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O-Benzoyl Pyridine Aldoxime and Amidoxime Derivatives: Novel Efficient DNA Photo-Cleavage Agents

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O-benzoyl derivatives of *meta*-, *ortho*- and *para*-pyridine aldoximes and amidoximes are novel efficient DNA photo-cleavage agents. In particular *O*-*p*-nitro-benzoyl derivatives **4**, **8** and **15** were effective in concentrations as low as 1 μM. Both aldoximes and amidoximes were active under aerobic and anaerobic conditions, with a ratio of double strand to single strand DNA cleavage up to 1. These results give prospects for multiple applications, including phototherapeutic treatment of solid tumors.

1. Introduction

Organic compounds with DNA cleaving activity are involved in biological diagnostic, therapeutic and mechanistic aspects such as gene and cancer therapy, DNA electron and energy transfer and design of DNA targeted drugs.[1-6] Photo-activated "chemical nucleases", known also as "photo-cleavers", interact with DNA and cause its cleavage using light. As a result, the need for external chemical initiators is eliminated, due to the fact that the chemical reaction is initiated only when the mixture of the organic compound and DNA is irradiated. Light causes selective excitation of the photo-cleavaging agents,[6,7] which via various mechanistic pathways lead to single-strand (ss) DNA damage, repairable by enzymatic processes,[8] and/or double-strand (ds) DNA photo-cleavage.[9-11] The latter, which is more difficult to repair, may trigger self-programmed cell death, featuring this approach as an efficient tool for cancer therapy.

Several organic compounds including certain enediynes,[10-12] pyrrolecarboxamide conjugated 4′-bromo-acetophenones,[13] riboflavins,[14] naphthalimides,[15] pyrenes,[16] anthraquinone based derivatives,[17,18] quinolines[19,20] and benzo[b][1,8] naphthyridines[21] were demonstrated as DNA photo-cleavaging agents.

Aldoximes, ketoximes and amidoximes (Figure 1, **I**, **II** and **III** respectively) retain a complementary position in drug design and discovery being considered individually as pharmacophores, and participating as parent compounds in multiple transformations.[22-25] However, the existence in amidoximes of the hydroxy-imino/amino tautomerization differentiates them from their other close relatives (Figure 1). Thus, amidoximes are bi-functional molecules which exhibit a rich chemistry, providing among others, one of the shortest ways to reach various heterocycles.[26-29] Amidoximes possess numerous and diverse biological activities, which makes them an important and attractive pharmacophore in medicinal chemistry. Furthermore, their ability to complex with metals, allow them to act as radiopharmaceuticals and anti-pollutants.[29]

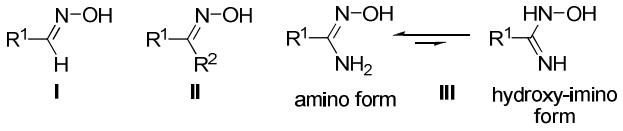


Figure 1.

The pyridine oxime moiety is rarely found in natural products,[30] however several synthetic derivatives possess various biological activities including cytotoxic, antiviral, analgesic, cardiovascular, anti-inflammatory, antidiabetic and antispasmodic activities.[24c] Additionally, charged[31,32] as well as uncharged derivatives[33-35] are efficient re-activators of nerve agent inhibited acetylcholonesterases aiming treatment from poisoning of organophosphorous compounds. Inspired from acetylcholinesterase re-activation, Terenzi et al. has examined the ability of oximes to cleave phosphate bonds in nucleic acids and act as metal-free artificial nucleases.[36] Compounds containing the N-O bond (aldoximes and ketoximes included) have been found to be efficient metal-free artificial DNA photo-cleavers[16-17,19,37-39] due to the homolysis of the weak N-O bond.[40]

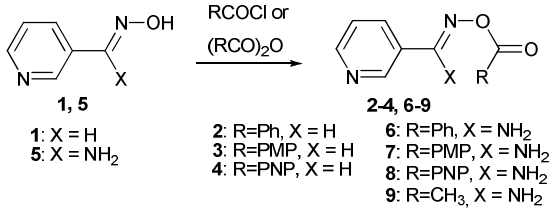
To the best of our knowledge amidoximes have never been tested as metal or metal-free artificial photo-cleavers. Our interest in the chemistry and biology of oximes,[29,41-43] has prompted us to synthesize and study several pyridine amidoxime ester conjugates and compare them with their corresponding aldoximes. The fact that the number of aldoximes acting as efficient photo-cleavers is limited,[19] prompted us to study novel scaffolds and provide a structure activity relationship based on electronic phenomena on the ester conjugates (electron donor or acceptor) as well as steric factors and hydrogen bonding capacities (H vs NH_2).

2. Results and discussion

2.1. Chemistry

meta-Pyridine aldoxime **1**[44] (Scheme 1) reacted with acyl anhydrides or acid chlorides in various solvents, to give *O*-acyl derivatives **2**-**4**. Under similar reaction conditions, *meta*-

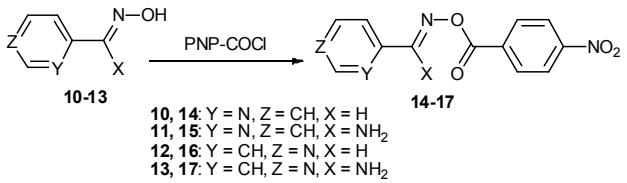
pyridine amidoxime **5**[45] (Scheme 1) delivered *O*-acyl derivatives **6-9**. It is known that the aliphatic acyl-oxy derivatives do not give radicals with the same efficiency as the aromatic ones probably due to the facile radical decarboxylation of the former which generates less reactive carbon centred radicals.[37] Since, however, amidoximes are tested for the first time, we needed to verify that the same applies to this class of oximes as well. Thus, amidoxime methyl-ester **9** has also been synthesized and tested.



Scheme 1. Synthesis of *meta*-pyridine derivatives **2-4** and **6-9**. Reaction conditions: acylating agent, Et_3N, solvent ($CHCl_3$ or THF or DMF), 0 °C to r.t., 74-89% yield for **2-4**, 50-72% yield for **6-9** [PMP = *p*-methoxy-phenyl, PNP = *p*-nitro-phenyl].

Interestingly, although several *meta*-pyridine *O*-acyl aldoximes and amidoximes are known,[46] derivatives **3**, **4**, **7** and **8** are new. Nevertheless, none of the rest had been fully characterized, either because their synthesis was very old, or because these particular amidoxime derivatives had been solely used as intermediates.

The photocleaving ability of the above compounds (*vide infra*) suggested that the *p*-nitro-benzoyl substituent is the most efficient, at low concentrations. Based on this observation, the *p*-nitrobenzoyl group is further linked to the *o*- and *p*-pyridine aldoxime and amidoxime scaffold, in an effort to examine the effect of the position of the pyridine ring nitrogen atom. A similar experimental protocol was adopted in order to obtain four new compounds, **14-17** (Scheme 2).

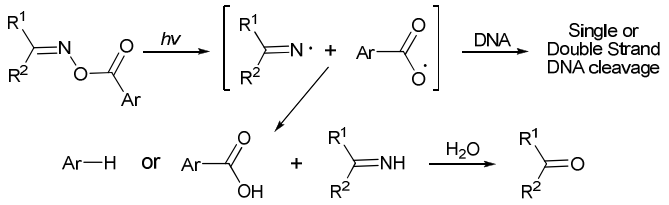


Scheme 2. Synthesis of *ortho*- and *para*-pyridine derivatives **14-17**. Reaction conditions: PNP-COCl, Et_3N, THF, 0 °C to r.t, 1.5h, 75-88% yield.

All reactions (Schemes 1 and 2) furnished a single product in good yield, without the need of chromatographic purification. The structure of all derivatives was fully assigned from their spectral data. Additionally, all compounds were found to absorb UV light at least partially over 300 nm, as it was deduced from their UV-Vis spectra (see electronic supporting information, ESI).

The stereochemistry of synthesized aldoxime esters was found to be *syn* (to the H atom). This was evidenced by a standard procedure, which involves the ester cleavage in alkaline methanolic solution, leading to the formation of the parent *syn*-oxime.[47] Additionally, all amidoxime derivatives have the *Z*-conformation in accordance to what had previously been reported.[27]

Photolysis causes homolytic cleavage of the N-O bond of *O*-aryl-oximes and production of iminyl and acyloxyl radicals (Scheme 3). The latter react with DNA, via several mechanistic pathways, to give single and double strand cleavages.



Scheme 3. Photo-cleavage of the N-O bond of oxime containing ester conjugates.

The reactive species are, according to the work of Theodorakis et al.,[37,38] the oxygen centred radicals. Irradiation of compounds in the absence of DNA under various conditions initially gives the iminyl radical ($R^1R^2C{=}N^{\cdot}$). This is eventually transformed to an imine which is further hydrolyzed to the corresponding ketone. The aroyloxyl radical ($ArCOO^{\cdot}$) gives the corresponding acid, whereas in some cases it is subsequently decarboxylated to the aryl radical. Other researchers observed photo-Beckman transformations, along with the expected ketone and acid products.[16]

In the case of amidoximes we have observed the same homolysis of the amidoxime N-O bond, which gave pyridine amidinyl radical (R^1 = pyridine, $R^2 = NH_2$) and *p*-nitro-benzoyl radicals (Ar = PNP), respectively, upon irradiation at 312 nm for 5 h in a $MeOH/H_2O$ solution or benzene containing 1,4-cyclohexadiene (see ESI).

2.2. Biological Assays

Compounds **1-9** and **14-17** were irradiated with UV light (312 nm, 90 W) at room temperature for 15 min, under aerobic conditions, at various concentrations in a DMF solution (1-20%) and in a Tris buffer solution (25 μM, pH=6.8) containing the supercoiled circular pBluescipt KS II DNA (Form **I**, 500 ng). Plasmid DNA was then analyzed by gel electrophoresis on 1% agarose stained with ethidium bromide. Plasmid DNA can exist in three conformations: supercoiled (Form **I**), relaxed or open-circular (Form **II**), and linear (Form **III**). For the same size, supercoiled DNA runs faster than relaxed circular DNA. Linear DNA sustains less friction than relaxed circular DNA, but more than supercoiled. We found that in the presence of the compounds the supercoiled plasmid DNA sustained single-strand nicks of the double helix, generating the relaxed circular DNA (Form **II**) found in many experiments, whereas in some cases the linear DNA (Form **III**) was formed as well, generated by double strand nicks. None of the tested compounds showed any activity towards DNA at the absence of UV irradiation. Blank experiments with DMF up to 20% did not show any effect on DNA and pH did not affect the activity of the cleavage (see ESI). Neither aldoxime **1** nor amidoxime **5** have shown any DNA photocleavage activity, a result that is in accordance with the lack of reactivity of other N-OH derivatives.[37a] All experiments were performed at least three times.

For the structure-activity relationships, we initially used concentrations of 100, 500 and 1000 μM, for all aldoxime and amidoxime derivatives (Figures 2 and 3, respectively). Aromatic derivatives **2-4** and **6-8** have shown conversion mainly at high concentrations, with derivatives **4** (Figure 2, lanes 5, 8, 11) and **8** (Figure 3, lanes 5, 9, 13), bearing an electron withdrawing substituent, to give the best results. The

latter compounds also promoted the formation of linear DNA (Form **III**), at levels of up to 36 % (at a concentration of 1 mM). In contrast, aliphatic *O*-acetyl derivative **9** showed very low conversion (Figure 3, lanes 6, 10, 14), in accordance to the literature.[37]

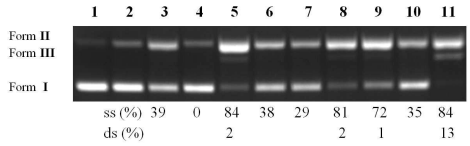


Figure 2. Comparative dose measurement results from DNA cleavage by the *meta*-pyridine oxime *O*-acyl derivatives **2-4**. Top: The gel electrophoresis data: Lane 1: DNA without UV irradiation; Lane 2: DNA with UV irradiation; Lane 3: DNA + **2** (100 μM); Lane 4: DNA + **3** (100 μM); Lane 5: DNA + **4** (100 μM); Lane 6: DNA + **2** (500 μM); Lane 7: DNA + **3** (500 μM); Lane 8: DNA + **4** (500 μM); Lane 9: DNA + **2** (1000 μM); Lane 10: DNA + **3** (1000 μM); Lane 11: DNA + **4** (1000 μM). Bottom: % Conversion of pBR322 DNA with derivatives **2-4**. For the calculation of ss and ds %, see ref 48.

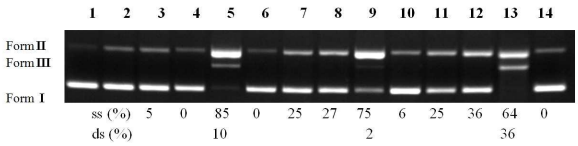


Figure 3. Comparative dose measurement results from DNA cleavage by the *meta*-pyridine amidoxime *O*-acyl derivatives **6-9**. Top: The gel electrophoresis data: Lane 1: DNA without UV irradiation; Lane 2: DNA with UV irradiation; Lane 3: DNA + **6** (100 μM); Lane 4: DNA + **7** (100 μM); Lane 5: DNA + **8** (100 μM); Lane 6: DNA + **9** (100 μM); Lane 7: DNA + **6** (500 μM); Lane 8: DNA + **7** (500 μM); Lane 9: DNA + **8** (500 μM); Lane 10: DNA + **9** (500 μM); Lane 11: DNA + **6** (1000 μM); Lane 12: DNA + **7** (1000 μM); Lane 13: DNA + **8** (1000 μM); Lane 14: DNA + **9** (1000 μM). Bottom: % Conversion of pBR322 DNA with derivatives **6-9**. For the calculation of ss and ds %, see ref 48.

From the comparison of aldoxime and amidoxime derivatives with the same *O*-substituent, we conclude that the electron withdrawing *p*-nitro-benzoyl conjugate strongly promotes DNA photo-cleavage in both classes of compounds. To the other end, for the less reactive compounds that bear an electron donating group (i.e. *p*-methoxy-derivatives), no significant difference is observed. However, benzoyl substituted aldoximes clearly showed an enhanced nicking effect relatively to the corresponding amidoximes. Since it was shown that the $S_0 \rightarrow S_1$ transition and the triplet state are localized on the oxime moiety, the mechanism of dissociation is most probably affected by the substituent effects.[40b]

All four *o*- and *p*-pyridine derivatives **14-17** interacted strongly with DNA under UV irradiation at the tested concentrations (Figure 4), and exhibited similar behaviour as the *m*-pyridine *p*-nitro-benzoyl derivatives **4** and **8**. Most importantly, all PNP derivatives regardless of the N position of the pyridine moiety caused not only single strand nicks, but also double strand DNA scission (50% for derivative **17** at the concentration of 1mM, Figure 4, lane 9).

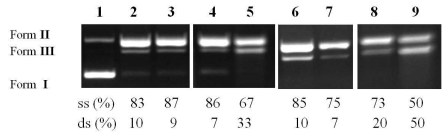


Figure 4. Comparative dose measurement results from DNA cleavage by the *ortho*- and *para*- pyridine derivatives **14-17**. Top: The gel electrophoresis data: Lane 1: DNA with UV irradiation; Lane 2: DNA + **14** (100 μM); Lane 3: DNA + **16** (100 μM); Lane 4: DNA + **14** (1000 μM); Lane 5: DNA + **16** (1000 μM); Lane 6: DNA + **15** (100 μM); Lane 7: DNA + **17** (100 μM); Lane 8: DNA + **15** (1000 μM); Lane 9: DNA + **17** (1000 μM); Bottom: % Conversion of pBR322 DNA with derivatives **14-17**. For the calculation of ss and ds %, see ref 48.

The above results have prompted us to examine the lowest concentrations, which are sufficient for DNA photo-cleavage for the PNP ester conjugates **4**, **8** and **14-17**. This concentration was determined as the amount of compound capable of cleaving at least 50% of the supercoiled plasmid DNA, or equally (Form **II** + Form **III**) / Form **I** ≥ 1. Three compounds, one aldoxime (**4**) and two amidoximes (**8** and **15**) were active even at concentrations as low as 1 μM (Figure 5, lanes 2, 5, 6 and Figure 6).

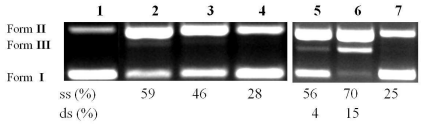


Figure 5. Comparative dose measurement results from DNA cleavage by the pyridine oxime **4**, **14**, **16** and amidoxime derivatives **8**, **15**, **17** at concentration 1 μM. Top: The gel electrophoresis data: Lane 1: DNA with UV irradiation; Lane 2: DNA + **4**; Lane 3: DNA + **14**; Lane 4: DNA + **16**; Lane 5: DNA + **8**; Lane 6: DNA + **15**; Lane 7: DNA + **17**; Bottom: % Conversion of pBR322 DNA with derivatives **4**, **8**, **14-17**. For the calculation of ss and ds %, see ref 48.

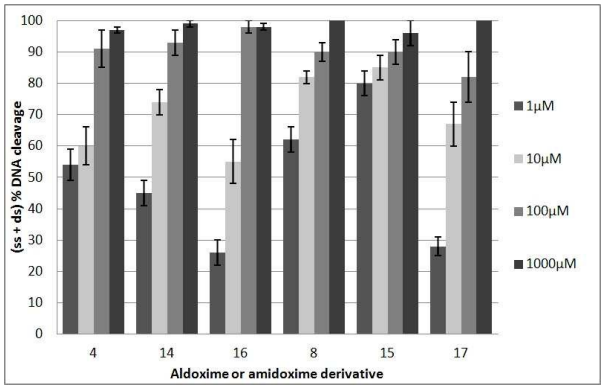


Figure 6. (ss+ds) % DNA cleavage caused by aldoximes **4**, **14**, **16**, and amidoximes **8**, **15**, **17** at concentrations of 1, 10, 100 and 1000 μM. Results presented in Figure are mean value ± SD of at least three runs.

All three pyridine amidoximes exhibited better photo-cleaving ability at concentration 10 μM comparing to their related aldoximes (**4** vs **8**, **14** vs **15** and **16** vs **17**, Figure 6). At higher concentrations both classes of oximes are highly efficient with their differences to stay within the statistical

error. Nevertheless, the percentage of the double strand photo-cleavage is much more enhanced in the case of amidoximes (**4** vs **8**, Figure 2, lane 11 and Figure 3, lane 13, respectively; **14** vs **15**, Figure 4, lane 4 and lane 8, respectively; **16** vs **17**, Figure 4, lane 5 and lane 9, respectively). It seems possible that NH_2 in amidoximes affects positively the affinity with DNA providing extra points for hydrogen bonding.

In an effort to understand the mechanistic pathways involved in the DNA cleavage we have also performed experiments under anaerobic conditions (argon atmosphere), and under aerobic conditions, in the presence of molecular oxygen with hydroxyl radical scavengers, like DMSO, and singlet oxygen quenchers, such as sodium azide for a representative aldoxime (**4**) and amidoxime (**8**) derivative. Results for these compounds are presented in Figures 7 and 8, respectively.

It seems that both compounds can react in anaerobic conditions and the mode of action most probably does not involve hydroxyl radicals (no reaction with DMSO). Their activity is notably reduced in the presence of sodium azide (Figure 7, lane 5 for compound **4**, Figure 8, lane 9 for compound **8**), which indicates that singlet oxygen is possibly involved. Nevertheless, the cleavage of plasmid DNA is not enhanced when D_2O was used as a solvent. It is known that D_2O increases the lifetime of singlet oxygen and thus leading to more effective cleavage when this radical is involved in the mechanism.[49]

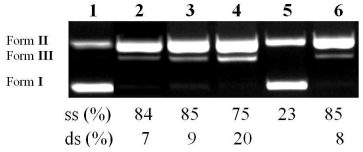


Figure 7. Mechanistic studies involved at the DNA cleavage by derivative **4** (100 μM). Top: The gel electrophoresis data: Lane 1: DNA with UV irradiation; Lane 2: DNA + **4**; Lane 3: DNA + **4** + argon; Lane 4: DNA + **4** + DMSO (20%); Lane 5: DNA + **4** + NaN_3 (20mM); Lane 6: DNA + **4** + D_2O. Bottom: % Conversion of pBR322 DNA with compound **4**. For the calculation of ss and ds %, see ref 47.

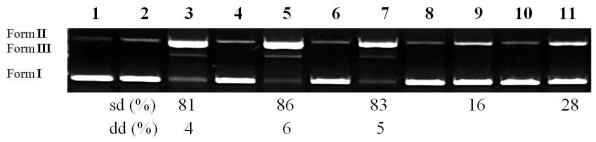


Figure 8. Mechanistic studies involved at the DNA cleavage by derivative **8** (100 μM);. Top: The gel electrophoresis data: Lane 1: DNA without UV irradiation; Lane 2: DNA with UV irradiation; Lane 3: DNA + **8**; Lane 4: DNA + argon; Lane 5: DNA + **8** + argon; Lane 6: DNA + DMSO (20%); Lane 7: DNA + **8** + DMSO (20%); Lane 8: DNA + NaN_3 (20 mM); Lane 9: DNA + **8** + NaN_3 (20mM); Lane 10: DNA + D_2O; Lane 11: DNA + **8** + D_2O. Bottom: % Conversion of pBR322 DNA with compound **8**. For the calculation of ss and ds, % see ref 47.

Summarizing these results, we believe that not a single mechanistic pathway is implicated in the cleavage and, certainly, the one involving singlet oxygen is not the dominant. Nitro substituted compounds have been studied for their ability to cleave DNA, thus, *p*-nitro-phenyl moiety may also contribute to the DNA photo-cleavage. It has been reported that radicals are formed upon irradiation of nitro compounds; nevertheless, their triplet state rapid deactivation may cause insufficient photochemistry. The mechanism of action of nitro-phenyl containing compounds is not fully clarified, since researchers have suggested either hydrogen abstraction from the deoxyribose DNA backbone and/or oxygen donation, or electron transfer, or hydrogen abstraction from the thymine methyl group. Nevertheless, other factors such as affinity of the compound with DNA is also a requirement.[6,50] The role of the substituent of the oxime or amidoxime moieties, as well as of the NO_2 group are currently investigated with the synthesis and evaluation of more derivatives.

3. Conclusions

We have found that *O*-benzoyl-pyridine aldoxime and amidoxime derivatives are DNA photo-cleavers. In particular, *meta*-, *ortho*- and *para*- pyridine *O*-*p*-nitrobenzoyl aldoximes and corresponding amidoximes are highly effective in concentrations as low as 1 μM, with amidoxime **15** being the most efficient. The fact that these compounds are able a) to react under anaerobic conditions and b) to cause relatively high ratios of ds/ss DNA cleavage may potentially lead to multiple applications, including phototherapeutic treatment of solid tumors and cancer therapy in general. Finally, we believe that amidoximes in particular, may be recognized as a novel class of "photo-cleavers" along with aldoximes and ketoximes.

4. Experimental

Mps were measured on a Kofler hot-stage apparatus or a melting point meter M5000 KRÜSS, and are uncorrected. FT-IR spectra were obtained in a Perkin-Elmer 1310 spectrometer using potassium bromide pellets. For the UV spectra a reader TECAN M1000 has been used. NMR spectra were recorded on an Agilent 500/54 (500 MHz and 125 MHz for ^1H and ^{13}C respectively) spectrometer using $CDCl_3$, and/or DMSO-d_6 as solvent. *J* values are reported in Hz. High resolution mass spectra (HRMS) were recorded on micrOTOF GC-MS QP 5050 Shimadzu single-quadrupole mass spectrometer. Mass spectra were determined on a Shimadzu LCMS-2010 EV system under Electrospray Ionization (ESI) conditions. All reactions were monitored on commercial available pre-coated TLC plates (layer thickness 0.25 mm) of Kieselgel 60 F_{254}. Yields were calculated after recrystallization. Samples containing plasmid DNA were irradiated in a Macrovue 2011 transiluminator LKB BROMMA at 312 nm, 90 W, 0.225 W/cm^2, 10 cm distance, whereas samples without the plasmid were irradiated with Phillips 2 x 9W/12/2P UV-B broadband lamps at 312 nm.

Synthesis of pyridine aldoxime derivatives.

4.1. Synthesis of nicotinaldehyde O-benzoyl oxime (2).[46a]

Aldoxime **1**[44] (122 mg, 1 mmol) was dissolved in tetrahydrofuran (0.2 M) and triethylamine (0.15 mL, 1.1 mmol) was added at 0 °C under an Ar atmosphere, followed by benzoic anhydride (249 mg, 1.1 mmol). The mixture was stirred for 12 h, then water (30 mL) was added and the mixture was extracted with ethyl acetate (2x30 mL). After drying with Na_2SO_4 the organic solvents were removed in a rotary evaporator and the crude residue was recrystallized to give 170 mg (75%) of oxime **2**. White crystals, m.p. 151-153 °C (ethyl acetate), IR (KBr): 1736, 1603; ^1H NMR (500 MHz, DMSO-d_6) δ 7.53–7.62 (m, 3H), 7.74 (tt, J = 7.5, 1.2 Hz, 1H), 8.08 (br dd, J = 8.4,

1.3 Hz, 2H), 8.23 (br dt, *J* = 8.0, 1.7 Hz, 1H), 8.74 (bd, *J* = 4.7, 1.6 Hz, 1H), 8.95 (br d, *J* = 1.2 Hz 1H), 8.99 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 124.3, 126.3, 127.9, 129.0, 129.3, 134.0, 134.8, 149.6, 152.5, 156.0, 163.0; HRMS (ESI) Calc $C_{13}H_{11}N_2O_2$ $[M+H]^+$ 227.0815; found 227.0817.

4.2. Synthesis of nicotinaldehyde O-4-methoxybenzoyl oxime (3).

Aldoxime **1**[44] (122 mg, 1 mmol) was dissolved in dry $CHCl_3$ (0.2 M) and triethylamine (0.15 mL, 1.1 mmol) was added at 0°C under an Ar atmosphere, followed by 4-methoxy-benzoyl chloride (188 mg, 1.1 mmol) and DMAP (0.05%). The mixture was stirred for 2 h and filtered off. The solid residue gave after recrystallizations 228 mg (89%) of oxime **3**. Off-white crystals, m.p. 133-135 °C (ethanol), IR (KBr): 1736, 1606 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.87 (s, 3H), 6.96 (d, *J* = 5.4 Hz, 2H), 7.39 (dd, *J* = 4.8, 2.9 Hz, 1H), 8.07 (d, *J* = 5.4 Hz, 2H), 8.26 (dt, *J* = 4.8, 1.0 Hz, 1H), 8.57 (s, 1H), 8.70 (dd, *J* = 2.9, 1.0 Hz, 1H), 8.86 (d, *J* = 0.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.5, 113.9, 120.3, 123.8, 126.6, 131.9, 134.5, 150.0, 152.4, 153.5, 163.4, 163.9; HRMS (ESI) Calc $C_{14}H_{13}N_2O_3$ $[M+H]^+$ 257.0921; found 257.0917.

4.3. Synthesis of nicotinaldehyde O-4-nitrobenzoyl oxime (4).

Aldoxime **1**[44] (244 mg, 2 mmol) was dissolved in tetrahydrofuran (0.15 M) and triethylamine (0.30 mL, 2.2 mmol) was added at 0 °C under an Ar atmosphere, followed by 4-nitro-benzoyl chloride (408 mg, 2.2 mmol). The mixture was stirred for 1.5 h. Water (100 mL) was added and the mixture was extracted with ethyl acetate (2x100 mL). The organic layers were further washed with water (100 mL), dried with Na_2SO_4, and the solvents were evaporated to dryness. The crude residue was recrystallized to give 445 mg (83 %) of oxime **4**. Off-white crystals, m.p. 161.2 °C (ethyl acetate), IR (KBr): 1739, 1614 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.58 (dd, *J* = 7.9, 4.9 Hz, 1H), 8.24 (dt, *J* = 8.0, 1.8 Hz, 1H), 8.31 (dd, *J* = 8.9, 1.8 Hz, 2H), 8.42 (dd, *J* = 8.9, 1.8 Hz, 2H), 8.76 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.95 (d, *J* = 1.6 Hz, 1H), 9.07 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 124.1, 124.3, 126.0, 130.8, 133.4, 134.8, 149.7, 150.5, 152.7, 156.8, 161.6; HRMS (ESI) Calc $C_{13}H_{10}N_3O_4$ $[M+H]^+$ 272.0666; found 272.0656.

4.4. Synthesis of picolinaldehyde O-4-nitrobenzoyl oxime (14).

From aldoxime **10**[44] following the procedure used for **4**. The crude residue was recrystallized to give 452 mg (84 %) of oxime **14**. Light yellow crystals, m.p. 178-180 °C (ethyl acetate), IR (KBr): 1755, 1696, 1607 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (dd, *J* = 6.6, 4.9 Hz, 1H), 7.82 (dt, *J* = 7.8, 1.3 Hz, 1H), 8.18 (d, *J* = 7.9 Hz, 1H), 8.31 (d, *J* = 8.9 Hz, 2H), 8.35 (d, *J* = 8.9 Hz, 2H), 8.67 (s, 1H), 8.71 (d, *J* = 4.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 122.3, 123.8, 125.8, 130.9, 133.8, 136.8, 149.5, 150.1, 150.9, 158.2, 161.9; HRMS (ESI) Calc $C_{13}H_{10}N_3O_4$ $[M+H]^+$ 272.0666; found 272.0662.

4.5. Synthesis of isonicotinaldehyde O-4-nitrobenzoyl oxime (16).

From aldoxime **12**[44] following the procedure used for **4**. The crude residue was recrystallized to give 475 mg (88 %) of oxime **16**. Pale yellow crystals, m.p. 194-196 °C (ethyl actate), IR (KBr): 1760, 1608 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.75 (d, *J* = 5.1 Hz, 2H), 8.29 (d, *J* = 8.5 Hz, 2H), 8.41 (d, *J* = 8.5 Hz, 2H), 8.76 (d, *J* = 5.1 Hz, 2H), 9.03 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 122.0, 124.1, 130.9, 133.3, 137.1, 150.6, 150.7, 157.4, 161.5; HRMS (ESI) Calc $C_{13}H_{10}N_3O_4$ $[M+H]^+$ 272.0666; found 272.0661.

Synthesis of pyridine amidoxime derivatives.

4.6. Synthesis of N′-(benzoyloxy)nicotinimidamide (6).[51]

Amidoxime **5**[45] (274 mg, 2 mmol) was dissolved in a mixture of chloroform and DMF (ratio 30/1, 0.15 M) and cooled to 0 °C under an Ar atmosphere. Triethylamine (0.28 mL, 2 mmol) was added, followed by benzoic anhydride (452 mg, 2 mmol) and the mixture was stirred for 6 h. Water (70 mL) was added and the mixture was extracted with dichloromethane (3x70 mL) and ethyl acetate (3x70 mL). After drying with Na_2SO_4 the organic solvents were removed in a rotary evaporator and the residue gave after recrystalizations 343 mg (69%) of amidoxime **6**. White crystals, m.p. 200-202 °C (ethanol); IR (KBr): 3425, 3331, 1727, 1637, 1605; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.17 (br s, 2H), 7.58 – 7.48 (m, 3H), 7.67 (tt, *J* = 7.4, 1.3 Hz, 1H), 8.15 (dt, *J* = 8.3, 1.8 Hz, 1H), 8.21 (dd, *J* = 8.2, 1.8 Hz, 2H), 8.71 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.95 (dd, *J* = 2.3, 0.8 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 123.5, 127.8, 128.6, 129.3, 129.5, 133.1, 134.7, 147.8, 151.4, 155.2, 163.5. HRMS (ESI) Calc $C_{13}H_{12}N_3O_2$ $[M + H]^+$, 242.0924; found 242.0920.

4.7. Synthesis of N′-((4-methoxybenzoyl)oxy)nicotinimidamide (7).

Amidoxime **5**[45] (137 mg, 1 mmol) was dissolved in a mixture of dry chloroform and DMF (ratio 10/1, 0.2 M) and cooled to 0 °C under an Ar atmosphere. Triethylamine (0.15 mL, 1.1 mmol) was added followed by 4-methoxy-benzoyl chloride (188 mg, 1.1 mmol) and DMAP (0.05%). The mixture was stirred for 12 h and filtered off. The crude solid gave after recrystallizations 196 mg (72%) of amidoxime **7**. White crystals, m.p. 201-203 °C (ethanol); IR (KBr): 3410, 3327, 1716, 1638, 1604 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.85 (s, 3H), 7.05 (dd, *J* = 8.9, 1.9 Hz, 2H), 7.11 (br s, 2H), 7.51 (dd, *J* = 7.9, 4.8 Hz, 1H), 8.12 (dt, *J* = 8.0, 1.8 Hz, 1H), 8.16 (dd, *J* = 8.9, 2.0 Hz, 2H), 8.70 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.93 (dd, *J* = 2.2, 0.6 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.6, 113.9, 121.4, 123.5, 127.9, 131.7, 134.7, 147.8, 151.3, 154.9, 163.1, 163.2; HRMS (ESI) Calc $C_{14}H_{14}N_3O_3$ $[M + H]^+$, 272.1035; found 272.1027.

4.8. Synthesis of N′-((4-nitrobenzoyl)oxy)nicotinimidamide (8).

Amidoxime **5**[45] (137 mg, 1 mmol) was dissolved in tetrahydrofuran (0.1 M) and triethylamine (0.15 mL, 1.1 mmol) was added at 0 °C under an Ar atmosphere, followed by 4-nitro-benzoyl chloride (204 mg, 1.1 mmol). The mixture was stirred for 4 h and filtered off. The crude solid gave after recrystalizations 188 mg (65%) of amidoxime **8**. Yellow crystals, m.p. 226-228 ° C (ethanol); IR (KBr): 3398, 3323, 3198, 1741, 1640 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.33 (br s, 2H), 7.53 (dd, *J* = 7.8, 4.8 Hz, 1H), 8.14 (dt, *J* = 8.0, 1.8 Hz, 1H), 8.34 (d, *J* = 8.8 Hz, 2H), 8.45 (d, *J* = 8.8 Hz, 2H), 8.72 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.94 (d, *J* = 1.7 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 123.5, 123.6, 127.5, 131.1, 134.7, 134.8, 147.8, 150.1, 151.5, 155.8, 162.1; HRMS (ESI) Calc $C_{13}H_{11}N_4O_4$ $[M + H]^+$, 287.0775; found 287.0772.

4.9. Synthesis of N′-acetoxynicotinimidamide (9).[46c]

Amidoxime **5**[45] (137 mg, 1 mmol) was dissolved in tetrahydrofuran (0.15 M) and triethylamine (0.15 mL, 1.1 mmol) was added at 0 °C under an Ar atmosphere, followed by acetic anhydride (0.1 mL, 1.1 mmol). The mixture was stirred for 3 h, and since the product was highly water soluble, the mixture was evaporated to dryness and the crude residue was recrystallized to give 90 mg (50%) of amidoxime **9**. White crystals, m.p. 150-152 °C (ethyl acetate/ethanol); IR (KBr): 3399, 3341, 3223, 1751, 1647, 1605 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.14 (s, 3H), 6.99 (br s, 2H), 7.49 (ddd, *J* = 8.0, 4.9, 0.9 Hz, 1H), 8.06 (ddd, *J* = 8.0, 2.3, 1.8 Hz, 1H), 8.68 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.87 (dd, *J* = 2.3, 0.8 Hz 1H); ^{13}C NMR (125 MHz,

DMSO-d_6) δ 19.8, 123.5, 127.6, 134.4, 147.6, 151.3, 154.7, 168.4; HRMS (ESI) Calc $C_8H_{10}N_3O_2$ $[M + H]^+$, 180.0768; found 180.0764.

4.10. Synthesis of N′-((4-nitrobenzoyl)oxy)picolinimidamide (15).

Amidoxime **11**[52] (274 mg, 2 mmol) was dissolved in tetrahydrofuran (0.15 M) and triethylamine (0.30 mL, 2.2 mmol) was added at 0 °C under an argon atmosphere, followed by 4-nitro-benzoyl chloride (408 mg, 2.2 mmol). The mixture was stirred for 1.5 h, and the mixture was filtered off to give part of the crude product. The filtrate was extracted with ethyl acetate (2x70 mL) and water (70 mL). The organic phases were collected, dried with Na_2SO_4, and evaporated to dryness. The crude residues were recrystallized to give 450 mg (79 %) of amidoxime **15**. Yellow crystals, m.p. 205-207 °C (ethanol); IR (KBr): 3475, 3361, 1726, 1633 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 7.18 (br s, 1H), 7.36 (br s, 1H), 7.58 (t, J = 5.3 Hz, 1H), 7.96 (t, J = 7.1 Hz, 1H), 8.02 (d, J = 7.9 Hz, 1H), 8.34 (d, J = 8.7 Hz, 2H), 8.47 (d, J = 8.7 Hz, 2H), 8.69 (d, J = 4.4 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 121.3, 123.7, 126.0, 131.2, 134.7, 137.5, 148.1, 148.9, 150.3, 155.1, 162.1; HRMS (ESI) Calc $C_{13}H_{11}N_4O_4$ $[M + H]^+$, 287.0775; found 287.0772.

4.11. Synthesis of N′-((4-nitrobenzoyl)oxy) isonicotinimidamide (17).

From amidoxime **13**[53] following the procedure used for product **15**. The crude residue was recrystallized to give 428 mg (75 %) of amidoxime **17**. Yellow crystals, m.p. 180.2 ° C (ethanol); IR (KBr): 3470, 3372, 1726, 1630 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 7.36 (br s, 2H), 7.75 (dd, J = 6.0, 0.4 Hz, 2H), 8.35 (dd, J = 9.0, 2.1 Hz, 2H), 8.45 (dd, J = 9.0, 2.0 Hz, 2H), 8.72 (dd, J = 6.0, 1.4 Hz, 2H); ^{13}C NMR (1255 MHz, DMSO-d_6) δ 121.3, 123.7, 131.2, 134.7, 139.1, 150.2, 150.3, 155.8, 162.1; HRMS (ESI) Calc $C_{13}H_{11}N_4O_4$ $[M + H]^+$, 287.0775; found 287.0770.

Molecular Biology

Cleavage of supercoiled circular pBR322 DNA by acyl aldoximes and amidoximes.

The reaction mixtures (20 µL) containing supercoiled circular pBluescipt KS II DNA stock solution (Form I, 50 µM/base pair, ~500 ng), compounds, and Tris buffer (25 µM, pH 6.8) in Pyrex vials were incubated for 30 min at 37 °C, centrifuged, and then irradiated with UV light (312 nm, 90W) under aerobic conditions at room temperature for 15 min. After addition of the gel-loading buffer (6X Orange DNA Loading Dye 10 mM Tris-HCl (pH 7.6), 0.15% orange G, 0.03% xylene cyanol FF, 60% glycerol, and 60 mM EDTA, by Fermentas), 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 (65V for 1h). The gel was visualized by 312nm UV transilluminator and photographed by an FB-PBC-34 camera vilber lourmat. Quantitation of DNA-cleaving activities was performed by integration of the optical density as a function of the band area using the program "Image J" available at the site http://rsb.info.nih.gov/ij/download.html.

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† Electronic Supplementary Information (ESI) available: [^1H and ^{13}C-NMR spectra of all compounds, photochemistry and pH experiments, and UV spectra are available]. See DOI: 10.1039/b000000x/

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48 ss % = Form II/(Form I + Form II + Form III) x100; ds % = Form III/(Form I + Form II + Form III) x100. As Form II we consider Form II of each series minus Form II of the irradiated control DNA. As Form I we consider Form I of each series. The same applies for the calculation of Form III/(Form I + Form II + Form III). Gel data analysis is performed with the program "Image J" available at the site http://rsb.info.nih.gov/ij/download.html.

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O-Benzoyl Pyridine Aldoxime and Amidoxime Derivatives: Novel Efficient DNA Photo-Cleavage Agents

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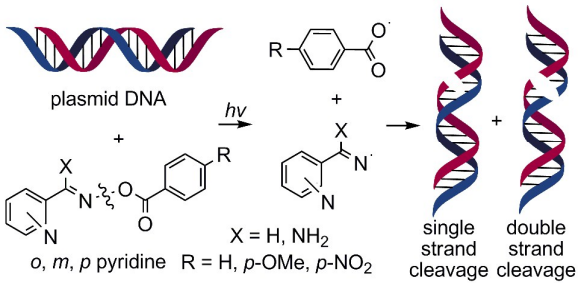

Pyridine oxime esters are effective DNA photocleavers, causing single/double strand DNA cleavage at concentrations as low as 1 µM.