Dyes and Pigments 125 (2016) 241–248

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Studies of the binding interactions of dicationic styrylimidazo[1,2-a] pyridinium dyes with duplex and quadruplex DNA

Zeynel Seferoğlu [a, b, *], Mohamed M.A. Mahmoud [b], Heiko Ihmels [b]

[a] Gazi University, Department of Chemistry, 06500 Teknikokullar, Ankara, Turkey
[b] University of Siegen, Organic Chemistry II, Adolf-Reichwein-Str. 2, D-57068 Siegen, Germany

ARTICLE INFO

Article history:
Received 22 September 2015
Received in revised form
8 October 2015
Accepted 10 October 2015
Available online 28 October 2015

Keywords:
Double cationic styryl dyes
Imidazo[1,2-a]pyridines
Nucleic acids
ct DNA
Quadruplex DNA
DNA-ligand interactions

ABSTRACT

The dicationic styrylimidazo[1,2-a]pyridinium dyes were synthesized and characterized using ^{1}H NMR, ^{13}C NMR, mass spectral techniques and elemental analysis. In addition, their photophysical and DNA-binding properties were investigated. The dyes absorb in the UV region with maxima at 342–358 nm, and the emission spectra of these derivatives show a maximum at 409–475 nm with moderate Stokes shift. It was shown with spectrophotometric, spectrofluorimetric and viscometric titrations as well as with Circular dichroism spectroscopic analysis that the dicationic styrylimidazo[1,2-a]pyridinium dyes associate as groove binders with double-stranded DNA with binding constants in the range of 10^{5} –10^{6} M^{-1}. Preliminary experiments revealed that these ligands also bind with reasonable affinity to telomeric quadruplex DNA.

1. Introduction

The identification and development of DNA-binding ligands is still a highly topical research field [1]. Firstly, such ligands serve as useful and versatile probe for the investigation of fundamental factors that govern the interactions between biomacromolecular host molecules and small guest ligands. More importantly, DNA binders raise particular interest as a class of latent bioactive compounds, specifically with a high potential as DNA-targeting drugs, because they may interfere with the biological activity of the nucleic acid. In this context, several classes of DNA-binding compounds have been established whose investigation contributed to the development of DNA-targeting drugs. Notably, in addition to regular double-stranded DNA, non canonical forms such as triplex or quadruplex DNA [2] have been suggested as promising targets in chemotherapy, because the application of triplex- or quadruplex selective DNA-targeting drugs has been proposed to exhibit less adverse effects on healthy tissue [3].

Among the established DNA-binders are the styryl dyes [4]. This class of compounds is well known for its application as functional dyes that are employed as fluorescent probes, materials with non-linear optical properties (NLO), organic light-emitting dieodes (OLEDs), laser dyes, etc. [5]. In particular, *ionic* styryl dyes are useful DNA binders that have a number of favorable properties. They are fluorescent, have high photostability, and by variation of the substitution pattern they may cover an absorption and emission spectrum from the UV to near infrared region. Moreover, several styryl dyes have been shown to exhibit a strong enhancement of fluorescence intensity on association with nucleic acids, thus operating as efficient DNA-sensitive fluorescent light up probes [6,7]. In our search for novel styryl-type functional dyes we have observed that styrylimidazo[1,2-a]pyridine derivatives exhibit some favorable properties [8]. Namely, these styryl dyes are synthetically easily available, they exhibit absorption and emission properties in the UV or visible region with moderate Stokes shifts, and their emission properties depend on the surrounding medium leading to fluorosolvatochromism and fluoroacidochromism. These results tempted us to explore the propensity of these dyes to operate as DNA binders and to monitor the complex formation by means of absorption and emission spectroscopy. However, due to their limited solubility in water these compounds are rather unsuitable for studies in biologically relevant media. To overcome this obstacle we intended to introduce a permanent charge by

* Corresponding author. Gazi University, Department of Chemistry, 06500 Teknikokullar, Ankara, Turkey. Tel.: +90 312 2021525; fax: +90 312 2122279.
E-mail address: znseferoglu@gazi.edu.tr (Z. Seferoğlu).

http://dx.doi.org/10.1016/j.dyepig.2015.10.008
0143-7208/

alkylation of the dye. This modification should lead to sufficient water solubility of the dye. Moreover, it is also well known that the cationic nature of a DNA binder will increase its affinity to DNA, mainly by inducing a counter-ion release from the DNA backbone [1]. Herein, we will show that the reaction of alkylaminostyrylimidazo[1,2-a]pyridines with methyl iodide leads to a novel class of dicationic styryl dyes that bind to the minor groove of double-stranded DNA. Since it has been demonstrated already that styryl dyes also bind to quadruplex DNA and that these ligands may also be used as optical probes for quadruplex DNA [9], we additionally investigated the interactions of the dicationic alkylaminostyrylimidazo[1,2-a]pyridine derivatives with quadruplex DNA and present these preliminary results as well.

2. Experimental

2.1. Materials and equipments

All reagents and solvents were obtained from commercial sources and used as received. Purified water with resistivity $\geq$18 MΩ cm^{-1} was used for preparation of buffer solutions and spectrometric measurements. The oligonucleotides **22AG** [d($AG_3T_2AG_3T_2AG_3T_2AG_3$)] and **F21T** [fluo-$G_3T_2AG_3T_2AG_3T_2AG_3$-TAMRA] were purchased (HPLC purified) from Metabion Int. AG (Planegg/Martinsried). BPE buffer (6.0 mM Na_2HPO_4, 2.0 mM NaH_2PO_4, 1.0 mM Na_2EDTA; total Na^+ concentration 16.0 mM; pH 7.0) was used for DNA titrations study. K-phosphate buffer (25 mM K_2HPO_4, 70 mM KCl; adjusted with 25 mM KH_2PO_4 to pH 7.0) was used for CD spectroscopic measurements of the quadruplex DNA. Na-cacodylate buffer (10 mM $Na(CH_3)_2AsO_2 \cdot 3H_2O$, 10 mM KCl, 100 mM LiCl; pH 7.2–7.3) was used for fluorimetric thermal denaturation studies. Melting points were determined with a Büchi 510 K melting point apparatus and are not corrected. Mass spectra were taken on a Waters Micromass ZQ (Waters Corporation, Milford, MA) connected with Waters Alliance HPLC using ESI(+) method with C-18 column. NMR spectra were measured on Bruker Avance 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) spectrometers at 293 K; chemical shifts are given in ppm (δ) relative to TMS (δ = 0.00 ppm). Spin–spin coupling constants (J) were determined with accuracy of 0.1 Hz. Signals are abbreviated as follows: singlet, s; doublet, d; doublet–doublet, dd; triplet, t; multiplet, m. Elemental microanalysis of the compounds was performed with a HEKAtech EuroEA combustion analyzer by Mr. H. Bodenstedt and Rochus Breuer (Organische Chemie I, Universität Siegen).

2.2. Synthesis

2.2.1. General procedure for the synthesis of dicationic styrylimidazo[1,2-a]pyridine (**2a**–**f**)

A mixture of 1.0 mmol of corresponding neutral styrylimidazo [1,2-a]pyridine and 2.5 mmol of methyl iodide in 10 ml of acetonitrile was stirred under reflux for 3–10 h under nitrogen atmosphere. Diethyl ether was added and the mixture was filtered off. Precipitate was washed thoroughly several times with acetone, ethyl acetate and used without additional purification.

(*E*)-1-methyl-7-{4-(1-methylpiperidin-1-ium-1-yl)styryl}-2-phenylimidazo[1,2-a]pyridin-1-ium iodide (2a). 0.59 g, Yield 89%. Mp 298–299 °C, color: yellow powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.60 (m, 4H, piperidinyl 2 × CH_2), 1.95 (m, 2H, piperidinyl CH_2), 3.46 (s, 3H), 3.85–3.90 (m, 2H, piperidinyl CH_2 adjacent to the cationic nitrogen), 3.97 (s, 3H), 4.40–4.43 (m, 2H, piperidinyl CH_2 adjacent to the cationic nitrogen), 7.69 (m, 3H), 7.75 (m, 3H), 7.93 (m, 2H), 7.98 (d, 2H, J = 8.7 Hz), 8.05–8.07 (d, 2H, J = 9.1 Hz), 8.47 (br s, 1H), 8.57 (s, 1H), 8.94 (d, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 20.6 (3 × CH_2), 32.2 (CH_3), 57.1 (CH_3), 62.6 (2 × CH_2), 108.5, 113.3, 115.7, 122.8, 125.7, 127.8, 129.3, 129.4, 129.7, 130.0, 131.0, 133.6, 137.6, 138.1, 140.7, 141.9; LC-MS (ESI$^+$): m/z (%) = 204.8 (100%) $[M-2I]^{2+}$, 536.3 $[M-127\ (-I)]^{2+}$. Anal. Calcd for $C_{28}H_{31}I_2N_3$: C, 50.70; H, 4.71; N, 6.33%. Found: C, 50.66; H, 4.68; N, 6.24%.

(*E*)-2-(4-methoxyphenyl)-1-methyl-7-(4-(1-methylpiperidin-1-ium-1-yl)styryl)imidazo[1,2-a]pyridin-1-ium iodide (2b). 0.63 g, Yield 91%. Mp 260–261 °C, color: dark yellow powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.60 (m, 4H, piperidinyl 2 × CH_2), 1.95 (m, 2H, piperidinyl CH_2), 3.46 (s, 3H), 3.88 (s, 3H for $-CH_3$ attached imidazo $-N^+$ and m, 2H, piperidinyl CH_2 adjacent to the cationic nitrogen), 3.98 (s, 3H), 4.42 (m, 2H, piperidinyl CH_2 adjacent to the cationic nitrogen), 7.21 (d, 2H, J = 8.8 Hz), 7.68 (d, 2H), 7.70 (d, 1H, J = 16.1 Hz), 7.90 (m, 2H), 7.98 (d, 2H, J = 8.7 Hz), 8.06 (d, 2H, J = 9.1 Hz), 8.46 (br s1H), 8.50 (s, 1H), 8.92 (d, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 20.6 (3 × CH_2), 32.2 (CH_3), 55.4 (OCH_3), 57.3 (CH_3), 62.6 (2 × CH_2), 108.4, 112.7, 115.1, 115.6, 117.6, 122.8, 127.8, 129.1, 129.2, 131.6, 131.5, 137.6, 138.1, 140.5, 141.6, 161.3; LC-MS (ESI$^+$): m/z (%) = 219.8 (100%) $[M-2I]^{2+}$. Anal. Calcd for $C_{29}H_{33}I_2N_3O$: C, 50.23; H, 4.80; N, 6.06%. Found: C, 49.96; H, 4.72; N, 5.98%.

(*E*)-7-(4-(diethyl(methyl)ammonio)styryl)-1-methyl-2-phenylimidazo[1,2-a]pyridin-1-ium iodide (2c). 0.57 g, Yield 87%. Mp 232–233 °C, color: light yellow powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.04 (t, 6H), 3.52 (s, 3H), 3.87 (s, 3H for $-CH_3$ attached imidazo $-N^+$ and m, 2H, ethyl CH_2 adjacent to the cationic nitrogen), 3.98 (s, 3H), 4.07 (m, 2H, ethyl CH_2 adjacent to the cationic nitrogen), 7.67 (m, 4H), 7.73 (m, 2H), 7.90–7.98 (m, 6H), 8.44 (br s, 1H), 8.57 (s, 1H), 8.94 (d, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.67 (2 × CH_3), 32.7 (CH_3), 63.7 (2 × CH_2), 108.4, 113.3, 115.6, 123.3, 125.7, 127.9, 129.5, 129.7, 129.9, 130.1, 130.8, 131.4, 133.6, 137.7, 138.1, 141.5, 141.9; LC-MS (ESI$^+$): m/z (%) = 198.5 (100%)$[M-2I]^{2+}$. Anal. Calcd for $C_{27}H_{31}I_2N_3$: C, 49.79; H, 4.80; N, 6.45%. Found: C, 49.66; H, 4.74; N, 6.28%.

(*E*)-7-(4-(diethyl(methyl)ammonio)styryl)-2-(4-methoxyphenyl)-1-methylimidazo[1,2-a]pyridin-1-ium iodide (2d). 0.57 g, Yield 94%. Mp 198–199 °C, color: light orange powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.02–1.06 (t, 6H), 3.52 (s, 3H), 3.61–3.88 (s, 3H for $-CH_3$ attached imidazo $-N^+$ and m, 2H, ethyl CH_2 adjacent to the cationic nitrogen), 3.97 (s, 3H), 4.07 (m, 2H, ethyl CH_2 adjacent to the cationic nitrogen), 7.21 (d, 2H, J = 8.8 Hz), 7.68 (d,2H), 7.70 (d, 1H, J = 16.1 Hz), 7.89–7.98 (m, 6H), 8.43 (br s, 1H), 8.50 (s, 1H), 8.92 (d, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.67 (2 × CH_3), 32.6 (CH_3), 55.9 (OCH_3), 63.7 (2 × CH_2), 108.4, 112.7, 115.2, 115.5, 117.6, 123.3, 127.9, 129.0, 129.2, 131.6, 133.5, 137.7, 138.1, 141.4, 141.6, 161.3; LC-MS (ESI$^+$): m/z (%) = 213.6 (100%) $[M-2I]^{2+}$. Anal. Calcd for $C_{28}H_{33}I_2N_3O$: C, 49.36; H, 4.88; N, 6.17%. Found: C, 49.40; H, 4.75; N, 6.24%.

(*E*)-1-methyl-2-phenyl-7-(4-(trimethylammonio)styryl)imidazo[1,2-a]pyridin-1-ium iodide (2e). 0.57 g, Yield 91%. Mp 299–301 °C, color: light orange powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.64 (s, 9H, $+N(CH_3)_3$), 3.95 (s, 3H), 7.67 (m, 3H), 7.75 (m, 3H), 7.91–7.97 (m, 4H), 8.08–8.10 (d, 2H, J = 9.1 Hz), 8.47 (br s 1H), 8.58 (s, 1H), 8.94 (d,1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 32.7 (CH_3), 56.7 (3 × CH_3), 108.4, 113.3, 115.7, 121.7, 125.7, 127.7, 128.8, 129.3, 129.7, 130.0, 131.0, 133.6, 137.8, 138.1, 140.7, 141.9, 147.5; LC-MS (ESI$^+$): m/z (%) = 184.6 (100%) $[M-2I]^{2+}$. Anal. Calcd for $C_{25}H_{27}I_2N_3$: C, 48.17; H, 4.37; N, 6.74%. Found: C, 48.03; H, 4.35; N, 6.53%.

(*E*)-2-(4-methoxyphenyl)-1-methyl-7-(4-(trimethylammonio)styryl)imidazo[1,2-a]pyridin-1-ium iodide (2f). 0.59 g, Yield 90%. Mp 277–278 °C, color: yellow powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.61 (s, 9H, $+N(CH_3)_3$), 3.83 (s, 3H), 3.94 (s, 3H), 7.21 (d, 2H, J = 8.5 Hz), 7.68 (m, 3H), 7.88–7.95 (m, 4H), 8.08 (d, 2H, J = 8.6 Hz), 8.43 (br s 1H), 8.50 (s, 1H), 8.91 (d,1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 32.6 (CH_3), 55.9 (OCH_3), 56.9 (3 × CH_3), 106.2, 106.9, 112.7, 115.2, 115.6, 117.6, 121.6, 127.8, 128.8, 129.2, 131.6, 133.5, 137.8,