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Regiospecific photocyclization of mono- and bis-styryl substituted N-heterocycles: A synthesis of DNA-binding benzo[*c*]quinolizinium derivatives

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Abstract

Regiospecific C–N photocyclization of mono- and bis-styryl substituted N-heterocycles was investigated. We demonstrated that the C–N regiospecificity of the photoinduced electrocyclization is a general feature of *ortho*-styryl substituted N-heterocycles comprising one and two nitrogen atoms. This phototransformation provides a straightforward synthesis of pharmaceutically important benzo[*c*]quinolizinium cation and its aza-analogues. Noticeably, bis-styryl derivatives undergo only one-fold cyclization with the second styryl fragment remaining uninvolved in the cyclization process. Photocyclization products of mono-styryl derivatives intercalate into calf thymus DNA (ct DNA), whereas photocyclization products of bis-styryl derivativatives possess a mixed binding mechanism with ct DNA. The results can be used for development of novel DNA-targeting chemotherapeutics based on benzo[*c*]quinolizinium derivatives.



Introduction

Benzo[c]quinolizinium derivatives represent a pharmacologically important class of heteroaromatic compounds that is applied in clinical practice.¹ In particular, benzo[c]quinolizinium (often referred to as MPBs) drugs are used as pharmacological chaperons² that promote folding and channel activation of the mutant CFTR protein (cystic fibrosis transmembrane conductance regulator) in the treatment of mucoviscidosis.³ As efficient CFTR activators benzo[c]quinolizinium compounds produce a relaxing effect on tracheal⁴ and aortic⁵ smooth muscle cells, thus inducing bronchodilation and vasorelaxation. Because of these favorable properties a range of benzo[c]quinolizinium derivatives has been patented for the use in the treatment of diseases that are linked to smooth muscle cell constriction.⁶ In addition, DNA-binding properties of benzo[c]quinolizinium ions⁷ as well as their inhibitory activity for protein kinase CKII⁸ make them promising lead structures for the development of novel anticancer chemotherapeutic agents.

The first synthesis of the benzo[c]quinolizinium system was performed in 1958 by Glover and Jones from cyanoquinolines in three steps.⁹ Later, Fozard and Bradsher obtained a series of benzo[c]quinolizinium derivatives by thermal cyclization of cis-2'-chloro-2-stilbazoles.¹⁰ Arai et al. developed a photochemical approach towards cyclization of chloro-substituted stylbazoles.¹¹ Modified Ca²⁺-assisted thermal cyclization of halogenated phenylazopyridine and related Schiff bases was applied for the synthesis of 5-aza, 6-aza, and 5,6-diaza analogues of benzo[*c*]quinolizinium.¹² Another approach synthesize to a family of 5.6-diazabenzo[*c*]quinolizinium compounds is based on proton-catalyzed cyclization of 2-(arylazo)pyridines.¹³ A series of substituted benzo[c]quinolizinium derivatives was prepared by condensation of 2-picolyllithium and ortho-halogenobenzonitrile with subsequent thermally induced cyclization.^{3a} Some benzo[*c*]quinolizinium derivatives can be obtained by a ring-closing metathesis (RCM) reaction.¹⁴ More recently, a range of novel benzo[c]quinolizinium salts was synthesized by interaction of α -oxoketene S,S-acetals with 2-methylquinolyllithium or 2,4dimethyl-6-methoxyquinolyllithium followed by BF₃-assisted cycloaromatization.¹⁵ However, all

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the reported methods possess at least one of the following significant drawbacks, namely performance of the reactions at high temperature, requirement of a catalyst (acid, Ca^{2+}), need of halogenation of the substrates at certain positions, or multi-step reaction sequences.

We are developing regiospecific C-N photocyclization of hetarylphenylethenes resulting in the formation of charged polycyclic heteroaromatics.¹⁶⁻²¹ In this reaction, a new C–N bond is formed through the photoinduced electrocyclic ring closure followed by in situ oxidation of the resulting dihydrohetarene intermediate by air oxygen. The process takes place at mild conditions at room temperature, does not require the use of catalysts and allows to obtain fused polycyclic cations with good yields. Recently, we have shown that the C-N photocyclization of 2-styrylpyridine²¹ and 2styrylquinolines¹⁹ leads to the efficient and selective formation of benzo[c]quinoliziniumwe performed the photogeneration of derivatives. Notably, the DNA-binding 8.9dimethoxybenzo[c]quinolizinium derivative **6** as a key process in a five-component supramolecular cascade. In this system, the light-controlled transport of the *in situ* modified ligand 6 between cyclodextrin, duplex DNA, and cucurbituril was accomplished; thus demonstrating biocompatibility of this reaction (Scheme 1).²¹

Scheme 1. Photocyclization of mono-styryl derivatives 1a–1e and chemical structures of annelated quinolizinium derivatives 6 and 7



Analysis of our previous data as well as the recent report of Budyka et al. on the photocyclization of styrylbenzoquinolines²² led us to the assumption that the regioselective C–N bond formation in the

photochemical cyclization may be a general chemical reactivity of *ortho*-styryl-substituted Nhetarenes containing donor substituents in the styryl residue. If that is the case, this simple reaction would open a straightforward synthesis of a wide range of biologically active benzo[c]quinolizinium derivatives.

As a proof of our hypothesis we describe herein the regiospecific C–N photocyclization of monoand bis-dimethoxystyryl-substituted N-heterocycles to the corresponding benzo[c]quinolizinium derivatives (Schemes 1 and 2). The substrates **1a–1e** and **3a**, **3b** were prepared by Knoevenagel condensation of the corresponding methyl-substituted heterocycles with veratraldehyde in the presence of KOBu^{*t*} in DMF (Scheme S1, Supporting information). The methoxy groups were introduced because our previous studies showed that these substituents provide high efficiency of the photocyclization.¹⁹ Since annelated quinolizinium ions represent important key structures for the design of DNA-targeting ligands²³ we particularly focused our attention on the interactions of the photocyclization products with DNA.

Scheme 2. Photoreactions of bis-styryl derivatives 3a and 3b.



Results and discussion

Photocyclization of mono-styrylazines 1a-1e

The photocyclization reactions of mono-styryl derivatives **1a–1e** (Scheme 1) were carried out in airsaturated solutions in acetonitrile or water upon irradiation with direct or filtered light of a highpressure Hg vapor lamp; and the course of the photoreaction was monitored photometrically. Direct irradiation of dimethoxystyryl derivatives of pyrazine **1a**, pyridazine **1d** and isoquinoline **1e** in

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acetonitrile led to the appearance of new red-shifted absorption bands at 350-450 nm (Figures S1, S4, S5, Supporting information). These bands were assigned to the photocyclization products 2a, 2d and **2e** by analogy to our previous reports.¹⁶⁻²¹ The regioselective formation of a new C–N bond in all cases was unambiguously proven by the ¹H NMR spectroscopic data based on the characteristic downfield shifts of the aromatic proton signals that clearly indicate the formation of the quinolizinium structure (Figures S11-S34, Supporting information). No products of C-C cyclization were detected. The remarkable regiospecificity of this photoreaction is caused by the significantly lower enthalpy of formation of the C-N cyclization product in comparison to that of C-C cyclization product formation, as was described previously for 2-styrylquinoline derivatives.¹⁹ Moreover, no positional isomerism of the methoxy substituents was detected for photocyclization products 2a, 2d, 2e, E-4a and E-4b. This means that formation of a new bond takes place only between N and C6' atoms of the styrylheterocycles (Schemes 1 and 2). Considering that the electrocyclization occurs from the Z-form of 1 and 3 the stability of different conformations of the corresponding Z-isomers influences the structure of the resulting polycyclic product. Thus, in the conformation of the Z-isomer that results N-C2' cyclization one of the methoxy groups in the phenyl ring is directed towards the heterocyclic fragment. This arrangement, however, leads to increased steric repulsion and destabilizes the corresponding conformation. On the other hand, in the conformation of the Z-isomer that leads to N-C6' cyclization the steric interactions are minimized so that the formation of a new bond between N and C6' atoms is energetically favored. The mono-styryl derivatives 1b, 1c, 1d, the bis-styryl derivative 3b and all photocyclization products 2a, 2d, 2e, E-4a and E-4b are novel compounds and were fully characterised by NMR spectroscopy and HRMS.

Photolysis of **1a**, **1d** and **1e** in water under similar conditions resulted in formation of more intense absorption bands of products **2a**, **2d** and **2e** (Figure 1) as compared to the photoreaction in acetonitrile. This fact points out higher efficiencies of the photocyclization in aqueous solution. Optimization of the conditions revealed that in case of compound **1a** the use of a cut-off filter ($\lambda >$ 290 nm) leads to an enhanced yield of photoproduct **2a**. However, the removal of the shortwavelength light increased the conversion time of **1a** by ca. 2.7. Heteroaromatic compounds **2a**, **2d** and **2e** were successfully isolated as perchlorate salts in moderate yields (**2a**: 39%; **2d**: 57%; **2e**: 50%) by recrystallization from MeOH with addition of equimolar amount of HClO₄.



Figure 1. Spectral changes during the photolysis of **1a**, **1d** and **1e** ($c = 20 \mu$ M, water, high-pressure Hg lamp, 20 °C): A: **1a**, $\lambda > 290$ nm, 95 min; B: **1d**, no filter, 16 min; C: **1e**, no filter, 15 min.

In contrast, direct irradiation of styrylpyrimidine derivatives **1b** and **1c** in acetonitrile with led to broad, almost shapeless spectra indicating extended photodecomposition that was confirmed by the NMR-spectroscopic analysis of the photolysate (Figures S2, S3, S9, S10, Supporting information). To improve the yield of the desired photoproducts we irradiated **1b** and **1c** in aqueous solution. The absorption spectra obtained after photolysis in water revealed pronounced red-shifted bands in the characteristic absorption range of the benzo[*c*]quinolizinium (Figure 2). Nevertheless, the isolation of the products **2b** and **2c** from the photolysate was not successful. For this reason, the obtained solutions were analyzed by HPLC (Figures S6 and S7, Supporting information). Unfortunately, the complexity of the product mixtures, comprising at least four components with close retention times, did not allow unambiguous determination of their content in the complex mixtures was very low and any attempts of their isolation had no preparative meaning. The most probable reason for this observation is the photochemical instability of compounds **2b** and **2c** that – unlike **2a**, **2d** and **2e** – undergo side reactions or secondary photoreactions in the course of the photolysis. In particular, it is known that pyrimidine derivatives possess low photostability and may undergo a [2+2]

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photocycloaddition reaction resulting in the formation of the intermolecular dimers.²⁴ To check this assumption a control experiment was performed with the photolytic mixture obtained after irradiation of styrylpyrazine derivative **1a** in water ($\lambda > 290$ nm) (Figure 1A). In this case only two fractions were separated by HPLC with significantly different retention times (Figure S8, Supporting information). The absorption spectra of the obtained fractions coincide well with the absorption spectra of substrate **1a** and its photocyclization product **2a**. This observation confirmed the low stability of pyrimidine derivatives during the photolysis in comparison to other monostyrylazines from the employed series.



Figure 2. Spectral changes during the photolysis of **1b** and **1c** in water ($c = 20 \mu$ M, high-pressure Hg lamp, 20 °C): A: **1b**, $\lambda > 290$ nm, 35 min; B: **1c**, no filter, 15 min. *Photocyclization of bis-styrylazines 3a and 3b*

Fused bis-styryl derivatives of pyrazine 3a and pyrimidine 3b, with both styryl moieties located in the *ortho*-positions to the nitrogen atoms of the central azine ring are potentially capable of two-fold C–N cyclization (Scheme 2). Two possible types of C–N electrocyclization products for 3a, 3b can be suggested with only one or both styryl fragments involved in the cyclization yielding corresponding mono- and dicationic heteroaromatics 4a, 4b and 5a, 5b. It should be noted that the dimethoxystyryl fragment of monocationic species 4a and 4b can potentially exist in *E*- or *Z*-form. Direct irradiation of the acetonitrile solutions of bis-styrylpyrazine 3a and bis-styrylpyrimidine 3bunder aerobic conditions resulted in the formation of the red-shifted absorption bands that indicate the extension of a conjugated aromatic system in the course of the photoreaction (Figure 3). The photolysate was purified by recrystallization from MeOH in the presence of HClO₄. As a result, pure *E*-isomers of monocyclization products **4a**, **4b** were isolated as perchlorate salts with low to moderate yields (*E*-**4a**: 25%; *E*-**4b**: 51%). Neither *Z*-isomers of **4a**, **4b** nor dicationic species **5a**, **5b** were detected (Figure 4). Interestingly, irradiation of **3a** and **3b** with filtered light ($\lambda = 405$ nm for **3a** or $\lambda = 365$ nm for **3b**) did not lead to the formation of polycyclic products. The poor solubility of **3a** and **3b** in water did not allow us to test the photocyclization process in aqueous medium.



Figure 3. Spectral changes during the photolysis of the bis-styryl derivatives in acetonitrile ($c = 20 \mu$ M, unfiltered light of a high-pressure Hg vapor lamp, 20 °C): A: **3a**, 30 min; B: **3b**, 16 min.



Figure 4. ¹H NMR spectra (400 MHz, range of the aromatic and olefin bond protons) of bis-styryl derivative E-**3a** (top) and its photocyclization product E-**4a** (bottom) in CD₃CN. The signals of the *trans*-double bonds are highlighted in blue. Dashed lines show shifting of the signals of the styryl fragment protons of photoproduct E-**4a** in comparison to the ones of the substrate **3a**.

The inhibition of the photoinduced E-Z-isomerization in **4a** and **4b** unambiguously points out the presence of an alternative dominating relaxation pathway in the excited state. Indeed, compounds

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4a and **4b** resemble cationic styryl dyes with a highly polar donor-acceptor structure that upon photoexcitation in polar media readily undergo non-radiative relaxation through rotation about single bonds and formation of twisted excited states.²⁵ In such systems, *E-Z*-isomerization of the C=C double bonds is usually significantly suppressed. Therefore, we assume that the absence of *E*-*Z*-isomerization may be a consequence of the pronounced dipolar character of **4a**, **4b** that promotes relaxation through the twisted excited states. However, the detailed investigation of the photophysical behavior of **4a** and **4b** is beyond the scope of the present study.

The exclusive formation of 4a and 4b in the *E*-form provides an explanation why two-fold cyclization to 5a, 5b does not occur, because the cyclization can take place only from the *Z*-form of a styrylheterocyclic species.¹⁹ Since the *Z*-isomers of 4a and 4b do not form, the cyclization process cannot proceed further and stops on the monocyclization stage. An additional reason for the impeded second cyclization may be the electrostatically unfavorable accumulation of two positive charges within one six-membered ring in 5a and 5b.

DNA-binding properties of photocyclization products 2a, 2d, 2e

The photoproducts **2a**, **2d** and **2e** possess characteristic features of DNA-intercalators, namely, planar heteroaromatic structure with a permanent positive charge.²⁶ To assess DNA-binding properties of these compounds their interaction with calf thymus DNA (ct DNA) was examined by spectrometric titrations. Thus, addition of ct DNA to the solutions of compounds **2a**, **2d**, and **2e** produced a strong hypochromic effect along with a pronounced red shift of the absorption of the ligand that is indicative of a strong interaction with DNA (Figure 5A, Table 1). In all cases, an isosbestic point is maintained during the titrations that indicates one almost exclusive binding mode. Fitting of the binding isotherms obtained from the photometric titrations to the neighbor-exclusion model²⁷ gave the binding constants for the complexes of **2a**, **2d**, and **2e** with ct DNA (Table 1). The binding constant of 3-azabenzo[*c*]quinolizinium derivative **2a** ($K_b = 1.1 \times 10^5 \text{ M}^{-1}$) is very close to the one reported for 8,9-dimethoxybenzo[*c*]quinolizinium **6** ($K_b = 1.2 \times 10^5 \text{ M}^{-1}$)²¹ which can be explained by close structural resemblance of these compounds. At the same time, the

DNA-binding affinity of structurally similar 1-azabenzo[c]quinolizinium derivative 2d is slightly lower ($K_b = 6.6 \times 10^4 \text{ M}^{-1}$). The binding constant of 2,3-dimethoxy-isoquinolinoquinolinium derivative 2e ($K_b = 4.6 \times 10^5 \text{ M}^{-1}$) is of the same order of magnitude as the one of the parent compound 7 ($K_b = 1.9 \times 10^5 \text{ M}^{-1}$).²⁸ Overall, the values of the binding constants of ligands 2a, 2d, and 2e with ct DNA are in good agreement with the reported data for DNA-intercalators of similar structure and size. The fluorescence of 2a, 2d and 2e was efficiently quenched upon addition of ct DNA with almost no changes in the positions of the emission maxima (Figure 5B, Table 1), which usually indicates the photoinduced electron transfer (PET) reaction between the bound ligand and the DNA bases, specifically the easily oxidized guanine residues.²⁹ Complementary polarimetric titrations revealed that in the presence of ct DNA the achiral ligands 2a, 2d and 2e display induced circular dichroism (ICD) signals that resemble the long-wavelength absorption of the ligands in the presence of DNA (Figure 5C). The tricyclic compounds 2a and 2d possess weak positive ICD signals whereas the tetracyclic derivative 2e exhibits a strong bisignated ICD signal that may indicate aggregation of 2e in the presence of DNA or two energetically close orthogonal transition dipoles.³⁰ In particular, the positive ICD signals result from an alignment of the transition dipole of the ligands perpendicular to the long axis of the binding pocket, i.e. the direction of the transition dipole of the DNA bases.³⁰ At the same time, it may be assumed that the transition dipole moment of the long-wavelength absorption band of the ligands 2a, 2d and 2e is essentially pointing along their long-molecular axes, i.e. from the methoxy sustituents to the pyridinium unit (Scheme 1). As a result, similarly as demonstarted for 7 already in detail,²⁸ it can be deduced from the positive ICD band that the ligands intercalate into DNA with their long molecular axes pointing almost perpendicular relative to the long axis of the intercalation pocket.



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Figure 5. A: Spectrophotometric titration of ct DNA to **2a** (A1, $c_{2a} = 50 \ \mu\text{M}$, $c_{DNA}/c_{2a} = 0-21$); **2d** (A2, $c_{2d} = 50 \ \mu\text{M}$, $c_{DNA}/c_{2d} = 0-23$); **2e** (A3, $c_{2e} = 20 \ \mu\text{M}$, $c_{DNA}/c_{2e} = 0-24$). B: Fluorimetric titration of ct DNA to **2a** (B1, $c_{2a} = 20 \ \mu\text{M}$, $c_{DNA}/c_{2a} = 0-59$, $\lambda_{ex} = 418 \ \text{nm}$); **2d** (B2, $c_{2d} = 20 \ \mu\text{M}$, $c_{DNA}/c_{2d} = 0-57$, $\lambda_{ex} = 396 \ \text{nm}$); **2e** (B3, $c_{2e} = 10 \ \mu\text{M}$, $c_{DNA}/c_{2e} = 0-14$, $\lambda_{ex} = 410 \ \text{nm}$). C: CD spectra of mixtures of ct DNA ($c_{DNA} = 50 \ \mu\text{M}$, in base pairs) with ligands **2a** (C1), **2d** (C2), **2e** (C3) at ligand-DNA ratio LDR = 0, 0.3, 0.6, 0.8, 1.0, 1.5. In all cases: BPE buffer (pH 7.0) with 2% v/v DMSO, $T = 20 \ ^{\circ}\text{C}$.

	$\lambda_{\rm abs}$ / nm a		$\lambda_{ m fl}$ / nm b		V . / M -1
	free	bound	free	bound	$-\mathbf{K}_{b}/\mathbf{W}$
2a	405	415	470	472	$1.1 \times 10^{5 c}$
2d	382	393	463	460	$6.6 \times 10^{4 c}$
2e	403	418	442	443	$4.6 \times 10^{5 c}$
<i>E-</i> 4 a	460	477	456	500	_ d
<i>E-</i> 4b	452	475	547	550	$5.0 imes 10^{5 e}$

Table 1. Absorption and emission maxima of free and DNA-bound ligands **2a**, **2d**, **2e**, *E*-**4a**, *E*-**4b** and binding constants of their complexes with ct DNA in BPE buffer (pH 7.0) with 2% v/v DMSO (for **2a**, **2d**, **2e**) or 5% v/v DMSO (for *E*-**4a**, *E*-**4b**).

^{*a*} Long-wavelength absorption maxima of free and DNA-bound ligands. ^{*b*} Fluorescence maxima of free and DNA-bound ligands. ^{*c*} Binding constant with ct DNA, obtained from the photometric data. ^{*d*} Binding constant was not determined. ^{*e*} Average binding constant with ct DNA, obtained from the fluorimetric data that reflects the combination of two binding modes. T = 20 °C.

To verify the proposed intercalative binding mode of the heteroaromatic photoproducts 2 with ct DNA, flow-linear dichroism (LD) spectroscopic analysis³⁰ was performed exemplarily with ligand 2e (Figure 6). The ct DNA without ligand gives a negative signal in the absorption region of the DNA bases (Figure 6A) as a result of the perpendicular alignment of the transition dipole moment

of the bases relative to the LD reference axis.³⁰ The addition of ct DNA to **2e** led to formation of a negative LD signal in the absorption range of the ligand (Figure 6A). This negative LD signal of **2e** results from a parallel alignment of the transition dipole of the bound ligand and the ones of the DNA bases, thus indicating the intercalative binding mode of the planar aromatic system of the ligand.³⁰ Further information about the relative orientation of the ligand in the binding site of DNA is provided by the reduced linear dichroism, LD_r, i.e. the wavelength dependent LD. In the case of DNA-bound **2e**, the LD_r values in the absorption regions of the DNA bases and of the ligand are negative and essentially independent of the absorption wavelength (Figure 6B), which confirms the intercalation of the ligand into the DNA helix.³⁰ Moreover, observation that the amplitude of the LD_r band of compound **2e** has almost the same magnitude than the one of the DNA bases.



Figure 6. Linear dichroism LD (A) and reduced linear dichroism LD_r (B) spectra of ligand **2e** in the presence of ct DNA ($c_{\text{DNA}} = 50 \ \mu\text{M}$, in base pairs) at LDR of 0 (---) and 0.04 (--) in BPE buffer (pH 7.0) with 2% v/v DMSO, $T = 20 \ ^{\circ}\text{C}$.

DNA-binding properties of photocyclization products E-4a,4b

Spectrophotometric titrations of the photoproducts *E*-4a and *E*-4b with ct DNA revealed nonuniform changes in the absorption intensity with no isosbestic point (Figure 7A). In the first part of the titration (ligand-DNA ratio (LDR): 110 - 7 for *E*-4a; 344 - 9 for *E*-4b), the absorption decreases with a small red shift of the absorption maxima for *E*-4a or a pronounced blue shift for *E*-4b, whereas at smaller ligand-DNA ratio (< 7 for *E*-4a; < 9 for *E*-4b) the absorption band increases with a significant red shift. Such a behavior usually indicates the presence of at least two different

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binding modes. The complex changes observed during the photometric titrations of ct DNA to E-4a and E-4b represent a superposition of changes arising from the different binding modes that cannot be dissected. For this reason, calculation of the binding constant from the photometric data was not possible.

A special feature of the photometric titration of DNA to *E*-4**b** is the initial blue shift of the absorption maximum at high LDR, which is rarely observed, for example on H-aggregate formation of cyanine dyes along the DNA backbone.³¹ In the case of *E*-4**b**, however, the backbone aggregation is rather unlikely because other than the cyanine dyes this ligand does not have the molecular shape and flexibility such that its H-aggregates fit perfectly in the DNA groove. Therefore, we assume that the blue shift is a result of a particular conformation of the DNA-bound ligand. Because of its rather rod-like structure, *E*-4**b** may have significant twist about the alkenearyl bonds to provide a reasonable fit to the DNA binding sites. In this conformation, the styryl unit is no longer well conjugated with the quinolizinium chromophore, so that a reduced donor-acceptor interplay results a blue shift. Evidence for this assumption is provided by the observation that the absorption maximum is close to the one of the DNA-bound benzo[*c*]quinolizinium ligands **2a** and **2d** that lack the styryl substituent.

For further investigation of the binding modes, CD- and flow-LD-spectroscopic analyses were performed (Figure 7, C and D). In the case of ligand *E*-4a ICD bands were detected between 300 nm and 400 nm upon complexation with DNA (Figure 7, C1). Most notably, only a very weak ICD bands developed in the long-wavelength absorption range of the ligand. This observation indicates that the transition dipole moments of the DNA base pairs couple efficiently with the transition dipole of the ligand that is assigned to the S_0 - S_2 and/or S_0 - S_3 transition (see discussion above). In contrast, the coupling with the lower energy S_0 - S_1 transition appears to be very weak, presumably because of an unfavorable relative orientation of the respective transition dipoles towards each other.³⁰ In the flow-LD experiment, however, clear negative LD bands were recorded in the absorption region of *both* transitions of the ligand *E*-4a (Figure 7, D1), thus indicating the intercalation of aromatic ligands into DNA (see discussion above).³⁰ These observations clearly indicate that one binding mode of ligand *E*-4a is intercalation, as unambiguously confirmed by the negative LD bands of the ligand. In analogy to the ligands 2a and 2d (see above) the ligand *E*-4a binds to DNA with the benzo[*c*]quinolizinium fragment inserted between the DNA base pairs, whereas the dimethoxystyryl unit is pointing outside the DNA helix.

In the case of ligand E-4b, the addition of DNA led to the development of a positive ICD and a negative LD band only at ca. 450 nm (Figure 7, C2 and D2), that corresponds to the blue-shifted absorption band formed at high ligand-DNA ratios, LDR > 9 (Figure 7, A2). Like in the case of E-*E*-4a, these ICD and LD bands indicate the intercalation of the benzo[c]quinolizinium fragment. But remarkably, the absorption band that was observed during photometric titration at smaller LDR values (LDR < 9) is neither CD nor LD active. Such a lack of CD and LD spectroscopic response of a DNA ligand is usually the result of unspecific aggregation along the DNA backbone, however, this binding mode occurs at high LDR values and is usually negligible at low LDR. At the same time, groove binding may be considered as alternative binding mode like for related styryl dyes,³² but groove-bound ligands usually give very strong ICD bands. Hence, along with the interpretation of the unusual blue shift during photometric titration (see above), it may be proposed that at the beginning of the titration, i.e. at high ligand-DNA ratio, the DNA is fully loaded with intercalated molecules, each of which occupies several binding sites because a fairly large amout of the ligand points outside the binding site and hinders the approach of another ligand in its close vicinity. With decreasing LDR value the intercalating ligands are distributed less densely over the DNA molecule, such that the ligand can also form aggregates the associate unselectively along the DNA backbone. Apparently, the binding constants of both binding modes are in the same range so that they compete at low LDR.





Figure 7. A: Spectrophotometric titration of ct DNA to *E*-4a (A1, $c_{E-4a} = 50 \ \mu$ M, $c_{DNA}/c_{E-4a} = 0-13.3$); *E*-4b (A2, $c_{E-4b} = 50 \ \mu$ M, $c_{DNA}/c_{E-4b} = 0-23.8$). B: Fluorimetric titration of ct DNA to *E*-4a (B1, $c_{E-4a} = 25 \ \mu$ M, $c_{DNA}/c_{E-4a} = 0-48.7$, $\lambda_{ex} = 400 \ nm$); *E*-4b (B2, $c_{E-4b} = 25 \ \mu$ M, $c_{DNA}/c_{E-4b} = 0-48.0$, $\lambda_{ex} = 400 \ nm$). C: CD spectra of mixtures of ct DNA ($c_{DNA} = 0.1 \ \mu$ M, in base pairs) with ligands *E*-4a (C1), *E*-4b (C2) at ligand-DNA ratio LDR = 0, 0.3, 0.6, 1.2. D: LD spectra of mixtures of ct DNA ($c_{DNA} = 0.1 \ \mu$ M, in base pairs) with ligands *E*-4a (D1), *E*-4b (D2) at ligand-DNA ratio LDR = 0, 0.3, 0.6, 1.2. In all cases: BPE buffer (pH 7.0) with 5% v/v DMSO (for the photometric and fluorimetric titrations) or 7% v/v DMSO (for the CD and LD measurements), $T = 20 \ ^\circ$ C. Blue lines denote the initial absorption and fluorescence spectra of *E*-4a and *E*-4b; red lines denote the corresponding spectra of DNA-bound *E*-4a and *E*-4b at saturation. Arrows indicate changes of the bands upon addition of ct DNA.

Despite the structural similarity of compounds E-4a and E-4b, their emission properties in the

presence of ct DNA differ significantly. Thus, upon increasing the concentration of DNA, the fluorescence of *E*-4a was efficiently quenched with just residual increase at the end of the titration. In addition, the emission maximum exhibited a pronounced red shift in the presence of DNA ($\Delta \lambda$ = 44 nm) (Figure 7B, Table 1). By contrast, the fluorescence of *E*-4b lights up by a factor of 6.3 upon addition of ct DNA with a negligible red shift of the emission maximum ($\Delta \lambda = 3$ nm) (Figure 7B, Table 1). Most likely, this diverge behavior of the two ligands arises from a different contributions of the two above-mentioned DNA binding modes in each case. By analogy to benzo[c]quinolizinium intercalators 2a, 2d and 2e, whose fluorescence is completely quenched in the presence of ct DNA (Figure 5B), we assume that intercalation of the benzo[c]quinoliziniumfragment of E-4a and E-4b between the DNA base pairs is also accompanied by fluorescence quenching due to photoinduced PET processes, as described above.²⁹ On the other hand, the aggregation of the cationic styryl derivatives E-4b along the DNA backbone prevents the nonradiative rotational relaxation and subsequently leads to the enhancement of the fluorescence intensity, thus representing a special case of aggregation-induced enhancement of emission.³³ Analysis of the fluorimetric data allowed to determine an average binding constant with ct DNA for *E*-4b (Table 1) reflecting the combination of two binding modes.

Conclusion

To sum up, we have demonstrated that *ortho*-styryl substituted N-heterocycles comprising one and two nitrogen atoms generally undergo the regioselective C–N bond formation during photocyclization resulting in formation of the family of (aza)benzo[c]quinolizinium derivatives. The photocyclization of mono-styryl derivatives **1a**, **1d** and **1e** occurs more efficiently in aqueous solution than in organic solvents (acetonitrile) making the process environment-friendly. Bis-styryl derivatives **3a** and **3b** – although designed for the two-fold cyclization – cyclize only one styryl fragment, whereas the second styryl residue remains photochemically inert. DNA-binding studies with ct DNA revealed that all obtained photoproducts have a high affinity towards nucleic acids.

Interestingly, the photoproducts **2a**, **2d**, **2e** of mono-styrylheterocycles are typical DNAintercalators, while the photoproducts *E*-**4a** and *E*-**4b** of bis-styrylheterocycles demonstrate a mixed binding mode comprising intercalation and aggregation along the DNA backbone. Overall, the studied phototransformation is the most simple synthetic approach, so far, to the benzo[*c*]quinolizinium scaffold that may be applied in the development of new DNA-targeting chemotherapeutics.

All reagents and solvents were obtained from commercial sources and used as received. ¹H NMR spectra were recorded at 400 or 600 MHz, ¹³C NMR spectra were recorded at 101 or 151 MHz at ambient temperature using 5 mm tubes. Chemical shifts were determined with accuracy of 0.01 ppm and 0.1 ppm for ¹H and ¹³C spectra, respectively, and are given relative to the residual signal of the solvent that was used as internal reference. Spin-spin coupling constants for the proton spectra were determined with accuracy of 0.1 Hz. The ¹H NMR signal assignments were performed using COSY and NOESY 2D NMR techniques. The ¹³C NMR signal assignments were performed by means of HSQC and HMBC 2D NMR techniques. Electrospray ionization (ESI) mass spectra were detected in the mode of full mass scanning of positive ions on a tandem dynamic mass spectrometer equipped with a mass analyzer with an octapole ionic trap. High-resolution mass spectra were recorded on a time-of-flight instrument in a positive-ion mode using electrospray ionization method. TLC was performed on silica gel on Al foils, eluent: EtOAc. Preparation and handling of the solutions were carried out under red light. Photochemical reactions were carried out with a high pressure Hg vapor lamp (120 W) and an immersed Hg photoreactor (125 W). Electronic absorption spectra were measured on a fiber-optic spectrometer connected to a computer at $20\pm1^{\circ}$ C. Fluorescence spectra were measured on a spectrofluorimeter equipped with double-grating monochromators and a Xe flash lamp at 20±1°C. Circular dichroism (CD) and linear dichroism (LD) spectra were recorded on a CD spectrometer equipped with High Shear Couette Cell

Accessory (for LD measurements). The LD samples were oriented in a rotating couette with a shear gradient of 1200 s^{-1} . The reduced linear dichroism, LD_r, was calculated according to Eq. 1.

$$LD_r = LD / A_{iso}$$
 (Eq. 1)

where A_{iso} is the absorbance of an isotropic sample that is determined under the same conditions as the LD.

General Procedure for the Synthesis of mono-styryl derivatives (1a–1e) and bis-styryl derivatives (3a, 3b)

A mixture of 3,4-dimethoxybenzaldehyde (for mono-styryl derivatives: 300 mg, 1.80 mmol; for bisstyryl derivatives: 600 mg, 3.60 mmol), the corresponding methyl-substituted heterocycle [for **1a**: 2-methylpyrazine (0.16 mL, 1.80 mmol); for **1b**: 2-methylpyrimidine (0.17 mL, 1.80 mmol); for **1c**: 4-methylpyrimidine (0.16 mL, 1.80 mmol); for **1d**: 3-methylpyridazine (0.16 mL, 1.80 mmol); for **1e**: 1-methylisoquinoline (0.24 mL, 1.80 mmol); for **3a**: 2,5-dimethylpyrazine (197 μ L, 1.80 mmol); for **3b**: 4,6-dimethylpyrimidine (199 μ L, 1.80 mmol)] and potassium *tert*-butoxide (for mono-styryl derivatives: 202 mg, 1.80 mmol; for bis-styryl derivatives: 404 mg, 3.60 mmol) in abs. DMF (for mono-styryl derivatives: 4 mL; for bis-styryl derivatives: 2.5 mL) was stirred at room temperature for 2–4 h (mono-styryl derivatives) or 24 h (bis-styryl derivatives). The reaction mixture was diluted with water (2 mL) and concentrated in vacuo. The obtained solid was dissolved in water (30 mL) and extracted with dichloromethane (5 x 20 mL). The organic layers were combined and the solvent was removed.

(E)-2-(3,4-dimethoxystyryl)pyrazine (1a)

Recrystallized from MeOH; yellow solid, yield 51% (0.91 mmol, 220 mg), mp 103–105 °C (lit. mp 107–108 °C)³⁴. ¹H NMR (DMSO-*d*₆, 400 MHz) δ : 3.79 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 6.99 (d, 1H, H-5', *J* = 8.3 Hz), 7.20 (br.d, 1H, H-6', *J* = 8.3), 7.28 (d, 1H, H-a, *J*_{trans} = 16.1 Hz), 7.34 (br. s, 1H, H-2'), 7.72 (d, 1H, H-b, *J*_{trans} = 16.1 Hz), 8.45 (d, 1H, H-6, *J* = 2.4 Hz), 8.59 (br. s, 1H, H-5), 8.75 (br. s, 1H, H-3). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ : 55.5 (2C, O<u>C</u>H₃), 109.6, 111.7, 121.4, 122.2, 128.8, 134.4, 142.6, 143.5, 144.4, 149.0, 149.8, 151.1. Anal. Calcd for C₁₄H₁₄N₂O₂: C, 69.41; H, 5.82; N, 11.56; found: C, 69.20; H, 5.81; N, 11.49. ESI-MS **1a** in MeCN, *m/z*: calcd. 242.27; found 243.10 [**1a**+H]⁺.

(E)-2-(3,4-dimethoxystyryl)pyrimidine (1b)

Further purification was not required; white solid, yield 53% (0.95 mmol, 230 mg), mp 110-112

°C. ¹H NMR (CD₃CN, 600 MHz) δ: 3.83 (s, 3H, OCH₃), 3.87 (s, 3H, OCH₃), 6.96 (d, 1H, H-5', *J* = 8.3 Hz), 7.15 (d, 1H, H-a, *J*_{trans} = 15.9 Hz), 7.16 (t, 1H, H-5, *J* = 4.8 Hz, 9.6 Hz), 7.22 (d, 1H, H-6', *J* = 8.6 Hz), 7.30 (s, 1H, H-2'), 7.91 (d, 1H, H-b, *J*_{trans} = 15.9 Hz), 8.70 (d, 2H, H-4, H-6, *J* = 4.8 Hz). ¹³C NMR (CD₃CN, 151 MHz) δ: 56.6 (*m*-O<u>C</u>H₃), 56. 7 (*p*-O<u>C</u>H₃), 111.1 (C-2'), 112.8 (C-5'), 119.8 (C-5), 122.9 (C-6'), 126.8 (C-a), 130.2 (C-1'), 138.6 (C-b), 150.7 (C-3'), 151.7 (C-4'), 158.4 (2C, C-4, C-6), 166.1 (C-2). Anal. Calcd for C₁₄H₁₄N₂O₂: C, 69.41; H, 5.82; N, 11.56; found: C, 69.48; H, 5.89; N, 11.61. ESI-MS **1b** in MeCN, *m/z*: calcd. 242.27; found 243.60 [**1b**+H]⁺. HRMS (ESI-TOF) *m/z*: [**1b**-H]⁺ Calcd for C₁₄H₁₃N₂O₂ 241.0972; Found 241.0973.

(E)-4-(3,4-dimethoxystyryl)pyrimidine (1c)

Recrystallized from MeOH; yellow solid, yield 52% (0.94 mmol, 227 mg), mp 108–110 °C. ¹H NMR (CD₃CN, 600 MHz) δ : 3.86 (s, 3H, OCH₃), 3.90 (s, 3H, OCH₃), 6.99 (d, 1H, H-5', J = 8.4), 7.11 (d, 1H, H-a, $J_{trans} = 16.0$ Hz), 7.24 (d, 1H, H-6', J = 8.4 Hz), 7.31 (br. s., 1H, H-2'), 7.42 (d, 1H, H-3, J = 5.3 Hz), 7.88 (d, 1H, H-b, $J_{trans} = 16.0$ Hz), 8.66 (d, 1H, H-6, J = 5.1 Hz), 9.08 (s, 1H, H-4). ¹³C NMR (CD₃CN, 151 MHz) δ : 56.4 (2C, O<u>C</u>H₃), 110.9 (C-2'), 112.6 (C-5'), 119.6 (C-5), 122.9 (C-6'), 124.8 (C-a), 129.7 (C-1'), 137.8 (C-b), 150.5 (C-3'), 151.7 (C-4'), 158.5 (C-6), 159.7 (C-2), 163.5 (C-4). Anal. Calcd for C₁₄H₁₄N₂O₂: C, 69.41; H, 5.82; N, 11.56; found: C, 69.50; H, 5.84; N, 11.56. ESI-MS **1c** in MeCN, m/z: calcd. 242.27; found 243.50 [**1c**+H]⁺. HRMS (ESI-TOF) m/z: [**1c**-H]⁺ Calcd for C₁₄H₁₃N₂O₂ 241.0972; Found 241.0978.

(E)-3-(3,4-dimethoxystyryl)pyridazine (1d)

The residue was purified by column chromatography (SiO₂; ethylacetate; $R_f = 0.80$); pale yellow solid, yield 48% (0.86 mmol, 208 mg), mp 83–85 °C. ¹H NMR (DMSO-*d*₆, 600 MHz) δ : 3.80 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 7.01 (d, 1H, H-5', J = 8.4 Hz), 7.22 (dd, 1H, H-6', J = 8.4 Hz; 2.0 Hz), 7.35 (d, 1H, H-b, $J_{trans} = 16.1$ Hz), 7.36 (d, 1H, H-2', J = 2.0 Hz), 7.65 (dd, 1H, H-5, J = 8.4 Hz; 4.8 Hz), 7.73 (d, 1H, H-a, $J_{trans} = 16.5$ Hz), 7.90 (dd, 1H, H-4, J = 8.8 Hz; 1.6 Hz), 9.06 (dd, 1H, H-6, J = 4.8 Hz; 1.2 Hz). ¹³C NMR (DMSO-*d*₆, 151 MHz) δ : 55.50 (3'-O<u>C</u>H₃), 55.52 (4'-O<u>C</u>H₃), 109.8 (C-2'), 111.8 (C-5'), 121.2 (C-6'), 123.1 (C-a), 123.9 (C-4), 126.8 (C-5), 128.8 (C-1'), 134.5 (C-b), 149.0 (C-3'), 149.6 (C-6), 149.8 (C-4'), 158.1 (C-3). Anal. Calcd for C₁₄H₁₄N₂O₂: C, 69.41; H, 5.82; N, 11.56; found: C, 69.56; H, 5.89; N, 11.47. HRMS (ESI-TOF) *m/z*: [**1d**+H]⁺ Calcd for C₁₄H₁₅N₂O₂ 243.1128; Found 243.1132.

(E)-1-(3,4-dimethoxystyryl)isoquinoline (1e)

The residue was purified by column chromatography (SiO₂; benzene–ethylacetate going from 1:0 to 80:20; $R_f = 0.80$); yellow solid, yield 35% (0.63 mmol, 184 mg), mp 134–137 °C (lit. mp 136–137

°C)³⁴. ¹H NMR (acetone-*d*₆, 400 MHz) δ : 3.87 (s, 3H, OCH₃), 3.93 (s, 3H, OCH₃), 7.01 (d, 1H, H-5', *J* = 8.1 Hz), 7.33 (d, 1H, H-6', *J* = 8.5 Hz), 7.53 (br. s., 1H, H-2'), 7.65-7.69 (m, 2H, H-4, H-7), 7.75 (t, 1H, H-6, *J* = 7.2 Hz), 7.94 (d, 1H, H-5, *J* = 8.2 Hz), 8.04 (d, 1H, H-b, *J*_{trans} = 15.4 Hz), 8.15 (d, 1H, H-a, *J*_{trans} = 15.4 Hz), 8.52 (d, 1H, H-3, *J* = 5.5 Hz), 8.61 (d, 1H, H-8, *J* = 8.2 Hz). ¹³C NMR (acetone-*d*₆, 101 MHz) δ : 56.2 (*p*-O<u>C</u>H₃), 56.3 (*m*-O<u>C</u>H₃), 111.3 (C-2'), 112.8 (C-5'), 120.2 (C-4), 121.4 (C-a), 122.5 (C-6'), 125.6 (C-8), 127.5 (C-8a), 128.1 (C-7), 128.2 (C-5), 130.8 (C-6), 131.1 (C-1'), 136.6 (C-b), 137.8 (C-4a), 143.5 (C-3), 150.8 (C-3'), 151.4 (C-4'), 155.5 (C-1). Anal. Calcd for C₁₉H₁₇NO₂: C, 78.33; H, 5.88; N, 4.81; found: C, 78.28; H, 5.84; N, 4.75. ESI-MS **1e** in MeCN, *m/z*: calcd. 291.34; found 292.40 [**1e**+H]⁺.

(E,E)-2,5-bis(3,4-dimethoxystyryl)pyrazine (3a)

Recrystallized from methanol; orange solid, yield 20% (0.36 mmol, 142 mg), mp 222–224 °C (lit. mp 214 °C and 229 °C for two polymorphic forms)³⁵. ¹H NMR (DMSO-*d*₆, 600 MHz) δ : 3.79 (s, 6H, OCH₃), 3.84 (s, 6H, OCH₃), 7.00 (d, 2H, H-5', *J* = 8.3 Hz), 7.21 (d, 2H, H-6', *J* = 8.5 Hz), 7.31 (d, 2H, H-b, *J*_{trans} = 16.1 Hz), 7.35 (s, 2H, H-2'), 7.70 (d, 2H, H-a, *J*_{trans} = 16.0 Hz), 8.68 (s, 2H, H-2, H-5). ¹³C NMR (DMSO-*d*₆, 151 MHz) δ : 55.5 (O<u>C</u>H₃), 109.5 (C-5'), 111.7 (C-2'), 121.3 (C-6'), 122.3 (C-a), 129.1 (C-1'), 133.4 (C-b), 143.1 (C-3), 148.7 (C-3'), 149.0 (C-4'), 149.7 (C-2). Anal. Calcd for C₂₄H₂₄N₂O₄: C, 71.27; H, 5.98; N, 6.93; found: C, 71.30; H, 6.01; N, 6.98. ESI-MS **3a** in MeCN, *m/z*: calcd. 404.46; found 405.60 [**3a**+H]⁺.

(*E*,*E*)-4,6-bis(3,4-dimethoxystyryl)pyrimidine (**3b**)

Purified by column chromatography (SiO₂, benzene–ethylacetate 10:1, $R_f = 0.64$) and then recrystallized from methanol; yellow solid, yield 23% (0.41 mmol, 170 mg), mp 128–131 °C. ¹H NMR (acetone-*d*₆, 400 MHz) δ : 3.86 (s, 6H, OCH₃), 3.90 (s, 6H, OCH₃), 7.01 (d, 2H, H-5', *J* = 8.3 Hz), 7.12 (d, 2H, H-a, *J*_{trans} = 15.9 Hz), 7.23 (d, 2H, H-6', *J* = 8.2 Hz), 7.36 (s, 2H, H-2'), 7.48 (s, 1H, H-5), 7.92 (d, 2H, H-b, *J*_{trans} = 16.1 Hz), 8.97 (s, 1H, H-2). ¹³C NMR (acetone-*d*₆, 101 MHz) δ : 56.2 (-O<u>C</u>H₃), 111.0 (C-2'), 112.6 (C-5'), 116.7 (C-5), 122.7 (C-6'), 124.9 (C-a), 129.9 (C-3'), 137.5 (C-b), 150.7 (C-1'), 151.8 (C-4'), 159.4 (C-2), 164.0 (C-4). Anal. Calcd for C₂₄H₂₄N₂O₄: C, 71.27; H, 5.98; N, 6.93; found: C, 71.15; H, 5.95; N, 6.88. ESI-MS **3b** in MeCN, *m*/*z*: calcd. 404.46; found 405.50 [**3b**+H]⁺. HRMS (ESI-TOF) *m*/*z*: [**3b**–H]⁺ Calcd for C₂₄H₂₃N₂O₄ 403.1652; Found 403.1645.

General Procedure for the synthesis of the photocyclization products (2a, 2d, 2e, E-4a, E-4b)

A solution of the substrate (30.0 mg; **1a**: 0.12 mmol, **1d**: 0.12 mmol, **1e**: 0.10 mmol, **3a**: 0.07 mmol, **3b**: 0.07 mmol) in acetonitrile (1 mM) was irradiated in an immersion well with a Hg photoreactor

(125 W), and the reaction was monitored photometrically. When a maximum conversion was reached the solvent was removed in vacuum, the residue was washed with hexane and recrystallized from MeOH with addition of HClO₄ to give the photocyclization products **2a**, **2d**, **2e**, *E*-**4a**, *E*-**4b** as perchlorate salts.

8,9-dimethoxypyrazino[1,2-a]quinolin-11-ium perchlorate (2a)

Brownish solid, yield 39% (0.05 mmol, 17.0 mg), mp 280–282 °C (dest). ¹H NMR (CD₃CN, 600 MHz) δ : 4.11 (s, 3H, 3'-OCH₃), 4.22 (s, 3H, 4'-OCH₃), 7.79 (s, 1H, H-2'), 8.09 (s, 1H, H-5'), 8.34 (d, 1H, H-a, J = 8.7 Hz), 8.83 (d, 1H, H-b, J = 8.7 Hz), 9.00 (d, 1H, H-5, J = 4.8 Hz), 9.57 (d, 1H, H-6, J = 4.8 Hz), 9.79 (s, 1H, H-3). ¹³C NMR (CD₃CN, 151 MHz) δ : 57.8 (8-O<u>C</u>H₃), 58.4 (9-O<u>C</u>H₃), 99.2 (C-10), 109.7 (C-7), 120.0 (C-5), 123.2 (C-1), 126.9 (C-6a), 130.8 (C-10a), 135.2 (C-4a), 140.3 (C-6), 141.3 (C-2), 154.7 (C-4), 154.8 (C-8), 157.5 (C-9). Anal. Calcd for C₁₄H₁₃ClN₂O₆: C, 49.35; H, 3.85; N, 8.22; found: C, 49.31; H, 3.95; N, 8.30. HRMS (ESI-TOF) *m*/*z*: [**2a**]⁺ Calcd for C₁₄H₁₃N₂O₂ 241.0972; Found 241.0972.

8,9-dimethoxypyridazino[1,6-a]quinolin-11-ium perchlorate (2d)

Brown solid, yield 57% (0.07 mmol, 23.9 mg), mp 183–186 °C (dest). ¹H NMR (DMSO-*d*₆, 600 MHz) δ : 4.08 (s, 3H, 8-OCH₃), 4.15 (s, 3H, 9-OCH₃), 7.96 (s, 1H, H-7), 8.30 (dd, 1H, H-3, *J* = 8.8 Hz; 4.8 Hz), 8.39 (d, 1H, H-5, *J* = 8.8 Hz), 8.55 (s, 1H, H-10), 8.95 (d, 1H, H-6, *J* = 8.8 Hz), 9.05 (d, 1H, H-4, *J* = 8.8 Hz), 9.62 (d, 1H, H-2, *J* = 4.4 Hz). ¹³C NMR (DMSO-*d*₆, 151 MHz) δ : 56.6 (8-O<u>C</u>H₃), 56.8 (9-O<u>C</u>H₃), 98.5 (C-10), 108.0 (C-7), 120.5 (C-5), 124.5 (C-6a), 127.3 (C-3), 133.9 (C-10a), 135.8 (C-4), 138.7 (C-6), 140.5 (C-4a), 150.9 (C-2), 152.3 (C-8), 155.3 (C-9). Anal. Calcd for C₁₄H₁₃ClN₂O₆: C, 49.35; H, 3.85; N, 8.22; found: C, 49.59; H, 3.91; N, 8.38. HRMS (ESI-TOF) *m/z*: [**2d**]⁺ Calcd for C₁₄H₁₃N₂O₂ 241.0972; Found 241.0968.

2,3-dimethoxyisoquinolino[2,1-a]quinolinium perchlorate (2e)

Orange solid, yield 50% (0.05 mmol, 19.5 mg), mp 327–329 °C (dest). ¹H NMR (CD₃CN, 400 MHz) δ : 4.11 (s, 3H, 3-OCH₃), 4.25 (s, 3H, 2-OCH₃), 7.75 (s, 1H, H-4), 8.08 (s, 1H, H-1), 8.10 (t, 1H, *J* = 7.7 Hz, H-8), 8.16 (t, 1H, *J* = 7.0 Hz, H-9), 8.27 (d, 1H, *J* = 8.2 Hz, H-10), 8.31 (d, 1H, *J* = 7.7 Hz, H-11), 8.80 (d, 1H, *J* = 9.2 Hz, H-5), 9.01 (d, 1H, *J* = 8.2 Hz, H-7), 9.02 (d, 1H, *J* = 9.2 Hz, H-6), 9.52 (d, 1H, *J* = 7.7 Hz, H-12). ¹³C NMR (CD₃CN, 101 MHz) δ : 57.5 (3-O<u>C</u>H₃), 58.2 (2-O<u>C</u>H₃), 100.0 (C-1), 109.7 (C-4), 117.2 (C-6), 123.7 (C-11), 124.6 (C-4a), 127.1 (C-7, C-7a), 127.3 (C-12), 129.1 (C-10), 132.3 (C-8), 133.1 (C-10a), 133.3 (C-1a), 135.4 (C-9), 139.8 (C-5), 143.7 (C-6a), 153.0 (C-3), 157.2 (C-2). Anal. Calcd for C₁₉H₁₆ClNO₆: C, 58.55; H, 4.14; N, 3.59; found: C, 58.48; H, 4.09; N, 3.57. ESI-MS **2e** in MeCN, *m*/*z*: calcd. 290.34; found 290.10 [**2e**]⁺. HRMS (ESI-

TOF) m/z: [2e]⁺ Calcd for C₁₉H₁₆NO₂ 290.1176; Found 290.1172.

(E)-2-(3,4-dimethoxystyryl)-8,9-dimethoxypyrazino[1,2-a]quinolin-11-ium perchlorate (E-4a)

Red solid, yield 25% (0.02 mmol, 10.1 mg), mp 289–292 °C (dest). ¹H NMR (CD₃CN, 600 MHz) δ : 3.86 (s, 3H, 4'-OCH₃), 3.90 (s, 3H, 3'-OCH₃), 4.10 (s, 3H, 8-OCH₃), 4.26 (s, 3H, 9-OCH₃), 7.00 (d, 1H, H-5', J = 8.2 Hz), 7.27 (d, 1H, H-6', J = 8.2 Hz), 7.29 (s, 1H, H-2' Hz), 7.48 (d, 1H. H-b, $J_{\text{trans}} = 15.8$ Hz), 7.72 (s, 1H, H-7), 8.02 (d, 1H, H-a, $J_{\text{trans}} = 15.7$ Hz), 8.08 (s, 1H, H-10), 8.26 (d, 1H, H-5, J = 8.8 Hz), 8.65 (d, 1H, H-6, J = 8.7 Hz), 9.38 (s, 1H, H-1), 9.67 (s, 1H, H-4). ¹³C NMR (CD₃CN, 151 MHz) δ : 56.7 (3'-OCH₃), 56.8 (4'-OCH₃), 58.0 (8-OCH₃), 58.7 (9-OCH₃), 99.4 (C-10), 110.0 (C-7), 111.2 (C-2'), 113.0 (C-5'), 120.5 (C-1), 120.6 (C-b), 121.1 (C-a), 121.4 (C-5), 123.3 (C-6'), 127.3 (C-6a), 129.8 (C-1'), 130.9 (C-10a) 133.5 (C-4a), 139.0 (C-6), 150.2 (C-2), 150.8 (C-3'), 152.4 (C-4'), 155.0 (C-4), 155.9 (C-8), 157.5 (C-9). Anal. Calcd for C₂₄H₂₃ClN₂O₈: C, 57.32; H, 4.61; N, 5.57; found: C, 57.38; H, 4.59; N, 5.59. HRMS (ESI-TOF) m/z: [*E*-4a]⁺ Calcd for C₂₄H₂₃N₂O₄ 403.1652; Found 403.1656.

(E)-2-(3,4-dimethoxystyryl)-8,9-dimethoxypyrimido[1,6-a]quinolin-11-ium perchlorate (E-4b)

Red solid, yield 51% (0.04 mmol, 18.3 mg), mp 340–345 °C (dest). ¹H NMR (DMSO-*d*₆, 600 MHz) δ : 3.85 (s, 3H, 3'-OCH₃), 3.89 (s, 3H, 4'-OCH₃), 4.03 (s, 3H, 8-OCH₃), 4.17 (s, 3H, 9-OCH₃), 7.09 (d, 1H, H-5', *J* = 8.2 Hz), 7.40 (d, 1H, H-6', *J* = 8.0 Hz), 7.49 (s, 1H, H-2'), 7.56 (d, 1H. H-a, *J*_{trans} = 15.5 Hz), 7.88 (s, 1H, H-7), 8.10 (d, 1H, H-5, *J* = 8.2 Hz), 8.13 (d, 1H, H-b, *J*_{trans} = 15.5 Hz), 8.34 (s, 1H, H-4), 8.54 (s, 1H, H-10), 8.70 (d, 1H, H-6, *J* = 8.2 Hz), 10.84 (s, 1H, H-1). ¹³C NMR (DMSO-*d*₆, 151 MHz) δ : 55.7 (3'-O<u>C</u>H₃, 4'-O<u>C</u>H₃), 56.4 (8-O<u>C</u>H₃), 57.4 (9-O<u>C</u>H₃), 99.2 (C-10), 109.4 (C-7), 110.4 (C-2'), 111.8 (C-5'), 115.9 (C-4), 119.6 (C-5), 121.7 (C-a), 122.4 (C-6a), 123.6 (C-6'), 127.9 (C-1'), 129.3 (C-6), 139.9 (C-10a) 141.2 (C-b), 144.4 (C-4a, C-1), 149.2 (C-3'), 151.0 (C-8), 151.4 (C-4'), 154.6 (C-9), 156.3 (C-3). Anal. Calcd for C₂₄H₂₃ClN₂O₈: C, 57.32; H, 4.61; N, 5.57; found: C, 57.40; H, 4.64; N, 5.60. HRMS (ESI-TOF) *m*/*z*: [*E*-**4b**]⁺ Calcd for C₂₄H₂₃N₂O₄ 403.1652; Found 403.1648.

Photochemical studies

The solutions of **1a–1e** and **3a**, **3b** ($c = 20 \mu$ M) in acetonitrile or water were irradiated either with filtered light ($\lambda > 295$ nm) or with full light of a high pressure mercury vapor lamp (120 W). Individual lines of the lamp emission spectrum ($\lambda = 365$, 405 nm) were isolated with glass filters. All photoreactions were carried out in air-saturated solutions under stirring. The analysis of the

reaction progress was performed by absorption spectroscopy.

Associated content

Supporting Information

¹H and ¹³C NMR spectra, HRMS data for all new compounds, optical spectroscopy and photochemical studies data. This material is available free of charge via the Internet at <u>http://pubs.acs.org</u>.

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Notes

The authors declare no competing financial interest.

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