



AZONIA DERIVATIVES OF THE γ -CARBOLINE SYSTEM. A NEW CLASS OF DNA INTERCALATORS

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Abstract: 1-Methyl- γ -carboline derivatives were transformed into the corresponding N-aminoazinium salts, which were condensed with 1,2-dicarbonyl compounds (Westphal reaction) to afford azonia derivatives with a bridgehead quaternary nitrogen atom. Some of them show DNA intercalating properties. Copyright © 1996 Elsevier Science Ltd

Since DNA is an important cellular receptor, most anticancer agents exert their effects through binding to it. Intercalation is one of these modes of interaction, in which a molecule is inserted between two adjacent base pairs causing lengthening, stiffening and unwinding of the helix.¹ Planar polyheteroaromatic cations are particularly well adapted for intercalation between nucleic acid base pairs. While the positive charge in the chromophore seems to be essential for increasing their DNA affinity,² their orientation in the intercalative process is highly dependent on the electrostatic component of the stacking interaction. Favourable dipolar interactions between the chromophore and the base pairs contribute to explain the observed selectivity for CpG or TpA steps.³ For these reasons, alterations of the dipole moment of the chromophore is an important element to be considered when DNA bis-intercalators are being designed.

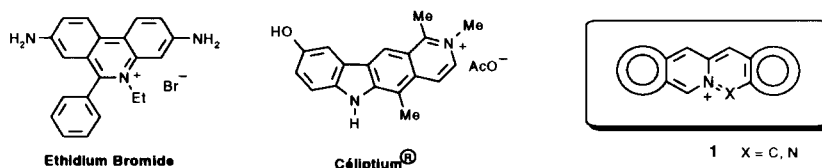
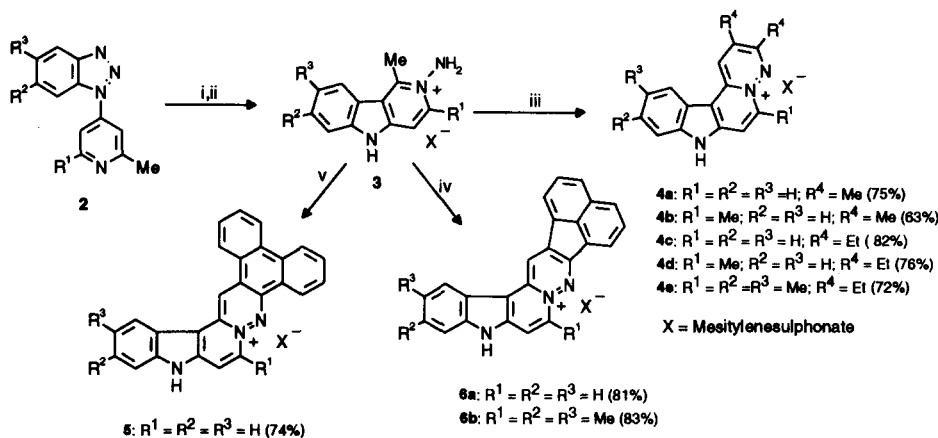


Figure 1

The above considerations led us to explore the DNA binding properties of type 1 azonia derivatives, incorporating as chromophore a polyheteroaromatic cation, in which the positive charge is not introduced by N-alkylation of the azaheterocycle, such is the case of well known DNA intercalators, such as ethidium bromide and some antitumour compounds of the ellipticine group (e. g. Céliptium®), but by the presence of a quaternary bridgehead nitrogen.⁴ In this communication we wish to report our initial results on the transformation of the γ -carboline system into tetracyclic and heptacyclic azonia derivatives, along with the DNA binding properties and charge distribution of a representative compound.

The 1-methylcarbolines were prepared by thermal or microwave decomposition of the corresponding pyridylbenzotriazoles **2** (Graebe-Ullmann reaction)⁵ as previously described.⁶ These derivatives were easily transformed into the salts **3** by amination with (O-mesitylenesulfonyl)hydroxylamine (MSH) in CH_2Cl_2 at room

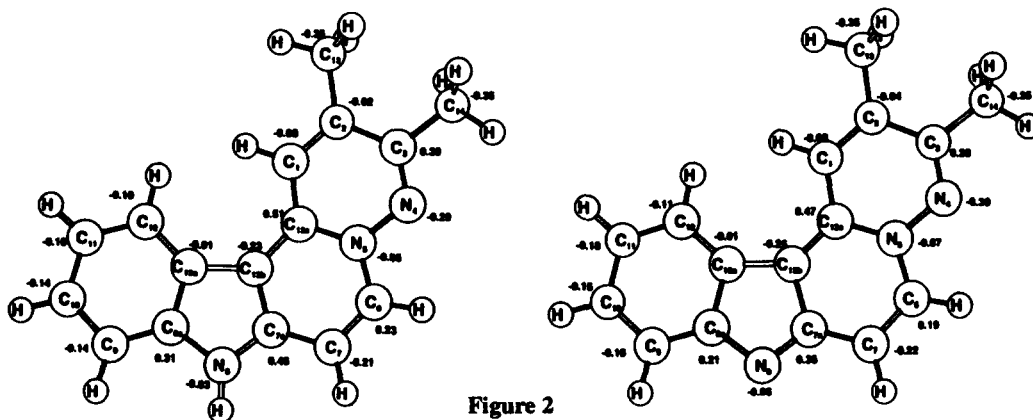
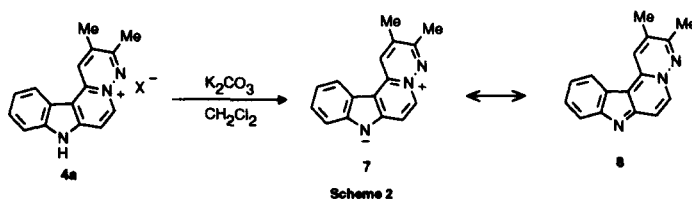
temperature. The reaction of **3** with various 1,2-dicarbonyl compounds (Westphal condensation)⁷ afforded several types of azonia derivatives (Scheme 1). Different reaction conditions were necessary for the condensation to be successful. Thus, to obtain the polycyclic cations **5** and **6** from the reaction of **3** with 1,2-acenaphthoquinone and 9,10-phenanthrenequinone, the condensation had to be carried out in sodium acetate/acetone whereas in the reaction of **3** with 2,3-butanedione or 3,4-hexanedione, **4a-d** could only be obtained if triethylamine/MeOH/ acetone was employed. The derivative **4e** required the use of dibutylamine/EtOH.



Scheme 1. i) 150–200 °C or MW, 160 watts; ii) MSH / CH₂Cl₂; iii) R⁴COCOR⁴, Et₃N / EtOH-acetone or Bu₂NH / EtOH; iv) 1,2-Acenaphthoquinone, NaOAc / acetone; v) 9,10-Phenanthrenequinone, NaOAc / acetone

Several techniques⁸ were used to evaluate the DNA binding properties of azonia derivatives **4–6**. Whereas the addition of compounds **5** and **6** to a sample of calf thymus DNA in TRIS-HCl buffer ([NaCl]=0.05 M, pH=7.5) induced hyperchromicity, probably due to dipole-dipole interactions, the cationic derivatives **4** gave hypochromic and bathochromic shifts in their UV spectra (see Table) accounted for by their binding to DNA.⁹ The unique binding mode with DNA was established by the appearance of isosbestic points for this series of compounds. From the spectral changes, nonlinear Scatchard binding isotherms were generated. The McGhee-Von Hippel¹⁰ treatment allowed the values of *K* (DNA affinity constant) and *n* (number of base-pairs occluded by each bound ligand molecule) to be determined. Intercalation was proved by measuring the viscosity of sonicated calf thymus DNA in the absence and presence of increasing concentrations of **4**. The slope¹¹ (*m*) obtained by plotting the relative increase in contour length (*L/L₀*) against the ligand binding ratio (*r*) gave values within the range of well known intercalators such as ethidium bromide,¹² used here as reference. In the Table are shown the values for each compound¹³ along with their cellular toxicity against colon carcinoma HT-29 cells.¹⁴ Although the number of analysed compounds is small, the presence and position of alkyl groups appears to have only a small effect on *K* values. EC₅₀ values indicate that inhibitory activity is enhanced by alkyl substitution, with the tetrasubstituted derivative **4e** being the most potent of this series whilst the monosubstituted derivatives **4a** and **4c** were significantly less potent.

To be used in the comparison with other DNA intercalators, the optimized geometries and atomic charges of the cation **4a** and the betaine **7** were determined using *ab initio* theoretical techniques¹⁵ (HF/6-31G**// HF/3-21G) and are presented in Figure 2. Calculations show that both are planar structures, determined as minima by frequency calculations. As a result, the easily formed and highly stable compound **7** seems better represented by the resonance hybrid **7-8** (Scheme 2).

**Table.** DNA Binding Properties of Azonia Derivatives 4

| Compd. No | λ_{free} | λ_{bound} | ϵ_{free} | ϵ_{bound} | λ_{IP} | $10^5 K$ | n | m | EC_{50} [μM] |
|-------------|------------------|-------------------|-------------------|--------------------|----------------|----------|-------------------|------|-----------------------|
| 4a | 354 | 364 | 7637 | 4845 | 388 | 1.10 | 4.75 | 1.16 | >10 |
| 4b | 358 | 368 | 7187 | 4461 | 388 | 2.39 | 2.84 | 0.97 | 2.0 |
| 4c | 352 | 362 | 7451 | 5056 | 390 | 1.27 | 3.98 | 0.95 | >10 |
| 4d | 356 | 366 | 7036 | 4651 | 388 | 2.62 | 3.56 | 0.87 | 2.1 |
| 4e | 368 | 384 | 5887 | 3547 | 398 | 1.45 | 3.00 | 0.77 | 0.5 |
| EtBr | -- | -- | -- | -- | -- | 12.0 | 2.00 ^a | 1.11 | -- |

K : affinity constant for DNA (M^{-1}). n : Number of base-pairs occluded by each bound ligand molecule. m : Helix extension slope measured by sonicated DNA viscosimetric lengthening; values within 0.09-0.13 error. λ_{free} and λ_{bound} : Wavelength of maximum absorption for free and DNA bound compounds. ϵ_{free} and ϵ_{bound} : Extinction coefficients for free and DNA bound compounds. λ_{IP} : Wavelength of isosbestic point. EC_{50} : Concentration of drug required to inhibit 50% of the cell growth after 72 h, of colon carcinoma HT-29. *In vitro* activity was measured as indicated in ref. 14 (doxorubicin, EC_{50} =2.5). EtBr: Ethidium bromide.

^a Value according to neighbor exclusion model (ref. 16)

In summary, this work presents a series of new pyridazino[1',6':1,2]pyrido[4,3-*b*]indol-5-ium salts which shown both batho- and hypochromicity in their UV spectra in the presence of DNA and increased viscosity in sonicated DNA. Transformation into bis-salts is now under way in order to improve on the observed affinity constants in these new DNA intercalators.

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