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N,O-Diacyl-4-benzoyl-*N*-phenylhydroxylamines as photoinduced DNA cleaving agents

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ABSTRACT

Photoinduced homolytic fission of nitrogen–oxygen bond in *N,O*-diacyl-4-benzoyl-*N*-phenylhydroxylamines using ⩾310 nm UV light for 10 min produced acylaminyl and acyloxy radicals, which resulted in single strand cleavage of DNA at pH 7.0. Further the DNA cleaving ability of *N,O*-diacyl-4-benzoyl-*N*-phenylhydroxylamines found to depend both on its concentration and acyl substituents.

Carbon and oxygen-centered organic radicals are of great importance in the field of bioorganic and medicinal chemistry due to its ability to induce DNA cleavage, which is an essential mechanism in many cancer therapies.[1–8]

Utility of nitrogen-centered stable organic radicals for DNA cleavage have been less explored, although some examples are recently reported. Hwu et al.,[9] showed photolytic fission of weak N–O bond of oxime esters of anthraquinone generated nitrogen-centered iminyl ($R_2C{=}N^{\cdot}$) radicals, which resulted in DNA cleavage. Further, the same group also demonstrated that arylaminyl ($R_2N^{\cdot}$) radicals generated through photoinduced cleavage of N–N single bond in arylhydrazones posses excellent DNA cleaving ability.[10]

Among nitrogen-centered aminyl radicals, acylaminyl radicals ($ROC(O)N^{\cdot}R$) are of interest due to their fascinating physical and chemical properties. Similar to arylaminyls, acylaminyls also exist in a π-electronic ground state but with slight delocalization of the unpaired electrons on the carbonyl group.[11,12] In spite of delocalization of unpaired electrons, all the chemical reactions of acylaminyls occurs at nitrogen.[13] Though, simple acylaminyls are short lived and sensitive to oxygen[12] it undergoes important reactions like Hofmann–Loffler intramolecular rearrangement,[14] facile hydrogen abstraction[15] and addition to unsaturated hydrocarbons.[14] Consequently, this prompted us to investigate the DNA cleaving ability of acylaminyl radicals.

In search of an organic molecule which can produce acylaminyl radicals via weak homolytic N–O bond cleavage led us to *N*-arylhydroxylamine derivatives. *N*-arylhydroxylamines are important class of compounds used as key intermediates in the synthesis of variety of natural products and useful biologically active compounds.[16] Moreover, they also exhibit a wide range of pharmacological and physiological activities.[17] Recently, *N*-arylalkyl-*N*-phenylhydroxylamines have been shown to act as efficient DNA cleaving agents.[18,19] The mechanism involves photochemical reaction of *N*-benzyl-*N*-phenylhydroxylamines with molecular oxygen to produce hydroxyl radicals which are responsible for DNA cleavage rather than homolytic fission of N–O bond.

On other hand, Sakurai et al.[20] showed that triplet sensitized photolysis of *N,O*-diacyl-*N*-phenylhydroxylamines undergoes an efficient homolytic N–O bond cleavage to generate acylaminyl ($ROC(O)N^{\cdot}R$) and acyloxy ($R_2COO^{\cdot}$) radicals, which in polar solvent escapes from the solvent cage and perform hydrogen abstraction to yield corresponding arene carboxanilides and carboxylic acids. Considering *N,O*-diacyl-*N*-phenylhydroxylamines ability to produce acylaminyl radicals under mild irradiation condition, led us to explore its application as DNA cleaving agents.

In this article, we report for the first time *N,O*-diacyl-*N*-phenylhydroxylamines with an inbuilt triplet sensitizer benzophenone as photoinduced DNA cleaving agents. The results indicated that acylaminyl, acyloxy and acyl radicals were mostly accountable for DNA cleavage. In addition, we also showed that acyl substituents as well as the concentration of *N,O*-diacyl-*N*-phenylhydroxyl amines have great influence on its DNA cleaving ability.

We have synthesized two series of derivatives of 4-benzoyl-*N*-phenylhydroxylamine (4-Bz-NPHA) as outlined in Scheme 1. The series-1 consists of *N*-benzoyl-*O*-acyl-4-Bz-NPHA derivatives (**3a**–**f**) and the series-2 has derivatives of *N*-acyl-*O*-benzoyl-4-Bz-NPHA (**5a**–**d**). The compounds of series-1 were synthesized, initially by carrying out N-benzoylation of 4-Bz-NPHA (**1**) with benzoyl chloride in presence of $NaHCO_3$ in dry DCM at 0 °C to yield *N*-ben-

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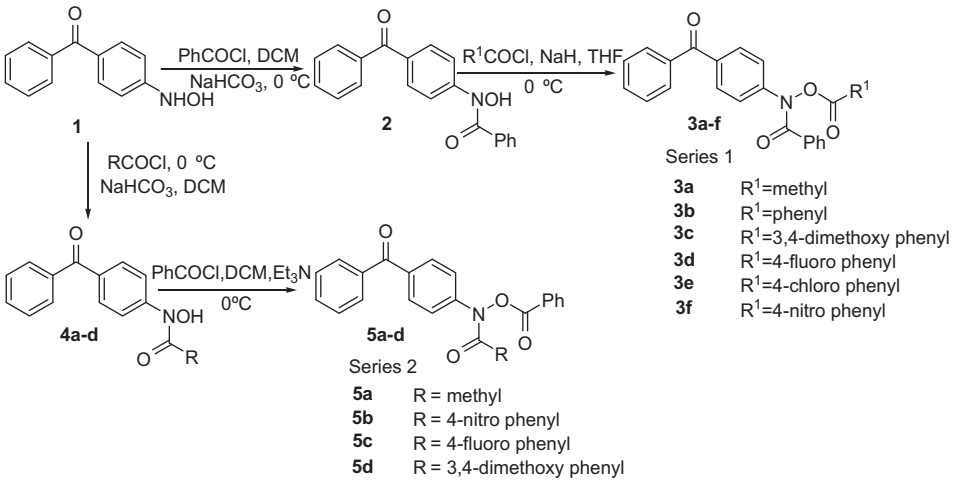


Scheme 1. Synthesis of *N*,*O*-diacyl-4-benzoyl-*N*-phenylhydroxylamines (**3a–j**, **5a–d**).

Table 1
Synthetic yield and UV–vis data of *N*,*O*-diacyl-4-benzoyl-*N*-phenylhydroxylamines (**3a–j**, **5a–d**)

Compound	Synthetic yield (%)	UV–vis λ_{max}			
		λ_1 (nm)	ε_1 (M^{-1} cm^{-1})	λ_2 (nm)	ε_2 (M^{-1} cm^{-1})
3a	85	235	27,945	287	18,967
3b	90	219	19,184	291	16,852
3c	92	223	29,940	294	21,960
3d	80	234	35,144	285	24,508
3e	82	241	33,041	281	18,078
3f	75	218	13,751	300	11,756
5a	90	234	37,379	281	27,454
5b	75	228	21,409	295	13,556
5c	82	232	34,717	285	22,747
5d	80	232	25,277	281	20,321

zoyl-4-Bz-NPHA (**2**).[21] Later, O-acylation of **2** with various acid chlorides in presence of sodium hydride in dry THF at 0 °C resulted in excellent yield of *N*-benzoyl-*O*-acyl-4-Bz-NPHA derivatives (**3a–f**). While, in the case of series-2, N-acylation of 4-Bz-NPHA (**1**) was carried out initially by treating with various acid chlorides to yield **4a–d**, which latter was O-benzoylated with benzoyl chloride to yield *N*-acyl-*O*-benzoyl-4-Bz-NPHA derivatives (**5a–d**). All the above compounds were characterized by ^1H, ^{13}C NMR, IR and mass spectral analysis.

The UV–vis absorption spectra of degassed 2.5×10^{-5} M solution of compounds (**3a–j**, **5a–d**) in methanol were recorded and the results were summarized in Table 1. The results clearly showed that all the compounds exhibited strong absorption maxima between 280–300 nm, thus makes them suitable for DNA cleavage.

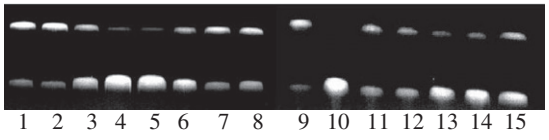


Figure 1. Single strand cleavage of supercoiled circular pBR322 DNA (form I) to relaxed circular DNA (form II) was carried out by irradiation of compounds (**3a–j**, **5a–d**) (250 μM) with 310 nm UV light in a sodium phosphate buffer (pH 7.0) under aerobic condition at room temperature for 10 min. The resultant products were subjected to electrophoresis on 1% agarose gel followed by ethidium bromide staining under UV light. (a) Series-1: lane 1, DNA alone; lane 2, DNA + **3a** (250 μM) in the dark; lane 3, DNA + **3a**; lane 4, DNA + **3b**; lane 5, DNA + **3c**; lane 6, DNA + **3d**; lane 7, DNA + **3e**; lane 8, DNA + **3f**; (b) series-2: lane 9, DNA + ascorbic acid; lane 10, DNA + **5a**; lane 11, DNA + **5b**; lane 12, DNA + **5c**; lane 13, DNA + **5d**; lane 14, DNA + **3a** + sodium azide; lane 15, DNA + **3a** bubbling with argon gas.

Table 2
Single-strand cleavage of supercoiled circular pBR322 DNA (form I) to relaxed circular DNA (form II) by photolysis of compounds (**3a–j**, **5a–d**) under aerobic conditions at room temperature with UV light ⩾310 nm for 10 min

Entry	Compounds	Time (10 min)		
		% Form I	% Form II	(Form II)/(Form I)
1[a]	—	85.47	14.53	0.17
2	**3a**[b]	83.67	16.33	0.19
3	**3a**	25.58	73.86	2.88
4	**3b**	13.16	86.84	6.59
5	**3c**	11.18	88.82	7.94
6	**3d**	37.14	62.86	1.69
7	**3e**	64.95	35.05	0.54
8	**3f**	56.59	43.41	0.77
9	**3a**[c]	76.58	23.42	0.31
10	**5a**	7.83	92.17	11.77
11	**5b**	63.67	36.33	0.57
12	**5c**	59.16	40.84	0.69
13	**5d**	22.92	77.08	3.36
14	**3a**[d]	24.05	75.95	3.18
15	**3a**[e]	26.08	72.95	2.79

[a] Refers to DNA alone.
[b] Refers to DNA + compound (**3a**) in the dark.
[c] Refers to DNA + compound (**3a**) + ascorbic acid.
[d] Refers to DNA + compound (**3a**) + (0.1 M) NaN_3 solution.
[e] Refers to DNA + compound (**3a**) bubbling with argon gas.

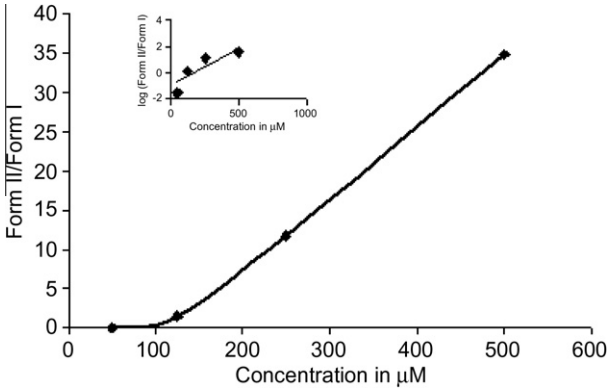
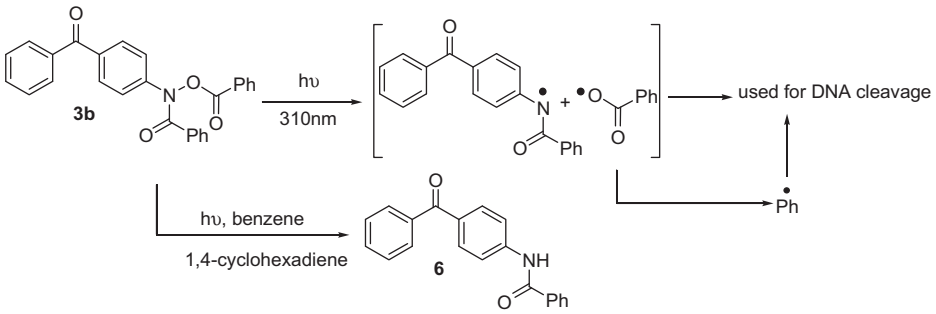


Figure 2. Effect of concentration versus DNA cleaving ability of compound **5a** in a sodium phosphate buffer (pH 7.0) upon irradiation of 310 nm UV light for 10 min.

In order to assess the DNA cleaving ability of *N*,*O*-diacyl-4-benzoyl-*N*-phenylhydroxylamines, we irradiated the compounds (**3a–j**, **5a–d**) with UV light (⩾310 nm, 125 W medium pressure mer-



Scheme 2. Mechanism for the photolysis of *N*,*O*-diacyl-4-benzoyl-*N*-phenylhydroxylamine (**3b**).

cury lamp) at the concentration of 250 μM dissolved in a sodium phosphate buffer (pH 7.0, 10 mM) with 10% DMSO containing the circular supercoiled pBR322 plasmid DNA (form I; 62.5 μg/ml) at room temperature for 10 min under aerobic condition.

By analyzing the results from gel electrophoresis on 1.0% agarose gel with ethidium bromide staining, we found that compounds (**3a**–**j**, **5a**–**d**) caused the single strand cleavage of DNA to give the relaxed circular DNA (form II). The ratios of (form II)/(form I) ranged from 0.54 to 11.77 (Fig. 1, Table 2).

To find out the requirement of UV light by *N*,*O*-diacyl-4-Bz-NPHA for DNA cleavage, we carried out a control experiment in the dark with 250 μM of **3a** with DNA. We observed the ratio of (form II)/(form I) to be 0.17 in the dark (entry 2, Table 2) compared to 2.88 with UV light (Table 2, entry 3), which clearly suggest that UV light is an essential requirement to initiate the DNA scission process. Secondly, to understand the role of molecular oxygen in the DNA cleavage process, we carried out the irradiation of DNA with compound **3a** under argon (argon was bubbled for a period of 15 min to reduce the concentration of dissolved oxygen), the ratio of (form II)/(form I) was found to be 2.79 (Table 2, entry 15) which is similar to the result obtained under aerobic condition (Table 2, entry 3) which rules out the involvement of oxygen in the DNA nicking process. In another experiment, we also carried out the irradiation of DNA with **3a** by adding sodium azide[22,23] (scavenger of singlet oxygen) and the ratio of (form II)/(form I) was found to be 3.18 (Table 2, entry 14), which excludes the possibility of DNA cleavage by singlet oxygen.

Analytical results from gel electrophoresis showed that acyl substituents have great influence on the DNA cleaving ability of *N*,*O*-diacyl-4-Bz-NPHA derivatives. Among series-1, compound **3c** having electron donating methoxy group attached to the *O*-benzoyl substituent exhibited best DNA cleaving ability while, compound **3f** with electron withdrawing nitro group showed poor performance. The above fact can be attributed to the stability of aryloxy radicals.[9]

In the case of series-2, compound **5a** having *N*-acetyl substituent showed excellent DNA cleaving ability compared to compound **3a** (series-1) with *N*-benzoyl substituent. Since, stronger electron withdrawing acyl substituent delocalize the unpaired electron more into carbonyl group, which reduces the spin density on the nitrogen atom of acyl aminyl radicals.[11,12] This is further confirmed by the fact that compound **5b** with electron withdrawing nitro group attached to the *N*-benzoyl substituent showed inferior cleaving ability compared to compound **5d** with electron donating methoxy substituent. Among both the series compound **5a** exhibited the best DNA cleaving ability.

To find out the optimum concentration required for DNA cleavage by *N*,*O*-diacyl-4-benzoyl-*N*-phenyl hydroxylamines, we carried out irradiation of DNA with different concentrations of **5a** (50–250 μM) for a period of 10 min. Results from the concentration study of **5a** showed that cleavage of DNA is depended upon its concentration (Fig. 2). We also noticed that DNA cleaving ability of **5a** enhanced with the increase of its concentration. For example, the ratio of (form II)/(form I) was 1.4 at 125 μM whereas it is 11.77 and 34.74 at 250 and 500 μM concentration.

Based on the literature precedence,[20] mechanism for the observed photochemistry of the *N*,*O*-diacyl-4-benzoyl-*N*-phenylhydroxylamines is outlined in Scheme 2. The mechanism proceeds through photoinduced homolytic N–O bond cleavage to generate acylaminyl and acyloxy radicals. The above generated radicals, then escape from the solvent cage to induce significant DNA cleavage. Further the acyloxy radicals produced can also undergo effective decarboxylation to yield aryl radicals, which are also known to posses' DNA cleaving ability.[24]

To confirm the DNA cleavage is due to the radicals generated from *N*,*O*-diacyl-4-Bz-NPHA, we irradiated DNA with **3a** in the presence of radical quencher ascorbic acid.[1a,25] We found that ascorbic acid completely inhibited the cleavage of DNA (lane 9, Fig. 1) and the ratio of (form II)/(form I) was 0.31 (Table 2, entry 9).

In order to trap the generated acylaminyl radicals, we irradiated **3b** (0.1 mmol) in benzene containing 1,4-cyclohexadiene[26] (1.1 mmol) using 310 nm UV light for 30 min. After completion of the reaction the photoproducts were isolated using column chromatography. We obtained 4-benzoylbenzanilide[27] (**6**) in 48% yield, which should have came from the abstraction of hydrogen atom by acylaminyl radicals from 1,4-cyclohexadiene.[28]

In summary, acylaminyl radicals produced via photo induced homolytic fission of weak N–O bond of *N*,*O*-diacyl-4-benzoyl-*N*-phenyl hydroxyl amines resulted in efficient DNA cleavage. Further strong electron withdrawing acyl substituent reduces the DNA cleaving ability of acylaminyl radicals. Among the derivatives, *N*-acetyl-*O*-benzoyl-4-benzoyl-*N*-phenylhydroxylamine **5a** showed best DNA cleaving ability at 250 μM concentration at 10 min of irradiation.

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Supplementary data

Supplementary data (general synthetic procedure, spectral data of compounds and HPLC chromatogram of the photolysis of compound **3b**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.116.

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28. Compound **3b** (50 mg, 0.1 mmol) was dissolved in dry benzene under argon atmosphere. Then, 1,4-cyclohexadiene (0.11 mL, 1.1 mmol) was slowly added via syringe. The solution was irradiated for 30 min at room temperature. Photoproduct (**6**) was isolated by using column chromatography.
Further the compound **3b** (20 mg, 0.047 mmol) was also irradiated in methanol under argon for 10 min at room temperature and the photolysate was analysed using HPLC (C18 reverse phase column (4.6 × 250 mm, 5 μm)mobile phase, acetonitrile/water = 80:20, flow rate 1 mL/ min, detection: UV at 254 nm). The HPLC chromatogram showed only two significant photoproducts namely 4-benzoylbenzanilide (**6**) and benzoic acid.