	83	17	0.20
None	6.0	92	8.3	0.091

[a] A 0.10 M sodium phosphate buffer (Na_2HPO_4 and NaH_2PO_4) containing 250 μM of a hydrazone, 50 μM/base pair of form I DNA (molecular weight 3.50×10^6, 5386 base pairs in length), and 10% EtOH.
[b] Analyzed by gel electrophoresis with 1% agarose and ethidium bromide staining.
[c] In the dark.

Sodium azide can function as a scavenger of singlet oxygen,[28,29] of which presence may contribute to DNA damage.[30,31] We investigated the percentages of DNA scission resulting from singlet oxygen during the photolysis of arylhydrazones. A series of control experiments were performed by addition of sodium azide into the photolytic solution containing hydrazone **1** and DNA. The results are summarized in Table 3.

The percentage of form II DNA decreased from 83% to 67% upon addition of sodium azide (rows 1 and 2 in Table 3). Furthermore, we detected the same percentage of form II DNA (i.e., 67%) by increasing the amount of sodium azide from 100 to 500 mM. These results indicate that singlet oxygen in the solution was destroyed completely by sodium azide in a concentration $\geqslant$100 mM. In comparison with the results from the experiment in the absence of hydrazone **1** (i.e., 9.9%, the last row in Table 3), the percentage of DNA cleavage resulting from **1** was 63% [$=(67\%-9.9\%)/(33\%+67\%-9.9\%)$]. On the other hand, the percentage of DNA scission caused by both **1** and singlet oxygen was calculated as 81% [$=(83\%-9.9\%)/(17\%+83\%-9.9\%)$]. Thus the contribution resulting from singlet oxygen to DNA cleavage was ~18% ($=81\%-63\%$).

Among various substituents onto the benzene ring in an arylhydrazone, our results from gel electrophoresis indicate that attachment of the electron-donating group OMe could enhance its DNA-cleaving activity. As shown in Figure 1 and Table 2, the methoxy-containing hydrazones (e.g., **2**, **6**, and **10**) exhibited appealing potency. Moreover, in a direct comparison of (form II)/(form I), we found that ratios were 5.4 for **2** and 16 for **6**. Thus, under the photolytic conditions, the corresponding methoxy-containing aminyl radical generated in situ acted more potent than the iminyl radical for DNA cleavage. Replacement of a benzene nucleus in a

Table 3. Influence of sodium azide on single-strand cleavage of supercoiled circular $\phi X174$ RFI DNA (form I) to relaxed circular DNA (form II) by photolysis of hydrazone **1** under aerobic conditions at room temperature with 350 nm UV light for 2.0 h

Hydrazone[a]	NaN_3 (mM)	% Form I[b]	% Form II[b]	(Form II)/(Form I)
1	0	17	83	4.9
1	100	33	67	2.0
1	500	33	67	2.0
None	0	90	9.9	0.11

[a] A 0.10 M sodium phosphate buffer (Na_2HPO_4 and NaH_2PO_4) containing 500 μM of a hydrazone, 50 μM/base pair of form I DNA (molecular weight 3.50×10^6, 5386 base pairs in length), and 10% EtOH.
[b] Analyzed by gel electrophoresis with 1% agarose and ethidium bromide staining.

benzaldehyde arylhydrazone with a naphthyl (cf. **11**), indolyl (cf. **12**), purinyl (cf. **14**), or chloropyrimidinyl (cf. **15**) unit did not perform superior potency (see Table 2). Their low solubility (34.8 mg/mL for **14** and 21.6 mg/mL for **15**) in aqueous solution caused the poor efficacy.

Compound **10**, in which the two aromatic rings on each side of =N—N— unit possess a methoxy group, exhibited the greatest cleaving activity (lane 12 of Fig. 1) among all of the tested arylhydrazones. Results from dose measurements of **10** (10–250 μM) reveal that cleavage of DNA depended upon its concentration (Fig. 2); it was able to cleave DNA with (form II)/(form I) = 1.4 at the concentration even as low as 50 μM.

Furthermore, we used polyacrylamide gel electrophoresis to analyze the DNA cleavage pattern resulting from arylhydrazones under photolytic conditions. We preincubated a mixture of 5′-^{32}P-end-labeled *Sal*I–*Sph*I double-helical DNA fragment of pBR322 and arylhydrazone **10** (250–1000 μM) in a sodium phosphate buffer (pH 6.0) containing 10% ethanol at room temperature for 30 min. The 93 base pair DNA fragment was obtained enzymically.[32,33] Photo-excitation (312 and 350 nm) of the mixtures at room temperature for 2.0 h followed by piperidine treatment at 95 °C for 30 min generated a sequence independent pattern on an autoradiogram of a 20% polyacrylamide/8.0 M urea gel (see Fig. 3). Use of piperidine as an external base assisted the vision of the fragmentation pattern. Its use caused DNA scission in the dark but to a limited extent. The results indicate that arylhydrazones could be useful for DNA footprinting.[34]

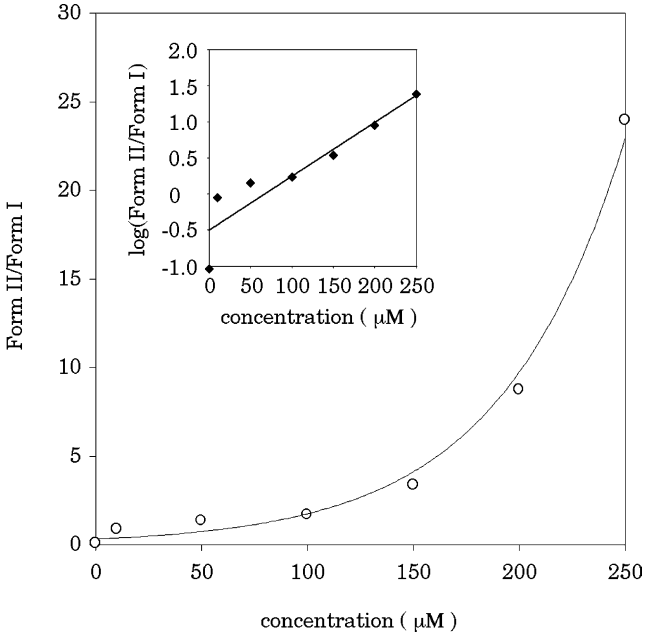


Figure 2. Dose measurements of arylhydrazone **10** for its DNA-cleaving ability in a sodium phosphate buffer (pH 6.0) upon irradiation with 350 nm UV light.

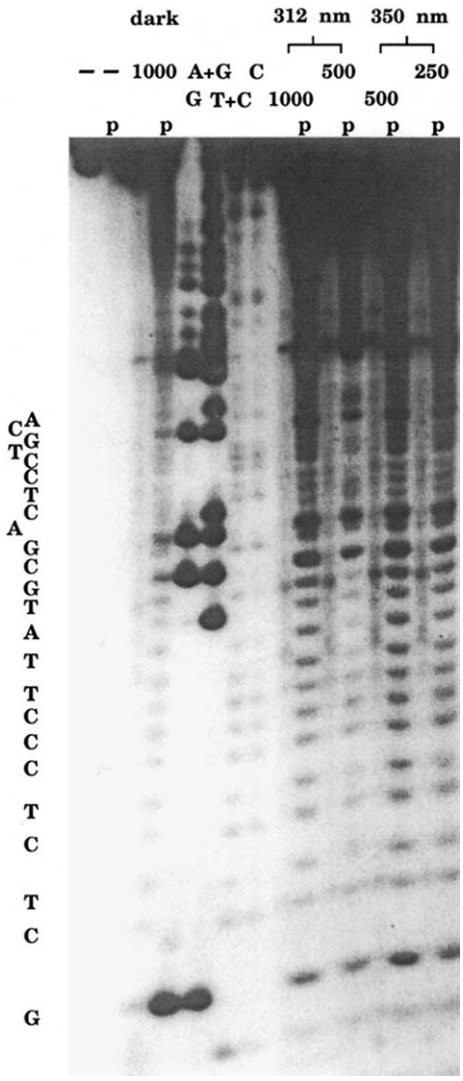


Figure 3. Autoradiogram of a denaturing polyacrylamide gel representing the cleavage of 5′-^{32}P-end-labeled *Sal*I–*Sph*I fragment of pBR322 by photolysis of arylhydrazone **10** (250–1000 μM): –, DNA alone; p: DNA fragments with piperidine treatment; G, A + G, T + C, C, nucleotide residues in sequence determined by Maxam–Gilbert chemical sequencing method. The nucleotide sequence of the labeled strand of the duplex is shown at the left.

3. Discussion

We carried out experiments of DNA cleavage by aminyl ($R_2N^{\cdot}$) and iminyl ($R_2C{=}N^{\cdot}$) radicals, which were generated by photolytic fission of the N–N single bond in arylhydrazones under aerobic conditions. Our results in Table 2 show that the efficiency, indicated by the ratio of (form II)/(form I), depended upon the substituents attached to both aryl units. In comparison with the parent hydrazone **1**, hydrazone **6** exhibited much greater cleaving ability (1.3 vs 16 for the ratio). Thus we conclude that an electron-donating (+M) group, such as OMe, attached to the aminyl nucleus can enhance its DNA-cleaving ability. An electron-withdrawing (−M) group NO_2 in **7** disabled its DNA-cleaving ability. The similar trend resulting from substituents on the iminyl

nucleus is reflected by the ratios of 5.4 and 0.42 for hydrazones **2** (OMe, +M) and **4** (NO_2, −M), respectively.

We believe that an arylaminyl or an aryliminyl group can be stabilized by bearing an electron-donating group. After photolytic fission occurs to the N–N single bond in arylhydrazones, the lifetime of the resultant nitrogen-centered radicals bearing a methoxy group could be long enough to allow themselves to attack DNA. Accordingly, the DNA scission takes place. Having a methoxy group on each of the aryl rings enabled hydrazone **10** to exhibit great efficiency for DNA cleavage; its ratio of (form II)/(form I) reached 24. This rationale is further supported by the results of DNA cleavage resulting from arylhydrazones with various substitutions on both aryl rings.

On the other hand, hydrazones **4** and **7**–**9** possess an NO_2 moiety, which is a strong electron-withdrawing group. Their ratios of (form II)/(form I) were between 0.31 and 0.42 at the concentration of 250 μM. Thus, the presence of an NO_2 group in hydrazones decreases their DNA cleaving ability significantly regardless the presence of an OMe group. Results from our systematic studies clearly indicate that the substituents on each of the aryl rings exerted influence on the DNA scission. Therefore we conclude that both the aminyl and the iminyl radicals are responsible for DNA cleavage.

The research teams of Wender,[35] Sortino,[16] Kawanishi,[36] and Davis[37] reported individually that aminyl radicals can damage DNA. An iminyl radical generated from oxime ester of anthraquinone by photolysis may also be responsible for DNA scissions.[38] Two possible mechanisms can be envisaged for DNA lesion by these nitrogen-centered radicals. The first is the hydrogen abstraction from the deoxyribose moieties.[39] The second is radical attack on the nucleobases to generate hot piperidine sensitive sites.[40] At the current stage, our data shown in Figure 1 (without piperidine treatment) and Figure 2 (with piperidine treatment) cannot differentiate these two possibilities. The cleaving mechanism in detail will be studied in due course.

To confirm the DNA cleavages by radicals from benzaldehyde phenylhydrazones, we performed control experiments by adding ascorbic acid[1a] and DMSO,[1a] respectively, as radical quenchers in the DNA media. Ascorbic acid was found to obstruct the scission process completely; DMSO degraded the scissions to a minimal extent. Ethanol can also function as a moderate radical quencher.[1a,41] Our experimental results however indicate that its addition to the samples of benzaldehyde phenylhydrazones **1**–**15** did not exert significant influence on their cleaving efficiency as displayed by gel electrophoresis. Thus we chose 10% ethanol as the co-solvent[42] for dissolving compounds **1**–**15** in the DNA cleaving experiments.

Autoxidation of benzaldehyde phenylhydrazones produces peroxy intermediates and free-radical species.[43,44] Thus phenylhydrazones **1**–**15** oxidized by molecular oxygen may generate radicals during photolysis under aerobic conditions.[45] Results from our control experiment involving the addition of sodium azide as a singlet oxygen scavenger indicate that the oxygen-centered radicals contributed up to ∼18% to the DNA scission.

4. Conclusion

Ability of arylhydrazones to cleave DNA was studied; these compounds could be also developed as photo-induced DNA-cleaving agents. Upon irradiation with 350 nm UV light, hydrazones bearing the electron-donating OMe group showed great cleaving activity. Our results from systematic studies provide evidence to show that the nitrogen-centered aminyl and iminyl radicals, generated by photolysis of arylhydrazones, are active species for DNA scission.

5. Experimental

5.1. General procedure

All reactions were carried out in oven-dried (120 °C) or autoclaved glassware and eppendorf tubes. Absolute ethyl alcohol was obtained from RDH Chemical Co. Hydrazine hydrate from Merck Inc. was dried and distilled from NaOH. Ammonium persulfate and boric acid were purchased from Serva Chemical Co. Bromophenol blue, salmon testing DNA, *t*-RNA, agarose, tetramethylethylenediamine, tris(hydroxymethyl)aminomethane, and xylene cyanol were purchased from Sigma Chemical Co. and used as received. Supercoiled circular $\phi X174$ RFI DNA, pBR322 DNA, calf intestinal alkaline phosphatase (*CIP*),T4 polynucleotide kinase as well as *Sal*I and *Sph*I restriction endonucleases were purchased from New England Biolabs. Maxam–Gilbert sequencing kit was purchased from NEN-DuPont Chemical Co. and deoxyadenosine 5′-[γ-^{32}P]-triphosphate (>5000 Ci/mmol) from Amersham Co. Acrylamide and *N*,*N*′-methylenebisacrylamide were purchased from Amersco Chemical Co.

Ultraviolet (UV) spectra were recorded on a Hitachi U-3300 spectrophotometer and λ_{max} was obtained in nm (ε) units. Infrared (IR) spectra were recorded on a Perkin–Elmer Paragon 1000 FTIR spectrophotometer. The wavenumbers reported are referenced to the 1601 cm^{-1} absorption of polystyrene. Proton NMR spectra were obtained on Bruker AC-200 (200 MHz) and AC-300 (300 MHz) spectrometers and ^{13}C NMR spectra were recorded on a Bruker AC-200 (50 MHz) or AC-300 (75 MHz) spectrometer. Chloroform-*d* or dimethylsulfoxide-d_6 was used as solvent; Me_4Si (δ 0.00 ppm) and the center of the $CDCl_3$ triplet (δ 77.00 ppm) were used as an internal standard for ^{1}H and ^{13}C NMR spectra, respectively. All NMR chemical shifts are reported as δ values in parts per million (ppm) and coupling constants (J) are given in hertz (Hz). The splitting pattern abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; br, broad; m, unresolved multiplet; dd, doublet of doublets.

Photolytic experiments were carried out at room temperature by use of a 16-Watt Rayonet 350 nm lamp, and equipped with Merry-go-round apparatus. The NIH 1.60 image program, provided by Dr. R. Wayne of National Institutes of Health, U.S.A., was used for the quantitative analysis of DNA cleavage.

5.1.1. Standard procedure for preparation of substituted *N*-aryl arylhydrazones. Arylaldehyde (1.1–1.2 equiv) was added into aqueous ethanol (20%) containing arylhydrazine (1.0 equiv) and a trace amount of glacial acetic acid. After the reaction mixture was stirred at room temperature in the dark for 3.0 h, the crystals of hydrazones were collected on a Büchner funnel. Their structures were determined by spectroscopic methods. Hydrazones **1**–**13** have been reported before;[46–58] their physical properties and spectroscopic characteristics are consistent with those in the literature.

5.1.1.1. Synthesis of benzaldehyde purin-6-yl-hydrazone (14). The standard procedure was followed by use of benzaldehyde (325 mg, 3.06 mmol, 1.2 equiv), aqueous ethanol (20%, 10 mL), and purin-6-ylhydrazine (383 mg, 2.55 mmol, 1.0 equiv). After the reaction mixture was stirred at room temperature for 3.0 h, it was worked up and the residue was recrystallized to give **14** as white crystals in 76% yield (462 mg, 1.94 mmol): mp 280–281 °C; δ_H (200 MHz, $CDCl_3$) 7.09–7.12 (m, 3H, ArH), 7.50–7.55 (m, 2H, ArH), 7.78–7.93 (m, 2H, N═CH + N═C(8)H), 8.14 (s, 1H, N═C(2)H); δ_C (50 MHz, DMSO-d_6) 127.24, 128.82, 129.41, 129.62, 134.34, 143.90, 142.24, 144.85, 150.03, 151.96; ν_{max} (KBr)/cm^{-1} 3410 (m), 3219 (m, N—H), 2796 (m, Ar—H), 1640 (s), 1604 (s), 1490 (w), 1320 (s), 1251 (m), 1141 (m), 941 (w); λ_{max} (EtOH)/nm (ε) 211 (25,000), 268 (18,173); m/z (EI) 238 (M^+; 8%), 210 (1), 161 (12), 155 (2), 135 (100), 128 (2), 119 (6), 108 (48), 103 (13), 89 (13), 77 (27), 65 (15), 51 (35); exact mass calcd for $C_{12}H_{10}N_6$ 238.0967, found 238.0968; Anal. Calcd for $C_{12}H_{10}N_6$: C, 60.50; H, 4.23; N, 35.27. Found: C, 60.48; H, 4.23; N, 35.28.

5.1.1.2. Synthesis of benzaldehyde 2-chloropyrimidin-4-ylhydrazone (15). The standard procedure was followed by use of benzaldehyde (558 mg, 5.26 mmol, 1.2 equiv), aqueous ethanol (20%, 10 mL), and 2-chloropyrimidin-4-ylhydrazine (482 mg, 4.38 mmol, 1.0 equiv). After the reaction mixture was stirred at room temperature for 3.0 h, it was worked up and the residue was recrystallized to give **15** as white crystals in 74% yield (754 mg, 3.24 mmol): mp 248–249 °C; δ_H (200 MHz, $CDCl_3$ + DMSO-d_6) 6.65–6.71 (m, 1H, ArH), 6.89–6.94 (m, 2H, ArH), 7.17–7.24 (m, 4H, ArH), 7.59 (s, 1H, N═CH), 7.66–7.69 (m, 1H, ArH); δ_C (50 MHz, DMSO-δ_6) 102.37, 126.80, 128.78, 129.84, 134.03, 144.68, 158.41, 159.28, 162.93; ν_{max} (KBr)/cm^{-1} 3461 (m), 3191 (m, N—H), 3048 (m), 2908 (m, Ar—H), 1594 (s), 1422 (s), 1380 (s), 1228 (m), 1119 (s), 829 (m); exact mass calcd for $C_{11}H_9N_4Cl$ 232.0516, found 232.0515; Anal. Calcd for $C_{11}H_9N_4Cl$: C, 56.78; H, 3.90; N, 24.08. Found: C, 56.80; H, 3.89; N, 24.09.

5.2. Cleavage of supercoiled circular $\phi X174$ RFI DNA by arylhydrazones

The reaction mixtures (10 μL) containing supercoiled circular $\phi X174$ RFI DNA stock solution (form I, 50 μM/base pair), an arylhydrazone (250 μM), phosphate buffers (0.10 M, pH 5.0–8.0), and 10% EtOH in a Pyrex vial were irradiated with UV light (350 nm, 16 W) under aerobic conditions at room temperature for 2.0 h. After addition of the gel-loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol), the reaction mixtures were loaded on a 1% agarose gel with ethidium bromide staining. The electrophoresis tank was attached to a power supply at a constant current (~100 mA). The gel was visualized by 312 nm UV transilluminator and photographed by an FB-PBC-34 camera. Quantitation of DNA-cleaving activities was performed by integration of the optical density as a function of the band area by use of a Microtek scanner and an NIH 1.60 image program.

5.3. Cleavage of ^{32}P-DNA by hydrazone 10

The reaction mixture containing 5′-^{32}P-end-labeled *Sal*I–*Sph*I dsDNA fragment of pBR322, a phosphate buffer (0.10 M, pH 6.0), and hydrazone **10** (250–1000 μM) in a Pyrex vial were preincubated at room temperature for 30 min. After irradiation with 312 or 350 nm UV light under aerobic conditions at room temperature for 2.0 h, the reaction mixtures were quenched with gel-loading buffer (80% formamide, 0.25% bromophenol blue, and 0.25% xylene cyanol) or 95% EtOH. The solutions were then subjected to piperidine treatment and ethanol precipitation. The DNA pellet was resuspended in aqueous piperidine solution (1.0 M, 60 μL) and maintained at 95 °C for 30 min. Subsequently, the reaction mixtures were sequentially lyophilized, treated with water (30 μL), lyophilized, and resuspended in the gel-loading buffer. The DNA solution and the Maxam–Gilbert markers were analyzed by use of 20% polyacrylamide/8.0 M urea gel. The electrophoresis was performed at a voltage of 500 V for 30 min and raised to 1000 V for another 2.0 h. The cleavage positions were visualized by use of Kodak X-Omat Ar-5 film at −70 °C for 24 h. Quantitation of the relative intensity for the cleavage sites was integrated by the optical density as a function of the band area by use of a Microtek scanner and NIH 1.60 image program.

Acknowledgements

For financial support, we thank Ministry of Education (Grant 89-B-FA04-1-4) of the Republic of China. We are also grateful to Dr. R. Wayne for providing the NIH 1.60 image program for the quantitative analysis of DNA cleavage. Tzu-Rong Su is a graduate student from Graduate Institute of Dental Sciences, Kaohsiung Medical University.

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