Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 5537–5542
Bioorganic & Medicinal Chemistry

New amino and acetamido monomethine cyanine dyes for the detection of DNA in agarose gels

Reda M. El-Shishtawy,[a,†] Cecília R. Santos,[b] Isabel Gonçalves,[b] Helena Marcelino[b] and Paulo Almeida[a,*]

[a]*Departamento de Química e Unidade de I&D de Materiais Têxteis e Papeleiros, Universidade da Beira Interior, 6201-001 Covilhã, Portugal*

[b]*CICS—Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior, 6201-001 Covilhã, Portugal*

Received 30 December 2006; revised 11 May 2007; accepted 18 May 2007
Available online 23 May 2007

Abstract—Some new monomethine cyanine dyes derived from quinoline and benzothiazole have been prepared and characterized by ^{1}H and ^{13}C NMR, FTIR, FABHRMS, and visible spectroscopy. The dyes containing amino and acetamido groups were conveniently synthesized by the condensation of two *p*-toluenesulfonate heterocyclic quaternary salts and were obtained in the forms of iodide, bromide, and tosylate counteranions. These dyes were compared to ethidium bromide as stains for DNA in electrophoretic gels. The overall results obtained for the sensitivity of these dyes suggest the suitability of acetamido moiety over the amine one and bromide as the counteranion when compared with iodide and tosylate, with a similar capacity of DNA detection in relation to the ethidium bromide stain over the concentration range of 1–3 ng.

1. Introduction

Cyanine dyes consist of two heterocyclic nuclei containing nitrogen centers linked through an odd number of methine bonds, which are normally in the *trans* configuration, in such a way that resonance occurs through the conjugated system between the tertiary and quaternary nitrogen atoms. The carbon atoms of the methine groups may be attached to groups other than hydrogen, or may be part of carbocyclic or heterocyclic ring systems. Due to their excellent staining properties, cyanine dyes have been utilized in biological applications such as flow cytometry,[1] DNA fragment sizing,[2] reporter groups in hybridization probes,[3–5] single DNA molecule fluorescence microscopy,[6] gel staining,[7] and in real-time PCR.[8–10] Thus, monomethine cyanine dyes are widely used as fluorescent probes for the detection of nucleic acids in fluorescent microscopy, gel electrophoresis, etc.[11,12]

The main advantage of these dyes is that they do not show fluorescence of their own, but become strongly fluorescent after complexing with DNA. Two possible types of interaction with nucleic acids, intercalation and electrostatic binding, have been proposed.[7] The huge enhancement in fluorescence upon binding to DNA is believed to originate from the loss of mobility around the methine bridge between the two heterocyclic moieties.[13]

DNA is commonly detected, in agarose gel electrophoresis with ethidium bromide (EtBr) staining, as fluorescent bands detected using UV irradiation.[14] However, this method has some disadvantages due to the strong mutagenic effects of EtBr that complicate handling and disposal of both the chemical and the stained gels and electrophoresis buffers.[15–17] These undesirable properties of EtBr stimulated many scientists to search for new more safe fluorescent monomethine cyanine probes envisioning the visualization of nucleic acids.[18–22] The purpose of the present research was the preparation of some new monomethine cyanine dyes and to explore

Keywords: Monomethine cyanine dyes; Ethidium bromide; DNA; Gel electrophoresis.
* Corresponding author. Tel.: +351 75 319761; fax: +351 75 319730; e-mail addresses: elshishtawy@hotmail.com; csantos@fcsaude.ubi.pt; paulo.almeida@ubi.pt
† On leave from National Research Centre, Textile Research Division, El-Behouth St. Dokki, Cairo, PO 12622, Egypt.

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.05.043

their possible use for DNA visualization in agarose electrophoretic gels.

2. Results and discussion

2.1. Synthesis

It has been reported that the exocyclic amines of EtBr are suitable for DNA binding, since the partial positive charges carried by the amino groups are effective for mediating electrostatic attraction and hydrogen bonding interactions with DNA's phosphate groups.[23–25] In addition to the reported mutagenicity of EtBr,[15–17] it is worth noting that the structure of EtBr (Fig. 1) reveals the presence of a benzidine group that renders the dye some carcinogenicity.[26,27] Therefore, new monomethine cyanine dyes containing amino and acetamido in different forms of counterions (Fig. 1) were prepared envisioning their use for DNA visualization in agarose electrophoretic gels as an alternative to the classical EtBr stain.

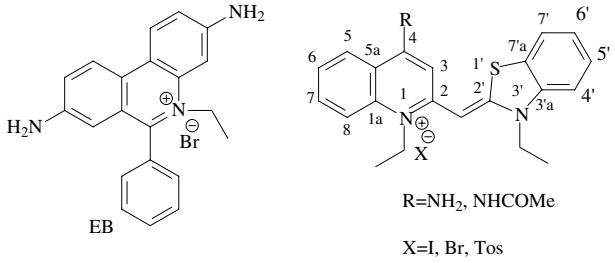
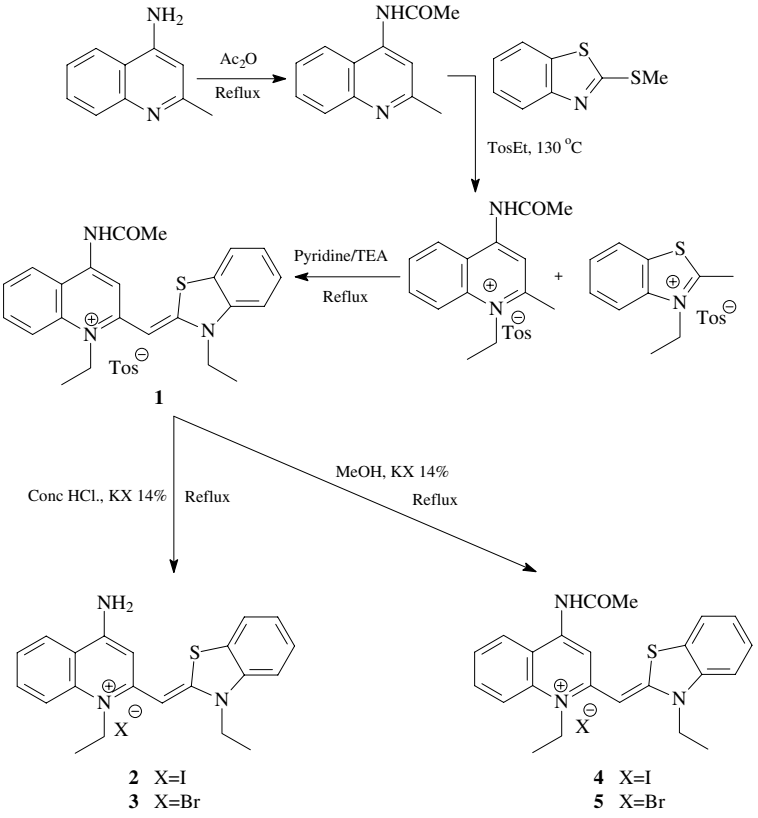


Figure 1. Ethidium bromide and monomethine cyanine dyes used.

Monomethine cyanine dyes are generally synthesized by the reaction of two heterocyclic quaternary salts, one bearing a methyl group and the other a thioalkyl as a good nucleofugic leaving group, both in the 2-position in relation to the heteroaromatic ammonium salt. Deprotonation of the first quaternary salt by a tertiary aliphatic amine gives the methylene derivative base, which acts as the nucleophilic reagent.[28] The synthetic route of monomethine cyanines **1**–**5** is shown in Scheme 1. Aminoquinaldine was refluxed with acetic anhydride for 2 h to afford 4-acetamido-2-methylquinoline in 87% yield. N-alkylation of a mixture (1:1 molar ratio) of 4-acetamido-2-methylquinoline and 2-methylthiobenzothiazole with ethyl *p*-toluenesulfonate at 130 °C afforded the corresponding tosylate salts as a viscous material, which was further used in the synthesis of dye **1** without purification. Treatment of the tosylate salts with a pyridine/triethylamine mixture (3:1 by volume) at 100–105 °C gave dye **1** as orange crystals in 39% yield. Generally, amino dyes like **2** and **3** are usually obtained from their corresponding acetyl derivative (dye **1**) in two-step processes, that is, acid hydrolysis followed by counteranion exchange. In the present work, dye **1** afforded dyes **2** and **3** quantitatively in one-pot process by undergoing acid hydrolysis in the presence of an excess of 14% potassium iodide and potassium bromide aqueous solutions, respectively. Counteranion



Scheme 1. Synthesis of monomethine cyanine dyes **1**–**5**.

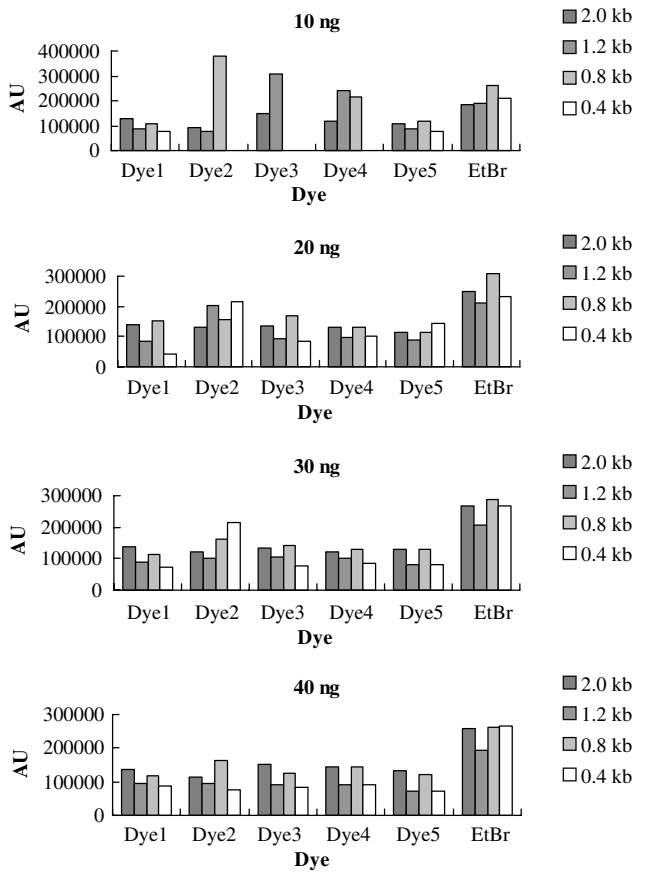


Figure 2. Agarose gel electrophoresis of DNA (10–40 ng) stained with monomethine cyanine dyes **1**–**5** and EtBr. Gels were exposed to UV light at 365 nm for 520 ms.

exchange of dye **1** was made with an excess of 14% potassium iodide and potassium bromide aqueous solutions to give the dyes **4** and **5**, respectively. The structures of the new dyes **1**–**5** were evidenced by their ^{1}H NMR, C^{13} NMR, FTIR, Vis, and FABHRMS data.

2.2. DNA visualization

The five asymmetric monomethine cyanine dyes presented in this work were assessed as DNA stains in comparison to EtBr. A direct comparison of the sensitivities between cyanine dyes' staining and EtBr staining was performed using serial dilutions of a commercial DNA marker as shown in Figure 2. The visualization of DNA patterns indicates that the relative sensitivity of cyanine dyes' staining compared with EtBr staining is decreasing with increasing the concentrations of DNA from 10 to 40 ng. The behavior of these dyes was different in relation to the size of DNA fragment analyzed. For the 2.0 kb DNA fragment, EtBr showed higher sensitivity than any of the other dyes ($p < 0.001$) at all DNA concentrations. For the 1.2 kb fragment, dyes **3** and **4** showed higher sensitivity than that of EtBr ($p < 0.001$) at 10 ng, and at 20 ng the sensitivity of dye **2** approached that of EtBr. For the 0.8 kb fragment, dye **2** sensitivity was higher than that of EtBr at 10 ng and the sensitivity of dye **4** was similar, whereas in the case of fragment 0.4 kb, only dye **2** approached the sensitivity of EtBr at 20 and 30 ng DNA concentrations.

In order to further explore the potential sensitivity of the dyes at lower concentrations of DNA, agarose gels were additionally run using 1 and 3 ng of DNA. Figure 3 shows the selected images of dyes **2** and **4** in comparison with the image of EtBr using 1 and 3 ng DNA at different illumination times. UV illumination of the gels of EtBr and dyes **2** and **4** at these low concentrations of DNA for 80 ms reveals the detection of 3 ng DNA bands using EtBr and dye **4** but not dye **2**. Nevertheless, the 1 ng bands are not visible with any of them. At 120 ms of exposure, EtBr staining allows the observation of a very faint band in the 1 ng lane which is not visible with any other dye, but too faint for further analysis (e.g., expression analysis by semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)). However if the exposure time is increased to 520 ms to allow a better visualization of DNA fragments, EtBr gel becomes photobleached due to over exposure of UV illumination, whereas dye **4** allows a fine view of the bands without photobleaching and dye **2** also allows the visualization of the DNA though with a weaker signal than dye **4**. These observations indicate a relative higher photostability of the monomethine cyanine dyes over EtBr.

Comparative sensitivity of monomethine cyanine dyes **1**–**5** in relation to EtBr for the detection of 1 and 3 ng DNA after UV illumination at 520 and 120 ms for the gels of dyes **1**–**5** and EtBr, respectively, is shown in Figure 4. At 3 ng DNA, the fragments were detected with a similar sensitivity for dyes **3**–**5** and EtBr, with a better sensitivity observed for the dye **4** when compared with EtBr at 0.8 DNA fragment. Dye **1** revealed a comparable sensitivity in relation to dyes **3**–**5** and EtBr at 1.2 and 2.0 kb DNA fragments but not at 0.8 kb. However, dye **2** showed a high sensitivity only for 2.0 kb DNA fragment when compared with all dyes. Further dilution of DNA concentration to 1 ng in the gels of the dyes including EtBr resulted in the detection of 2.0 and 1.2 kb DNA fragments using dyes **4**, **5** and EtBr in the following sensitivity order dye **5** > dye **4** ⩾ EtBr, whereas dyes **1**–**3** were not able to detect DNA at this concentration.

The overall results of dye **1**–**5** sensitivity suggest the suitability of amide moiety over the amine one as well as the bromide counteranion over the iodide and tosylate ones. This structural sensitivity difference might be explained by the DNA binding ability to the different dyes, being higher in the dyes which have amide groups than in those dyes containing amine ones, due to the increased hydrogen bonding between DNA with the former. Compared to EtBr, it appears that dyes **4** and **5** are promising tools for DNA analysis under UV light when its concentration is low overcoming the undesirable use of radioactivity normally applied in such limiting cases.

3. Conclusion

Some new monomethine cyanine dyes have been synthesized and fully spectroscopically characterized. A

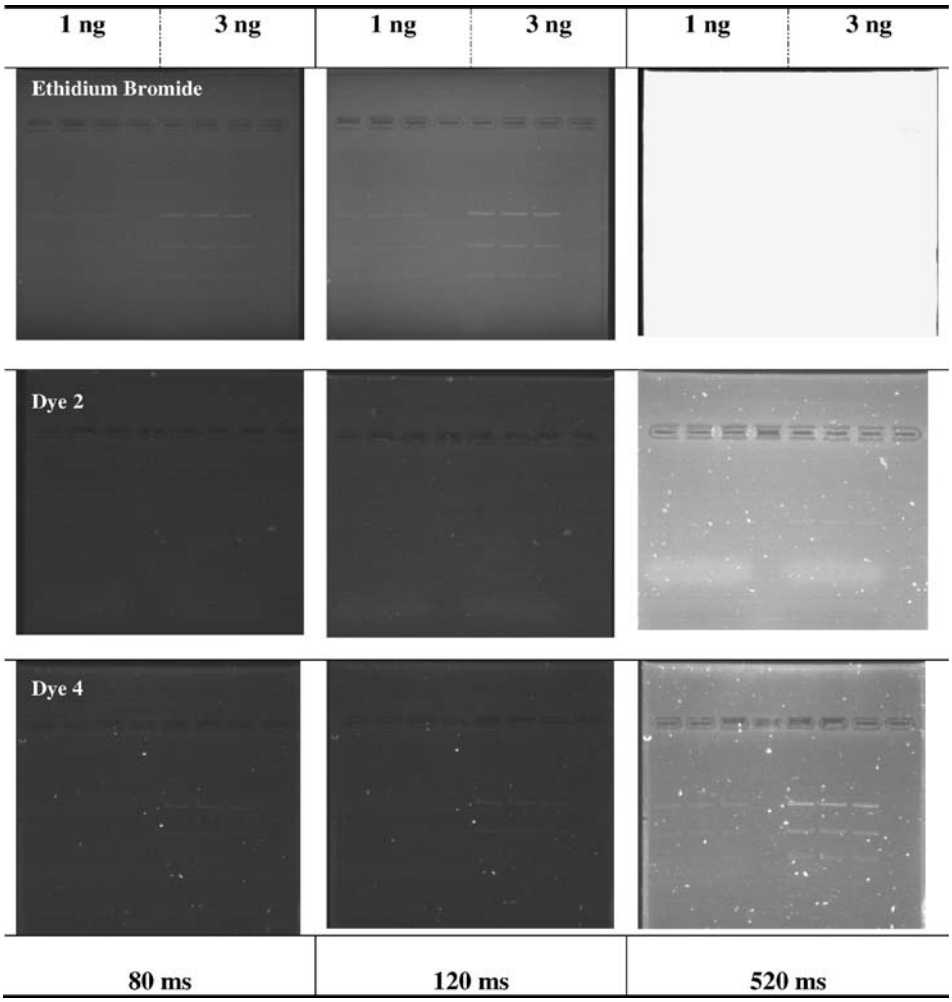


Figure 3. Agarose gel electrophoresis of DNA stained with monomethine cyanine dyes **2**, **4** and EtBr at 80, 120, and 520 ms exposure times. Images were acquired under UV light at 365 nm.

comparative study for DNA visualization between these dyes and the conventionally used EtBr stain was made using agarose gel electrophoresis. The acetamido dyes **4** and **5** are promising tools for DNA analysis at low levels of concentration when UV light is required for visualization. These new dyes may help overcome the use of the mutagenic EtBr stain or radioactivity for detecting low levels of DNA.

4. Experimental

4.1. General

Ethidium bromide and other reagents were of the highest purity available, purchased from Sigma–Aldrich Company and used as received. Solvents were of analytical grade. Agarose and DNA marker (low DNA mass ladder) were supplied by Eurobio and Invitrogen, respectively. All reactions were monitored by TLC on aluminum plates precoated with Merck silica gel 60 F_{254} (0.25 mm) using dichloromethane or dichloromethane/methanol (5–10%) and the spots examined under 254, 312, and 365 nm UV light. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 solutions on a Brücker ACP 250 (250.13 and 62.90 MHz) spectrometer. Chemical shifts are reported in parts per million relative to residual solvent signals or Me_4Si and coupling constants (J) are given in Hertz. Infrared spectra were performed on a Mattson 5000-FTS FTIR spectrometer. All samples were prepared by mixing FTIR-grade KBr with 1% (w/w) compound and grinding to a fine powder. Spectra were recorded over the 400–4000 cm^{-1} range without baseline corrections. More intensive bands are given in cm^{-1}. FABHRMS were recorded in a Micromass AutoSpec M, operating at 70 eV, using a matrix of 3-nitrobenzyl alcohol (3-NBA). All new dyes were determined to be >95% pure by ^{1}H NMR. Melting points were determined in open capillary tubes in a Büchi 530 melting point apparatus and are uncorrected.

4.2. Synthesis

4.2.1. *N*-(2-Methylquinolin-4-yl)acetamide. A solution of 4-aminoquinaldine (11.38 g, 72 mmol) in acetic anhydride (40 ml) was refluxed for 2 h. After cooling to room temperature the reaction mixture was treated with brine to precipitate the product, which was filtered off and re-dissolved in water. The aqueous layer was neutralized with sodium hydroxide (26 g) and extracted with ethyl

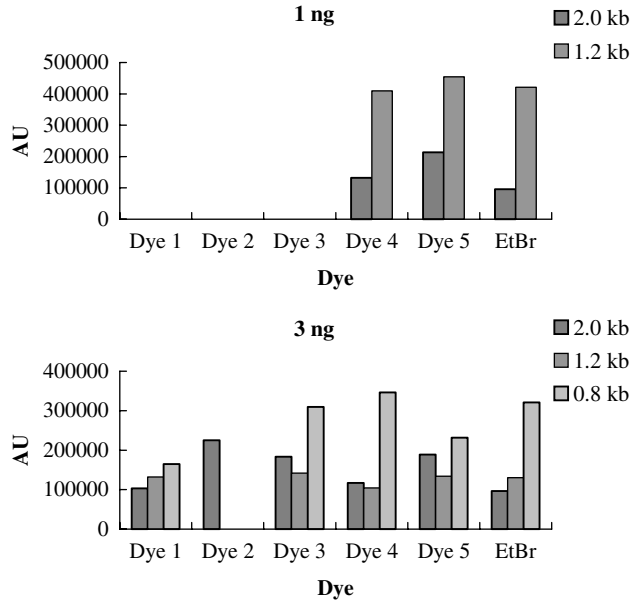


Figure 4. Agarose gel electrophoresis of DNA at 1 and 3 ng concentrations stained with monomethine cyanine dyes **1**–**5** and EtBr. Gels stained with dyes **1**–**5** and EtBr were exposed at 365 nm for 520 and 120 ms, respectively.

acetate and the combined extracts were dried with anhydrous sodium sulfate and evaporated to afford the product as a lemon yellow crystal (13.0 g, 90%). Mp 163–164 °C; ^{1}H NMR (250.13 MHz, DMSO-d_6): δ (ppm) 2.25 (3H, s, NHCOCH_3), 2.59 (3H, s, ArCH_3), 7.54 (1H, t, J = 7.6 Hz, ArH), 7.70 (1H, t, J = 7.6 Hz, ArH), 7.89 (1H, d, J = 8.4 Hz, ArH), 8.00 (1H, s, ArH), 8,29 (1H, d, J = 8.5 Hz, ArH), 10.14 (1H, s, NHCOCH$_3$); ^{13}C NMR (62.90 MHz, DMSO-d_6): δ (ppm) 24.27 (*C*H$_3$), 24.30 (*C*H$_3$), 112.04 (*C*H), 119.08 (*C*), 122.11 (*C*H), 124.96 (*C*H), 128.75 (*C*H), 129.38 (*C*H), 141.55 (*C*), 148.27 (*C*), 159.03 (*C*), 169.89 (C=O). IR (KBr) ν (cm^{-1}): 3274, 1669, 1621, 1563, 1535, 1381, 1348, 1277, 1256, 756. FABHRMS (3-NBA): (M+H$^+$); found: 201.1028, $C_{12}H_{13}N_2O$ requires 201.1061.

4.2.2. 1-Ethyl-4-acetamido-2-[(2,3-dihydro-3-ethylbenzothiazol-2-ylidene)methyl]quinolinium tosylate (1). A mixture of *N*-(2-methylquinolin-4-yl)acetamide (4.00 g, 20 mmol), 2-methylthiobenzothiazole (3.6 g, 20 mmol), and ethyl *p*-toluenesulfonate (8.4 g, 42 mmol) was heated at 130 °C for 6 h until practically total conversion into the corresponding quaternary ammonium salts was achieved. The resulting mixture of solid salts was heated at 100–105 °C in dry pyridine/triethyl amine solvent mixture (80 ml, 3:1 v/v) for 12 h. After cooling, the precipitate formed was collected by filtration, washed with acetone, and air-dried to afford **1** as an orange crystal (4.4 g, 39%). Mp 269.5–271.0 °C; ^{1}H NMR (250.13 MHz, DMSO-d_6): δ (ppm) 1.36 (3H, t, J = 6.8 Hz, CH$_2$CH_3), 1.47 (3H, t, J = 6.6 Hz, CH$_2$CH_3), 2.27 (3H, s, Tos-CH_3), 2.38 (3H, s, NHCOCH_3), 4.51 (2H, q, J = 7.3 Hz, CH_2CH$_3$), 4.67 (2H, q, J = 7.5 Hz, CH_2CH$_3$), 6.02 (1H, s, =CH–), 7.09 (2H, d, J = 7.7 Hz, Tos-H), 7.35 (1H, t, J = 7.8 Hz, ArH), 7.50 (2H, d, J = 7.7 Hz, Tos-H) 7.54 (1H, t, J = 7.8 Hz, ArH), 7.64 (1H, t, J = 7.0 Hz, ArH), 7.71 (1H, d, J = 7.1 Hz, ArH), 7.94 (1H, t, J = 8.4 Hz, ArH), 7.99 (1H, d, J = 7.9 Hz, ArH), 8.12 (1H, d, J = 9.0 Hz, ArH), 8.48 (1H, d, J = 8.0 Hz, ArH), 8.91 (1H, s, ArH), 10.55 (1H, s, NHCOCH$_3$); ^{13}C NMR (62.90 MHz, DMSO-d_6): δ (ppm) 11.72 (CH$_2$*C*H$_3$), 12.48 (CH$_2$*C*H$_3$), 20.82 (Tos-*C*H$_3$), 24.75 (NHCO*C*H$_3$), 41.04 (*C*H$_2$CH$_3$), 43.78 (*C*H$_2$CH$_3$), 84.27 (=*C*H–), 105.60 (*C*H), 112.43 (*C*H), 117.37 (*C*), 117.56 (*C*H), 122.79 (*C*H), 123.58 (*C*), 123.70 (*C*H), 124.43 (*C*H), 125.07 (*C*H), 125.52 (*C*H-Tos), 128.08 (*C*H, *C*H-Tos), 133.72 (*C*H), 137.62 (*C*-Tos), 139.38 (*C*), 139.90 (*C*), 144.05 (*C*), 145.78 (*C*-Tos), 152.32 (*C*), 159.36 (*C*),>171.03 (C=O). IR (KBr) ν (cm^{-1}): 3286, 1712, 1626, 1567, 1524, 1383, 1332, 1210, 751. UV/visible (MeOH): λ_{max} = 480 nm and ε_{max} = 51135 M^{-1} cm^{-1}. FABHRMS (3-NBA): M$^+$; found: 390.1632, $C_{23}H_{24}N_3OS$ requires 390.1640.

4.2.3. 1-Ethyl-4-amino-2-[(2,3-dihydro-3-ethylbenzothiazol-2-ylidene)methyl]quinolinium iodide (2) and bromide (3). A mixture of dye **1** (0.562 g, 1 mmol), hydrochloric acid (37%, 3.4 ml), and 32.3% potassium iodide or potassium bromide aqueous solution (2.6 ml) was boiled for 1 h. The resulting solution was allowed to cool to room temperature and neutralized with sodium hydroxide aqueous solution (8%, 20 ml). The resulting precipitated dye was filtered off, washed several times with cold water, and air-dried to afford **2** or **3**, respectively.

Dye **2** as a yellow solid (0.45 g, 95%). Mp > 300 °C; ^{1}H NMR (250.13 MHz, DMSO-d_6): δ (ppm) 1.32 (3H, t, J = 6.8 Hz, CH$_2$CH_3), 1.42 (3H, t, J = 6.9 Hz, CH$_2$CH_3), 4.32 (2H, q, J = 7.1 Hz, CH_2CH$_3$), 4.54 (2H, q, J = 7.1 Hz, CH_2CH$_3$), 5.79 (1H, s, =CH–), 7.05 (1H, s, ArH), 7.21 (1H, t, J = 7.0 Hz, ArH), 7.49–7.40 (2H, m, J = 7.7 Hz, ArH), 7.59 (1H, t, J = 7.5 Hz, ArH), 7.77 (1H, d, J = 7.8 Hz, ArH), 7.91 (1H, t, J = 7.7 Hz, ArH), 8.03 (1H, d, J = 8.7 Hz, ArH), 8.31 (1H, d, J = 8.4 Hz, ArH), 8.38 (2H, broad s, exchange with D$_2$O, NH_2); ^{13}C NMR (62.90 MHz, DMSO-d_6): δ (ppm) 11.27 (CH$_2$*C*H$_3$), 13.27 (CH$_2$*C*H$_3$), 38.69 (*C*H$_2$CH$_3$), 42.83 (*C*H$_2$CH$_3$), 82.17 (=*C*H–), 97.04 (*C*H), 110.92 (*C*H), 116.15 (*C*), 117.71 (*C*H), 122.19 (*C*H), 122.58 (*C*), 123.03 (*C*H), 123.88 (*C*H), 124.70 (*C*H), 127.51 (*C*H), 133.90 (*C*H), 139.37 (*C*), 140.47 (*C*), 152.37 (*C*), 154.89 (*C*), 155.15 (*C*). IR (KBr) ν (cm^{-1}): 3298, 3163, 1641, 1535, 1395, 1219, 746. UV/visible (MeOH): λ_{max} = 440 nm and ε = 29770 M^{-1} cm^{-1}. FABHRMS (3-NBA): M$^+$; found: 348.1532, $C_{21}H_{22}N_3S$ requires 348.1534.

Dye **3** as a yellow solid (0.41 g, 95%). Mp 275.3–269.8 °C; ^{1}H NMR, ^{13}C NMR, IR (KBr), and HRMS (FAB, 3-NBA) are the same as for dye **2**. UV/visible (MeOH): λ_{max} = 440 nm and ε_{max} = 37954 M^{-1} cm^{-1}.

4.2.4. 1-Ethyl-4-acetamido-2-[(2,3-dihydro-3-ethylbenzothiazol-2-ylidene)methyl]quinolinium iodide (4) and bromide (5). A solution of dye **1** (0.562 g, 1 mmol) in methanol (3 ml) was heated at the boil while excess of 14% potassium iodide or potassium bromide aqueous solution

(12 ml) was dropwisely added. After cooling, the resulting precipitated dye was filtered off, washed with water, acetone, and air-dried to afford **4** or **5**, respectively.

Dye **4** as an orange crystal (0.49 g, 95%). Mp 278.0–279.7 °C; ^{1}H NMR (250.13 MHz, DMSO-d_6): δ (ppm) 1.37 (3H, t, J = 6.9 Hz, CH_2CH_3), 1.48 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.38 (3H, s, $NHCOCH_3$), 4.52 (2H, q, J = 7.1 Hz, CH_2CH_3), 4.67 (2H, q, J = 7.6 Hz, CH_2CH_3), 6.03 (1H, s, =CH–), 7.37 (1H, t, J = 7.6 Hz, ArH), 7.56 (1H, t, J = 7.8 Hz, ArH), 7.67 (1H, t, J = 7.8 Hz, ArH), 7.72 (1H, d, J = 8.4 Hz, ArH), 7.95 (1H, t, J = 8.4 Hz, ArH), 8.00 (1H, d, J = 7.9 Hz, ArH), 8.14 (1H, d, J = 8.9 Hz, ArH), 8.48 (1H, d, J = 8.0 Hz, ArH), 8.92 (1H, s, ArH), 10.54 (1H, s, NHCOCH$_3$); ^{13}C NMR (62.90 MHz, DMSO-d_6): δ (ppm) 11.73 (CH_2CH_3), 12.49 (CH_2CH_3), 24.77 ($NHCOCH_3$), 41.08 (CH_2CH_3), 43.81 (CH_2CH_3), 84.29 (=CH–), 105.68 (CH), 112.44 (CH), 117.42 (C), 117.58 (CH), 122.80 (CH), 123.58 (C), 123.69 (CH), 124.46 (CH), 125.09 (CH), 128.10 (CH), 133.74 (CH), 139.41 (C), 139.92 (C), 144.10 (C), 152.39 (C), 159.40 (C), 171.03 (C=O). IR (KBr) ν (cm^{-1}): 3244, 1692, 1623, 1517, 1378, 1326, 1212, 751. UV/visible (MeOH): λ_{max} = 480 nm and ε = 48218 M^{-1} cm^{-1}. FAB-HRMS (3-NBA): M$^+$; found: 390.1632, $C_{23}H_{24}N_3OS$ requires 390.1640.

Dye **5** as an orange crystal (0.45 g, 95%). Mp 282.5–284.5 °C; ^{1}H NMR, ^{13}C NMR, IR (KBr), and FAB-HRMS (3-NBA) are the same as for dye **4**. UV/visible (MeOH): λ_{max} = 480 nm and ε_{max} = 43408 M^{-1} cm^{-1}.

4.3. Agarose gel electrophoresis

Dyes were dissolved in 50% aqueous dimethylformamide to a final concentration of 500 μg/ml. Ethidium bromide was diluted to the same concentration in distilled water. Agarose gels (1%) containing 0.5 μg/ml of each dye or ethidium bromide were prepared in Tris–Acetate–EDTA buffer (TAE). Six different dilutions of the DNA marker (corresponding to 1, 3, 10, 20, 30, and 40 ng for the detection of 2.0, 1.2, 0.8, and 0.4 kb fragments of DNA) were run in triplicate in each gel. Electrophoresis was carried out at constant voltage (100 V) until the front has migrated 8 cm. Gel Images were acquired under UV-illumination (Viber Lourmat) at wavelength of 365 nm. Exposure time (520 ms) was kept constant for each gel, except for DNA concentrations of 1 and 3 ng where 80 and 120 ms of exposure times were additionally tested. The volume of each of the observed bands was determined using BioID (Vilber Loumat). Statistical analysis was made for the volumes of the detected bands in each gel for all dyes in comparison with those detected in the ethidium bromide stained gel using the paired t-test analysis for all DNA concentrations. Significant differences were considered when $p < 0.05$.

Acknowledgments

Thanks are due to Fundação para a Ciência e a Tecnologia, Portugal, POCTI, and FEDER, for the funding of the Project 'Development of New Supports for Dye-Affinity Chromatography' (POCTI/2002/QUI/44776) and for granting Reda M. El-Shishtawy a Post-doctoral fellowship (SFRH/BPD/14618/2003).

References and notes

1. Hirons, G. T.; Fawcett, J. J.; Crissman, H. A. *Cytometry A* **1994**, *15*, 129.
2. Yan, X.; Grace, W.; Yoshida, T.; Habbersett, R.; Velappan, N.; Jett, J.; Keller, R.; Marrone, B. *Anal. Chem.* **1999**, *71*, 5470.
3. Svanvik, N.; Westman, G.; Wang, D.; Kubista, M. *Anal. Biochem.* **2000**, *281*, 26.
4. Ishiguro, T.; Saitoh, J.; Yawata, H.; Otsuka, M.; Inoue, T.; Sugiura, Y. *Nucleic Acids Res.* **1996**, *24*, 4992.
5. Seitz, O.; Bergmann, F.; Heindl, D. *Angew. Chem. Int. Ed.* **1999**, *38*, 2203.
6. Gurrieri, S.; Wells, K. S.; Johnson, I. D.; Bustamante, C. *Anal. Biochem.* **1997**, *249*, 44.
7. Rye, H. S.; Yue, S.; Wemmer, D. E.; Quesada, M. A.; Haugland, R. P.; Mathies, R. A.; Glazer, A. N. *Nucleic Acids Res.* **1992**, *20*, 2803.
8. Bengtsson, M.; Karlsson, H. J.; Westman, G.; Kubista, M. *Nucleic Acids Res.* **2003**, *31*, e45.
9. Yin, J. L.; Shackel, N. A.; Zekry, A.; McGuinness, P. H.; Richards, C.; Putten, K. V.; McCaughan, G. W.; Eris, J. M.; Bishop, G. A. *Immunol. Cell. Biol.* **2001**, *79*, 213.
10. Svanvik, N.; Stahlberg, A.; Sehlstedt, U.; Sjoback, R.; Kubista, M. *Anal. Biochem.* **2000**, *287*, 179.
11. Haugland, R. P. *Handbook of Fluorescent Probes and Research Products*, 9th ed.; Molecular Probes: Eugene, OR, 2002.
12. Lee, L. G.; Chen, C. H.; Chiu, L. A. *Cytometry* **1986**, *7*, 508–517.
13. Carlsson, C.; Larsson, A.; Jonsson, M.; Albinsson, B.; Nordén, B. *J. Phys. Chem.* **1994**, *98*, 10313.
14. Sharp, P. A.; Sugden, B.; Sambrook, J. *Biochemistry* **1973**, *12*, 3055.
15. Kantor, G. J.; Hull, D. R. *Biophys. J.* **1979**, *27*, 359.
16. Cerutti, P. A. *Science* **1985**, *227*, 375.
17. Marks, R. *Cancer* **1995**, *75*, 607.
18. Lartia, R.; Asseline, U. *Chem. Eur. J.* **2006**, *12*, 2270.
19. Moreda, W.; Forrester, A. R. *Tetrahedron* **1997**, *53*, 12595.
20. Karlsson, H. J.; Bergqvist, M. H.; Lincoln, P.; Westman, G. *Bioorg. Med. Chem.* **2004**, *12*, 2369.
21. Matselyukh, B. P.; Yarmoluk, S. M.; Matselyukh, A. B.; Kovalska, V. B.; Kocheshev, I. O.; Kryvorotenko, D. V.; Lukashov, S. S. *J. Biochem. Biophys. Methods* **2003**, *57*, 35.
22. Kovalska, V. B.; Tokar, V. P.; Losytskyy, M. Yu; Deligeorgiev, T. J.; Vassilev, A.; Gadjev, N.; Drexhage, K.-H.; Yarmoluk, S. M. *J. Biochem. Biophys. Methods* **2006**, *6*(8), 155.
23. Luedtke, N. W.; Liu, Q.; Tor, Y. *Bioorg. Med. Chem.* **2003**, *11*, 5235–5252.
24. Wakelin, L. P. G.; Waring, M. J. *Mol. Pharmacol.* **1974**, *10*, 544.
25. Luedtke, N. W.; Liu, Q.; Tor, Y. *Chem. Eur. J.* **2005**, *11*, 495.
26. Beyerbach, A.; Rothman, N.; Bhatnagar, V. K.; Kashyap, R.; Sabbioni, G. *Carcinogenesis* **2006**, *27*, 1600.
27. Golka, K.; Kopps, S.; Myslak, Z. W. *Toxicology Lett.* **2004**, *151*, 203.
28. Hamer, F. M. In *The Cyanine Dyes and Related Compounds—The Chemistry of Heterocyclic Compounds*; Weissberger, A., Ed.1971; Interscience: New York; Vol. 18.