The Triplet State of a *N*-Phenylphthalimidine with High Intersystem Crossing Efficiency: Characterization by Transient Absorption Spectroscopy and DNA Sensitization Properties

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The detailed triplet state characteristics of 2-(4-acetylphenyl)isoindolin-1-one (kINP), a *N*-phenylphthalimidine (PPI) derivative, have been studied in fluid solution at room temperature. The attachment of an acetyl group to the *N*-phenyl moiety of PPI has permitted to enhance the intersystem crossing quantum yield, generally low for such compounds. Upon 308-nm laser flash photolysis of kINP in acetonitrile, a triplet−triplet transition has been evidenced ($\lambda_{max} = 440$ nm). Further characterization of this transient at 440 nm gave a lifetime $\tau = 11$ μs, a molar absorption coefficient $\epsilon = 22\ 000\ M^{-1} \times cm^{-1}$, and an intersystem crossing quantum yield of 0.89. Moreover, a $\pi\pi^*$ nature has been found for this triplet state that lies at ca. 290 kJ $\times$ mol^{-1} above the ground state. In addition to providing fundamental information on the triplet state properties of PPI derivatives, its importance during a photobiological process has been evidenced. kINP is the key compound involved in thymine dimers formation during the photosensitization of DNA by indoprofen, a nonsteroidal antiinflammatory drug.

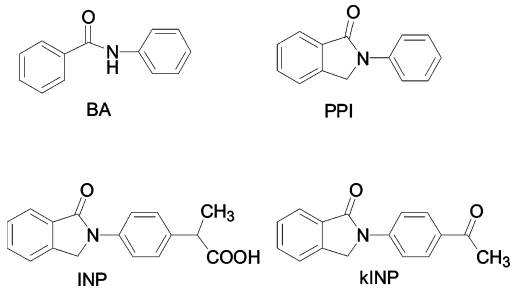
Introduction

The intriguing emission behavior of benzanilide (BA) (Chart 1) and its derivatives has received considerable attention.[1−5] In solution, at room temperature, BA is very weakly fluorescent in nonpolar media and essentially nonfluorescent in polar media. Under these conditions, no phosphorescence is observed. The same is true for *N*-phenylphthalimidine (PPI), a rigid lactam analogue of BA where rotation about the carbonyl-nitrogen bond is precluded.[5] Basically, all the energy absorbed by both compounds is wasted through radiationless decay, corresponding almost exclusively to internal conversion within the singlet manifold.[1]

Recent investigations on the phosphorescence of BA and its derivatives at 77 K, both in methyltetrahydrofuran (MTHF) glasses and in microcrystalline solids, have shown that emission takes place from a planar $\pi\pi^*$ triplet state.[5] This is in agreement with theoretical (ZINDO) calculations. The phosphorescence spectra of microcrystalline BA and PPI are strongly structured, and their maxima (460 and 430 nm, respectively) appear at longer wavelengths than in MTHF glasses (410 and 420 nm).

Thus, very little information is available on the triplet states of BA and PPI. In particular, the spectroscopic characterization and chemical behavior of these states in solution, at room temperature, has not yet been reported. A systematic investigation of these aspects would be interesting both from the fundamental point of view and in connection with the photobiological properties of the basic *N*-phenylphthalimidine chromophore present in the rigid derivative PPI.

CHART 1: Structures of Benzanilide (BA), *N*-Phenylphthalimidine (PPI), Indoprofen (INP), and Its Photoproduct kINP



This chromophore is the key substructure of the nonsteroidal antiinflammatory drug (NSAID) indoprofen (INP, 2-[4-(1-*oxo*-2-isoindolinyl)phenyl]propionic acid) that belongs to the arylpropionic acid family (Chart 1). Related NSAIDs are ketoprofen, tiaprofenic acid, naproxen, and suprofen, among others. Such NSAIDs have been repeatedly associated with photosensitivity reactions that generally involve drug-photosensitized damages to biomolecules.[6−9] In this context, DNA photosensitization has received special attention, as genome damage is considered to be the primary event for many changes of biological importance (photogenotoxicity, photogenomutagenicity, photocarcinogenesis).

One of the major alterations of DNA is formation of cyclobutane thymine dimers, which are thought to be predominant premutagenic damages.[10,11] For this photolesion to be initiated by the UVA region of sunlight, a photosensitizer must be able to participate in a triplet−triplet energy-transfer mech-

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anism. Only a few compounds are efficient in this process, since they have to combine a good intersystem crossing quantum yield with a triplet state energy higher than that of thymine (ca. 310 kJ × mol^{-1} in the free base or the nucleoside, but at least 10 kJ × mol^{-1} lower in DNA).[12,13] Thus, although DNA damage formation has been reported for several NSAIDs derived from arylpropionic acid,[14−19] only ketoprofen or indoprofen (Chart 1) appears to photosensitize a significant amount of dimers.[17−19] In INP, this was surprising since no direct triplet−triplet absorption can be detected by laser flash photolysis (LFP).[20] Thus, intersystem crossing to the triplet state does not take place in an efficient way, as expected from the close structural similarity between INP and PPI. An interesting hypothesis is that one of the INP photoproducts (rather than the parent drug) could be the key chemical entity involved in the DNA-photosensitization reaction. In agreement with this hypothesis, the photostable methyl ester of indoprofen, containing the same chromophore, does not photosensitize thymine dimers.[19]

All the photoproducts of indoprofen contain the same PPI substructure, but only one of them, namely, 2-(4-acetylphenyl)-isoindolin-1-one (kINP), includes the acetophenone chromophore (Chart 1). As acetophenone is able to produce thymine photodimerization,[13,21,22] kINP was considered as the potential photosensitizer of thymine dimers during irradiation of DNA in the presence of INP.

With this background, the purpose of the present work has been to perform a detailed photophysical and photochemical study on kINP. From the fundamental point of view, attachment of the acetyl substituent to the *N*-phenyl ring of PPI should greatly enhance intersystem crossing, providing an efficient route to the triplet state; this could in principle allow characterization of this excited state in solution, at room temperature, by LFP. As regards the photobiological properties, a combination of time-resolved studies on kINP in the presence of triplet quenchers (including DNA and its components) with gel sequencing experiments using repair enzymes was expected to provide a valuable tool to investigate the mechanism of DNA damage upon photosensitization with INP.

Materials and Methods

Synthesis of kINP. Although kINP was available in small amounts by photolysis of indoprofen, it was prepared at higher scale by reaction of 4-aminoacetophenone with 2-carboxybenzaldehyde in methanol, followed by reduction with $NaBH_4$.[23] Its characterization was in agreement with the previously published data.[20]

Laser Flash Photolysis Measurements. A pulsed Excimer Laser Systems with Xe/HCl/Ne mixture was used for excitation at 308 nm. The single pulses were ∼17 ns duration and the energy was ≤100 mJ/pulse. A pulsed Lo255 Oriel xenon lamp was employed as detecting light source. The laser flash photolysis apparatus consisted of the pulsed laser, the Xe lamp, a 77200 Oriel monochromator, an Oriel photomultiplier tube (PMT) system made up of a 77348 side-on PMT tube, 70680 PMT housing, and a 70705 PMT power supply. The oscilloscope was a TDS-640A Tektronix. The output signal from the oscilloscope was transferred to a personal computer. Indoprofen was dissolved in acetonitrile or in phosphate buffer (PBS) (10 mM in NaCl)/20% ethanol mixtures (to increase kINP solubility) and had an absorbance of 0.4 (∼2×10^{-5} M) at 308 nm. All the solutions were deaerated by nitrogen bubbling.

The molar absorption coefficient of triplet−triplet transition of kINP (ϵ (^{3}kINP)) was determined by the energy-transfer method using biphenyl (Biph) as triplet energy acceptor.[24] A solution of kINP (2×10^{-5} M) and biphenyl (10^{-3} M) in acetonitrile was studied; under these conditions, more than 95% of kINP triplet state was quenched by biphenyl. Then eq 1 was used:

$$\epsilon(^3\text{kINP}) = \epsilon(^3\text{Biph}(360)) \times \Delta A(^3\text{kINP}(440))/\Delta A(^3\text{Biph}(360)) \quad (1)$$

where $\Delta A(^3\text{kINP}(440))$ and $\Delta A(^3\text{Biph}(360))$ refer to the transient absorption of kINP triplet state ($\lambda = 440$ nm) at the beginning of the reaction and to the absorbance of biphenyl triplet state ($\lambda = 360$ nm) at the end of the process, respectively; $\epsilon(^3\text{Biph}(360))$ corresponds to the molar absorption coefficient of the biphenyl triplet at 360 nm, which is 42 800 $M^{-1} \times cm^{-1}$.[25]

The kINP triplet quantum yield ϕ_{isc}(kINP) was obtained by comparing the amount of ^{3}kINP populated after laser excitation with that for naphthalene (Naph) at the same absorbance. Thus, ϕ_{isc} was calculated by using eq 2:

$$\phi_{isc}(\text{kINP}) = \phi_{isc}(\text{Naph}) \times \Delta A(^3\text{kINP}(440)) \times \epsilon(^3\text{Naph}(415))/[\Delta A(^3\text{Naph}(415)) \times \epsilon(^3\text{kINP}(440))] \quad (2)$$

where ϕ_{isc}(Naph) corresponds to the intersystem crossing quantum yield of naphthalene; $\epsilon(^3\text{Naph}(415))$ refers to the molar absorption coefficient of naphthalene triplet at 415 nm; $\Delta A(^3\text{kINP}(440))$ and $\Delta A(^3\text{Naph}(415))$ refer to the transient absorption of ^{3}kINP at 440 nm and naphthalene triplet at 415 nm, respectively. The values of $\epsilon(^3\text{Naph}(415))$ and ϕ_{isc}(Naph) were 24 500 $M^{-1} \times cm^{-1}$ and 0.8, respectively.[24,25]

Phosphorescence Spectroscopy. Emission spectra were obtained on a Perkin-Elmer LS-50B spectrofluorimeter equipped with a xenon source (flash duration 8 μs) and a Hamamatsu R928 photomultiplier tube with the low-temperature accessory L2250136. The apparatus was operated in time-resolved mode, with a delay time of 0.15 ms. Excitation and emission monochromator band-passes of 4 nm were used. The sample was put in a capillary tube (2-mm diameter) and cooled to 77 K in a liquid nitrogen bath. Dry nitrogen circulation in the cell compartment avoided water condensation on the cell walls. The emission was obtained by exciting the sample at 308 nm in ethanol, using an absorbance of about 0.05 in a 10 mm cell.

Gel Sequencing Experiments. Oligonucleotides **1** and **2** and their complementary strands were synthesized and purified by polyacrylamide gel electrophoresis by Genosys (Cambridgeshire, U.K.). Phage T4 endonuclease V was purchased from TEBU (Le Perray-En-Yvelines, France):

25-mer **1**: 5′TGAGCGTTAGTTTAAGTCGGCTATC-3′

20-mer **2**: 5′-TGATCGGTGCGTCTGAGACT-3′

Preparation of ^{32}P End-Labeled Oligonucleotides. 25-mer **1** and 20-mer **2** were radiolabeled at the 5′-end using standard procedures. Typically, 15 μCi of [γ^{32}P] ATP and 5 pmol of oligonucleotide were added to the Ready-to-Go T4 Polynucleotide Kinase. After 30-min incubation at 37 °C, the reaction was stopped by addition of 5 μL of 250 mM ethylenediamine tetraacetic acid. Then, to remove free [γ^{32}P] ATP, the reaction mixture was purified using G-25 Microspin columns. Double-stranded DNA was prepared by hybridization of the 5′-end-labeled oligonucleotide with the same amount of its unlabeled complementary strand, heating 10 min at 70 °C and cooling slowly to room temperature.

Photosensitization Experiments. *Frank and Alkali-Labile Breaks.* All the solutions were prepared in 5 mM phosphate buffer with 10 mM NaCl, pH 7.4. Samples containing 5 μL of radiolabeled double-stranded oligonucleotides (5000 cpm/μL),

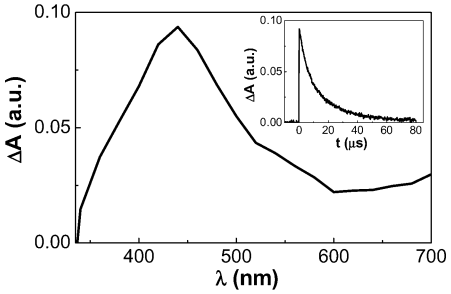

Figure 1. Transient absorption spectrum obtained 0.45 μs after laser excitation of 2×10^{-5} M kINP in acetonitrile (absorbance ~0.4 at 308 nm) under nitrogen. Insert: Decay of the signal at 440 nm.

5 μL of drug, 5 μL of unlabeled double-stranded oligonucleotides, and 5 μL of buffered solution were irradiated at 25 °C using a xenon lamp (Muller 450 W) equipped with a long pass filter $\lambda > 320$ nm (Oriel, WG-320). After irradiation, samples used for the detection of frank breaks were lyophilized. Alkali-labile sites were revealed using 20 μL of freshly prepared 1 M aqueous piperidine solution at 90 °C during 30 min. Piperidine was then removed by lyophilization.

Detection of Pyrimidine Dimers. The samples were prepared by the procedure described above but in a Tris/HCl buffer (5 mM in EDTA, 5 mM in $MgCl_2$, pH 7.5). After irradiation, they were incubated for 1 h at 37 °C with an excess of T4 endonuclease V and lyophilized.

Electrophoresis Analysis. Before electrophoresis, the samples were resuspended in 10 μL of formamide sequencing buffer. Frank breaks and alkali- and endonuclease-sensitive lesions were separated by 20% denaturating (7 M urea) polyacrylamide gel electrophoresis and visualized after exposure of autoradiograms overnight at −70 °C (Hyperfilm ECL). G[26] and T ≫ G[27] markers were prepared as described in the literature.

Results

The Triplet of kINP in Acetonitrile. Laser flash photolysis of kINP in acetonitrile was performed at 308 nm with a pulsed excimer laser. A well-defined transient absorption spectrum with a maximum at 440 nm was observed (Figure 1). The decay monitored at 440 nm (Figure 1, insert) showed a first-order kinetic corresponding to a lifetime of ca. 11 μs. It was tentatively assigned to the kINP triplet state by comparison with the spectrum obtained upon photosentization of the triplet state of indoprofen by acetone in aqueous medium ($\lambda_{max} = 450$ nm, $\tau = 25$ μs).[20]

This assignment was confirmed by quenching experiments. As expected for a triplet, it was efficiently quenched by oxygen. Moreover, experiments in the presence of naphthalene or biphenyl showed clearly formation of the corresponding triplet states (at $\lambda = 410$ and 360 nm, respectively) concomitantly with the decay of the ^{3}kINP signal (Figure 2a and 2b). The triplet−triplet energy-transfer mechanism at the origin of this efficient population of the quencher triplet state evidenced the triplet nature of the 440-nm transient.

TABLE 1: Bimolecular Rate Constants for the Interaction of KINP Triplet with Several Quenchers of Different Triplet Energy, in Acetonitrile

quencher	triplet state energy (kJ × mol^{-1})	k_q (M^{-1} × s^{-1})
oxygen	95	6.0×10^9
naphthalene	255	4.0×10^9
styrene	260	7.8×10^9
biphenyl	275	3.3×10^9
fluorene	285	3.4×10^9
benzophenone[a]	290	$<5 \times 10^7$
norbornene	300	1.6×10^6

[a] Because of the significant absorption of benzophenone at 308 nm, accurate kINP triplet lifetime measurements were not possible at quencher concentrations higher than 10^{-3} M.

To evaluate the triplet state energy of kINP, ^{3}kINP lifetime was measured in the presence of different amounts of several quenchers, and the corresponding quenching rate constants (k_q) were obtained by means of the Stern−Volmer plots. A clear effect of triplet state energy on the bimolecular rate constant was observed (Table 1) and would permit to situate ^{3}kINP by respect with the energy of triplet quenchers.

Representation of the logarithm of k_q against the triplet state energy of the quenchers (Figure 3) showed a "plateau" for energies up to 275−285 kJ × mol^{-1}; over this value the rate of energy transfer sharply decreased. As triplet−triplet energy transfer is nearly diffusion-controlled when the triplet energy of the donor is higher than that of the acceptor by at least 10 kJ × mol^{-1},[28] the energy of ^{3}kINP was situated in the range of 285−295 kJ × mol^{-1}. Furthermore, kINP phosphorescence emission in ethanol at 77 K (see Supporting Information) permitted to estimate a similar value (ca. 285 kJ × mol^{-1}). Thus, the triplet energy of kINP must be very close to that of benzophenone, but still somewhat lower. Actually, when a 10:1 mixture of benzophenone and kINP was submitted to LFP at 355 nm (where most of the energy is absorbed by the former compound) only the T−T absorption of kINP at $\lambda_{max} = 440$ nm was observed 1 μs after the laser pulse.

For further characterization of ^{3}kINP, its molar absorption coefficient was determined by using the energy-transfer method described in the Materials and Methods section (see eq 1) and is 22 000 M^{-1} × cm^{-1} (at $\lambda = 440$ nm). On the basis of this value, a high intersystem crossing quantum yield ($\phi_{isc} = 0.89$) was obtained by means of the comparative method also described in the Experimental Section (see eq 2). Hence, although INP does not produce significant amounts of triplet upon direct photolysis, attachment of the acetyl substituent to the phenyl ring results in a dramatic enhancement of intersystem crossing efficiency.

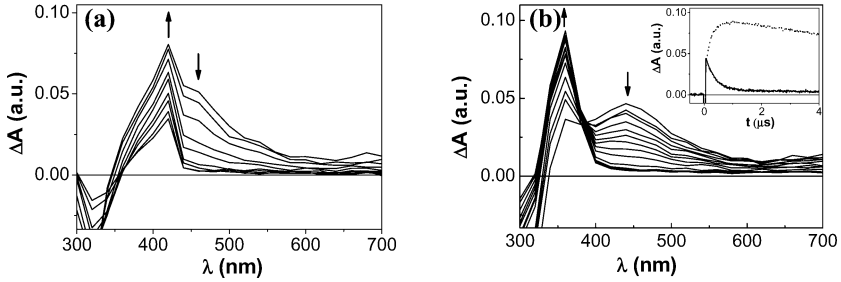

Figure 2. Time-dependent transient absorption spectra of nitrogen-purged solutions of 2×10^{-5} M kINP in acetonitrile in the presence of 10^{-4} M naphthalene (a) or 10^{-3} M biphenyl (b). Insert Figure 2b: Decay of ^{3}kINP at $\lambda = 440$ nm (—) and growth of biphenyl triplet at $\lambda = 360$ nm (···).

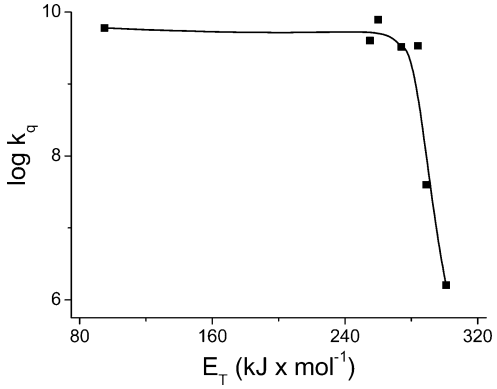

Figure 3. Plot of the logarithm of the bimolecular quenching rate constants of ^{3}kINP by different quenchers vs their triplet state energy.

Finally, the reactivity toward hydrogen abstraction was investigated as an indicator of the electronic nature ($n\pi^*$ vs $\pi\pi^*$) of ^{3}kINP. Thus, laser flash photolysis of kINP in acetonitrile solution was performed in the presence of increasing amounts of 2-propanol as hydrogen donor (from equimolar to 200-fold molar ratio). Even when the highest 2-propanol excess was used, no significant ^{3}kINP quenching was observed; the bimolecular rate constant was $<8 \times 10^5$ M$^{-1} \times$ s^{-1}.

The Triplet of kINP in PBS. The generation and reactivity of ^{3}kINP was also studied in PBS to determine its potential to induce DNA photosensitization in aqueous media. The same transient as in acetonitrile was observed at $\lambda_{max} = 440$ nm, but with a longer lifetime ($\tau = 35$ μs). This transient was efficiently quenched by oxygen and naphthalene, with bimolecular rate constants of 4×10^9 M$^{-1} \times$ s^{-1} and 1.5×10^9 M$^{-1} \times$ s^{-1}, respectively.

Further, the interaction of ^{3}kINP with key substructures of DNA was investigated; thymidine (dThd) and 2′-deoxyguanosine (dGuo) were chosen as model nucleosides because of their particular properties in DNA. Thus, thymine is the nucleobase with the lowest triplet energy,[12] being the most favorable acceptor site for an energy-transfer mechanism. By contrast, guanine is the preferred site for oxidation in DNA, owing to its low oxidation potential.[29,30] Laser flash photolysis of kINP was performed in PBS, in the presence of increasing amounts of dThd and dGuo. The lifetime of the resulting transient ^{3}kINP was determined under different conditions, and then the Stern−Volmer plots were recorded as usually. Operating in this way, bimolecular rate constants of 7.8×10^6 M$^{-1} \times$ s^{-1} and 1.6×10^9 M$^{-1} \times$ s^{-1} were obtained for quenching of ^{3}kINP by dThd and dGuo, respectively. The same procedure was employed to study quenching of ^{3}kINP by calf thymus DNA in aqueous medium. This process occurred with a rate constant of ca. 2×10^7 M$^{-1} \times$ s^{-1}, a value intermediate between those obtained for dThd and dGuo.

DNA Photosensitization. The photosensitizing properties of kINP toward DNA were investigated to conclude about its possible role during DNA damage upon irradiation in the presence of indoprofen. The experiments were carried out on two different 5′-^{32}P end-labeled synthetic double-stranded oligonucleotides, each of them containing specific sites in relation with the potentially involved mechanism:

(i) oligonucleotide **1** included adjacent thymines to investigate the possibility of energy transfer at the origin of the formation of thymine dimers,

(ii) oligonucleotide **2** contained several guanines included in different sequences to study the participation of electron transfer from a guanine base to the excited drug.

The two oligomers (8 μM in base pair) were UVA-irradiated in the presence of kINP (8 μM) and submitted to different treatments; the oligonucleotide **1** was treated with a specific DNA repair enzyme (T4 endonuclease V) to reveal thymine dimers, while alkali-labile products of guanine oxidation were studied by piperidine treatment of the oligonucleotide **2**.

Without piperidine or enzymatic treatment no direct cleavage of DNA occurred (Figure 4, lanes D_1 and K_1). However, after incubation with T4 endonuclease V, DNA irradiation in the presence of kINP gave rise to fragments at the TTT site (Figure 4, lane D_2) corresponding to an efficient formation of thymine dimers. Alkali-labile treatment revealed that kINP is also responsible for a specific guanine oxidation (Figure 4, lane K_2) in the 5′ site of the GG sequence. The efficiency of these reactions was similar to that observed using benzophenone and its derivatives as photosensitizers.[17]

Discussion

Overall, the obtained results have provided the first evidence for the triplet state of an isoindolinone derivative in fluid solution and at room temperature. Characterization by laser flash photolysis of this transient at 440 nm gave a τ of ca. 11 μs with a molar absorption coefficient $\epsilon = 22\,000$ M$^{-1} \times$ cm^{-1}, an intersystem crossing quantum yield of 0.89, an energy of 290 kJ $\times$ mol^{-1}, and a $\pi\pi^*$ nature in acetonitrile.

It is interesting that the triplet state is directly available from excitation of kINP; this is in sharp contrast with the case of indoprofen where acetone photosensitization was needed.[20] Hence, kINP may be at the origin of the thymine dimers formation previously described during photosensitization of DNA by INP.[19]

The spectroscopic properties of ^{3}kINP have been studied at room temperature and compared with results reported for similar compounds.[1,5,20] First, the similarity of the transient spectrum ($\lambda_{max} = 440$ nm) to that of the photosensitized triplet state of INP together with the similar values of the triplet state energy of kINP and PPI (i.e., 280 kJ $\times$ mol^{-1})[5] reveal the minor influence of substitution at the *N*-phenyl group on these properties. As triplet states of composite molecules (unlike singlets) prefer to be localized, it seems that the delocalized $\pi\pi^*$ singlet state undergoes intersystem crossing to form the acetophenone $n\pi^*$ triplet, which decays to the lower energy (*N*-phenyl)-isoindolinone $\pi\pi^*$ triplet.

The high intersystem crossing quantum yield indicates that the acetophenone moiety still plays an important role. Actually, the studies on INP and PPI described in the literature have shown that population of their triplets is quite ineffective; no transient was detected during the "direct" flash photolysis of INP,[20] while PPI has a low triplet yield ($\phi_{isc} < 0.05$).[1] It is clear that the well-known behavior of ketones plays a key role in kINP, where substitution on the isoindolinone ring by acetophenone (ϕ_{isc} close to 1)[25] facilitates intersystem crossing for the whole system.

The electronic nature of ^{3}kINP was studied by investigating its ability to abstract hydrogen atoms. In this context, efficient quenching of the triplet state by H-donors is associated with $n\pi^*$ triplet character (i.e., benzophenone $k_q = 2.9 \times 10^8$ M$^{-1} \times$ s^{-1});[31] by contrast, $\pi\pi^*$ states exhibit low bimolecular quenching rates (i.e., 4-methoxybenzophenone $k_q = 5 \times 10^5$ M$^{-1} \times$ s^{-1}).[32] On the other hand, the isoindolinone ring has been reported to be a H-donating (rather than H-accepting) group in photochemical reactions.[33] Thus, the low bimolecular rate constant found for quenching of ^{3}kINP by 2-propanol (ca. 8×10^5 M$^{-1} \times$ s^{-1}) points to the $\pi\pi^*$ nature of kINP triplet state.

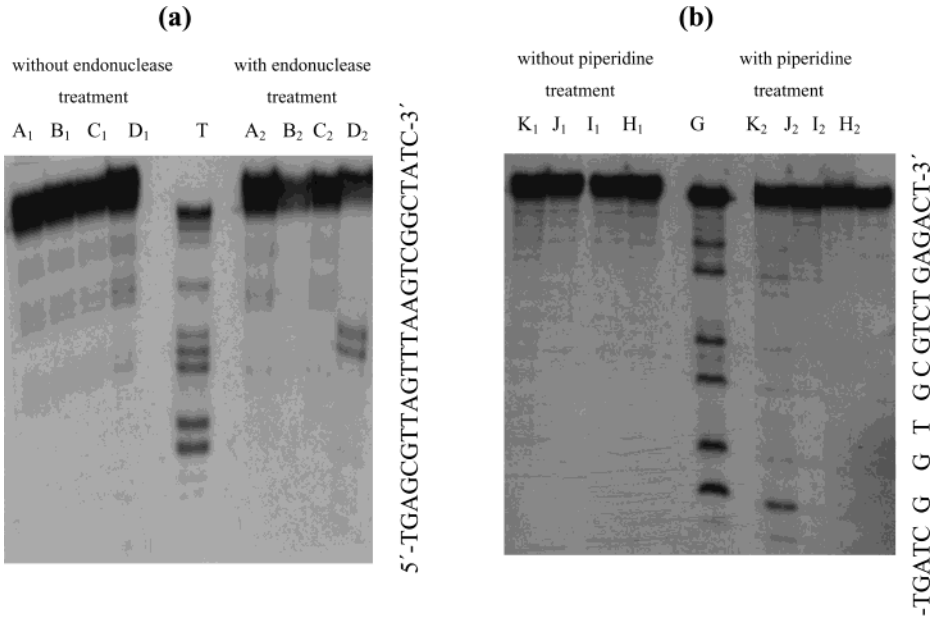

Figure 4. A 20% polyacrylamide gel showing DNA photosensitization by kINP. All experiments were carried out in phosphate buffer, 10 mM NaCl (pH 7.4), and the samples were UVA-irradiated ($\lambda > 320$ nm) for 50 min. (a) Duplex oligonucleotide **1** was submitted or not to enzymatic treatment to reveal thymine dimers formation. (b) Duplex oligonucleotide **2** was treated or not with hot piperidine to reveal guanine oxidations. Lanes A, H: nonirradiated; Lanes B, I: irradiated alone; Lanes C, J: nonirradiated in the presence of 8 μM kINP; Lanes D, K: irradiated in the presence of 8 μM kINP. G and T are guanine and thymine markers, respectively. The subscripts 1 and 2 refer to nontreated and treated (endonuclease or piperidine) DNA samples.

This is also in agreement with the low efficiency of INP as photosensitizer of lipid peroxidation, a process that generally involves abstraction of double allylic hydrogens from polyunsaturated fatty acids, as the initiation step of a radical chain mechanism.[34] Further support in favor of the $\pi\pi^*$ character of ^{3}kINP was provided by the lack of quenching by norbornene. In this case, energy transfer is not possible because of thermodynamic reasons (E_T of norbornene = 300 kJ $\times$ mol^{-1}); however, triplet quenching associated with a Paterno−Büchi cycloaddition should still be observable for a carbonylic $n\pi^*$ triplet.[35]

Laser flash photolysis of kINP was also performed in PBS to study the photosensitizing properties of ^{3}kINP toward DNA or some of its substructures, thymidine and 2′-deoxyguanosine. The weak interaction of ^{3}kINP with dThd (ca. 7.8 $\times$ 10^6 M^{-1} $\times$ s^{-1}) can be accounted for by comparing the triplet energies of the nucleoside (310 kJ $\times$ mol^{-1}) and kINP (ca. 290 kJ $\times$ mol^{-1}); this makes an energy-transfer mechanism endergonic and hence quite disfavored. As regards quenching of ^{3}kINP by dGuo, it can be attributed to an electron transfer from dGuo to the excited kINP. Similar guanine oxidations by phthalimide derivatives have been reported in the literature.[36]

Photosensitization of DNA by kINP gave rise to the formation of thymine dimers and guanine oxidation products. Thymine dimerization occurs by a triplet−triplet energy-transfer mechanism. Although this type of lesions was detected during INP photosensitization of DNA, the parent drug was not at the origin of the triplet−triplet energy transfer. Accordingly, it has been reported that no DNA damage occurs when photodecomposition of the drug is "blocked" by esterification, while preirradiated solutions containing the photodecomposition products give rise to thymine dimers.[19]

The DNA-photosensitization experiments now reported are in agreement with the data obtained by laser flash photolysis. Nevertheless, the efficiency of the reactions in DNA cannot be completely correlated with the reactivity of the isolated nucleosides. It is assumed that, like the lowering of base singlet excited state which occurs on going from mononucleotide to oligonucleotide, base triplet energy levels must be lowered in DNA.[13,37] Moreover, the determination of the triplet state of kINP permits to approach the triplet energy of thymine within DNA, which is not yet known. This value must be lower than that of kINP (290 kJ $\times$ mol^{-1}) since an energy-transfer mechanism takes place from the triplet state of kINP to thymine residues. As a matter of fact, an analogous energy transfer takes place from benzophenone and its derivatives,[17,38,39] leading to cyclobutadithymidine lesions in DNA. The frequency of thymine dimers formation is also influenced by adjacent sequence and will be favored if the 5′ neighbouring base is a thymine.[40]

In principle, thymine dimerization could also occur via a singlet energy-transfer mechanism. However, this possibility appears very unlikely in kINP, as its singlet energy (350 kJ $\times$ mol^{-1}) is much lower than that of the nucleobase (417 kJ $\times$ mol^{-1}).[25] Accordingly, the weak fluorescence of kINP was not quenched by addition of dThd (see Supporting Information).

In addition to this bipyrimidine lesion, the production of piperidine alkali-labile sites essentially at 5′G of a GG step has been evidenced. It is now well known that the presence of a GG site in DNA will act as a "potential trap", this specific oxidation being relevant of a mechanism involving an electron transfer from the guanine to the excited photosensitizer.[30,41]

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Supporting Information Available: UV, phosphorescence, and fluorescence spectra of kINP. This material is available free of charge via the Internet at http://pubs.acs.org.

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