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Selective interactions of perylene derivatives having different side chains with inter- and intramolecular G-quadruplex DNA structures. A correlation with telomerase inhibition

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Abstract—While the importance of the aromatic core in small organic molecules, studied as G-quadruplex mediated telomerase inhibitors, appears well studied by a number of researches, the role of side chains has been less well characterized. In this paper, we have studied the ability of six perylene derivatives with different side chains to induce both inter- and intramolecular G-quadruplex structures. The distance between the aromatic core and the positive charges in the side chains emerges as a significant molecular feature in G-quadruplex formation. Furthermore, the G-quadruplex formation appears also related to drugs 'self-association', influenced by the side chains basicity. The different efficiencies of the six perylene derivatives in interacting both with inter- and intramolecular G-quadruplex structures satisfactorily correlate with telomerase inhibition in cell-free systems.

Human telomeric DNA consists of tandem arrays of the short sequence TTAGGG on the 3′ ending strand, for several kilobases in length.[1] A single stranded overhang of about 200 nucleotides protrudes at the 3′ terminus of telomeric DNA. In the nonreplicative state, the 3′ single strand can invade the telomere duplex to form a t-loop structure[2] that stabilizes and protects the terminal end of chromosomes. This structure cannot be stable during the entire cell cycle:[3] in fact, the 3′ single stranded overhang represents the substrate of telomerase, a ribonucleoproteic reverse transcriptase enzyme, involved in the maintenance of telomere length in eukaryotic cells.[4] Since telomerase is not active in most somatic cells, while it is active in most human tumors, it has become a potentially highly selective target for several anti-tumor strategies.[5] In the last few years, a novel approach for anti-cancer drug design has been represented by the use of small organic molecules that bind the G-rich single strand and drive it into unusual DNA secondary structures inaccessible to telomerase, known as G-quadruplexes.[6] G-quadruplex DNA structures are characterized by stacked G-tetrads, stabilized by Hoogsteen G–G pairings to form a planar ring: the hole between the G-tetrads is well suited to co-ordinate monovalent cations (K^+ better than Na^+), stabilizing the structure.[7] G-quadruplex shows extensive polymorphism, basically dependent on the number and orientation of involved DNA strands, as well as positions of the loops. Moreover, the presence in the cell of proteins that bind and/or induce G-quadruplexes[8] and of helicases able to unwind specifically these structures[9] suggests that G-quadruplexes represent biologically important signaling structures: the recent evidence of the existence of G-quadruplex structure in vivo supports this hypothesis.[10]

So far, several classes of small organic molecules, that induce and/or stabilize G-quadruplex structures and inhibit telomerase have been characterized.[5] General features of most of such molecules include a large aromatic core, that favors stacking interactions with the G-tetrads, and positively charged side chains, that interact with the DNA grooves.

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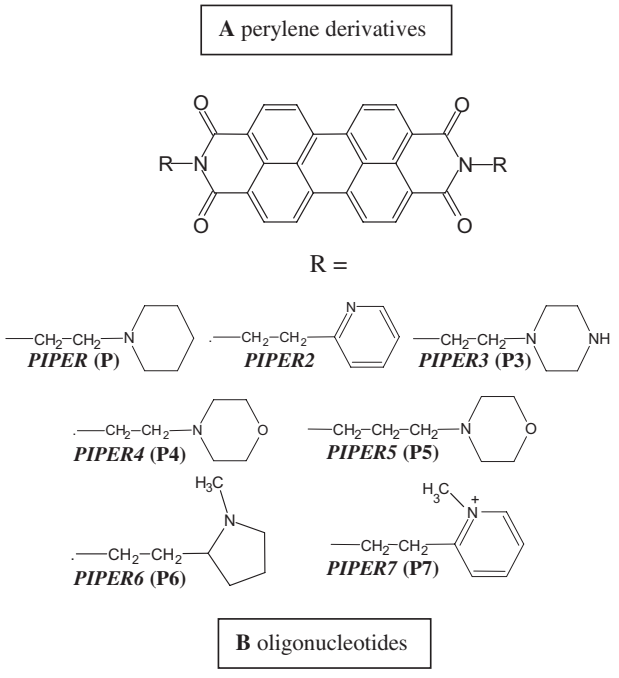
Recently, we have studied four *N*-*N*′-disubstituted perylene diimides having the same perylene core and different side chains. We have shown that electrostatic interactions between ligands side chains and DNA phosphates play a main role, not only in the formation of G-quadruplex structures, but also in selecting its topology.[11] Furthermore, such molecules show different abilities to inhibit telomerase, indicating the necessity to consider side chains in the design of efficient telomerase inhibitors.

On these bases, we have extended our analysis to a wider series of perylene derivatives with different side chains (Scheme 1A) to obtain a more satisfactory understanding of the role of different features of the side chains in the formation of both inter- and intramolecular G-quadruplex structures and in the inhibition of telomerase. From this study, the distance of the positive charges in the side chains from the aromatic core appears as an important molecular feature in inducing and stabilizing both inter- and intramolecular G-quadruplex structures. Furthermore, the side chains basicity plays a significant role in the drugs 'self-association', which is surely involved in their specific interactions with G-quadruplex structures.

We have considered six perylene derivatives[12] (Scheme 1A). PIPER and PIPER3 (*N*,*N*′-bis[2-(1-piperidino)ethyl]-3,4,9,10-perylenetetracarboxylic diimide and *N*,*N*′-bis[2-(1-piperazino)ethyl]-3,4,9,10-perylenetetracarboxylic diimide, respectively), with piperidine and piperazine in the side chains, were already present in our previous work.[11] The piperazine ring of PIPER3 has two nitrogen atoms, whose first dissociation constant is comparable to that of PIPER ($pK_a \approx 11$), while the second one is comparable to that of PIPER2[11] ($pK_a \approx 5.5$), so that a small fraction of molecules will be doubly charged in our experimental conditions, while in the molecules with a single charge, it can be positioned alternatively on one of the two nitrogen atoms. Four new molecules have been synthesized. Two compounds have a morpholine ring in the side chains: PIPER4 (*N*,*N*′-bis[2-(4-morpholino)ethyl]-3,4,9,10-perylenetetracarboxylic diimide) and PIPER5 (*N*,*N*′-bis[3-(4-morpholino)propyl]-3,4,9,10-perylenetetracarboxylic diimide); the expected pK_a for these compounds is about 8. They have ethyl and propyl linkers, respectively, to connect the morpholine nitrogen atom to the aromatic moiety; the oxygen atom on the morpholine ring leads to a greater polar chain than all the other considered compounds, except PIPER3. PIPER6 (*N*,*N*′-bis[2-(2-(1-methyl)pyrrolidino)ethyl]-3,4,9,10-perylenetetracarboxylic diimide) and PIPER7 (*N*,*N*′-bis[2-(2-(1-methyl)pyridino)ethyl]-3,4,9,10-perylenetetracarboxylic diimide) have not the charged nitrogen atom directly linked to the side chain; both of them have an ethyl linker and the expected pK_a for PIPER6 is about 10. This means that the distance of the nitrogen atom from the aromatic core is roughly the same as in the case of PIPER5, that is characterized by a propyl linker. The case of PIPER7 is particularly interesting, since it was synthetically[12] derived from PIPER2 (*N*,*N*′-bis[2-(2-pyridino)ethyl]-3,4,9,10-perylenetetracarboxylic diimide), that is basically uncharged and unable to induce any G-quadruplex structure.[11] Through the quaternization of the nitrogen atom on the pyridine ring, PIPER7 achieves a fully charged nitrogen atom and shows an activity comparable to the best perylene derivatives.



Scheme 1.

The role of different side chains of perylene derivatives in the formation of both inter- and intramolecular G-quadruplex structures was investigated by PAGE (PolyAcrylamide Gel Electrophoresis). The DNA oligonucleotides 2HTR and TSG4 (Scheme 1B) were used. 2HTR is able to form only dimeric and/or tetrameric intermolecular G-quadruplex structures and was also considered in our previous work;[11] TSG4 forms preferentially intramolecular G-quadruplex structures and can act as a substrate for telomerase elongation in a modified TRAP assay.[13] They were incubated with all perylene derivatives at increasing concentrations and the formation of G-quadruplex structures was investigated by PAGE analysis (Fig. 1A and B and Fig. 2A and B). Considering previous electrophoretic gel shift data obtained in similar experimental conditions[14] and the mobility standard obtained by G-quadruplex dimeric forms induced by the potassium ions,[11,15] we identified major electrophoretic bands as single stranded DNA (ss), dimeric (D), tetrameric (T), and monomeric (M) G-quadruplex structures. Intramolecular structure (M) corresponds to the band showing the highest mobility: in fact, its particular structure favors the running in the gel grid with respect to single stranded DNA.[16] To probe that electrophoretic bands correspond to inter- and intramolecular G-quad-

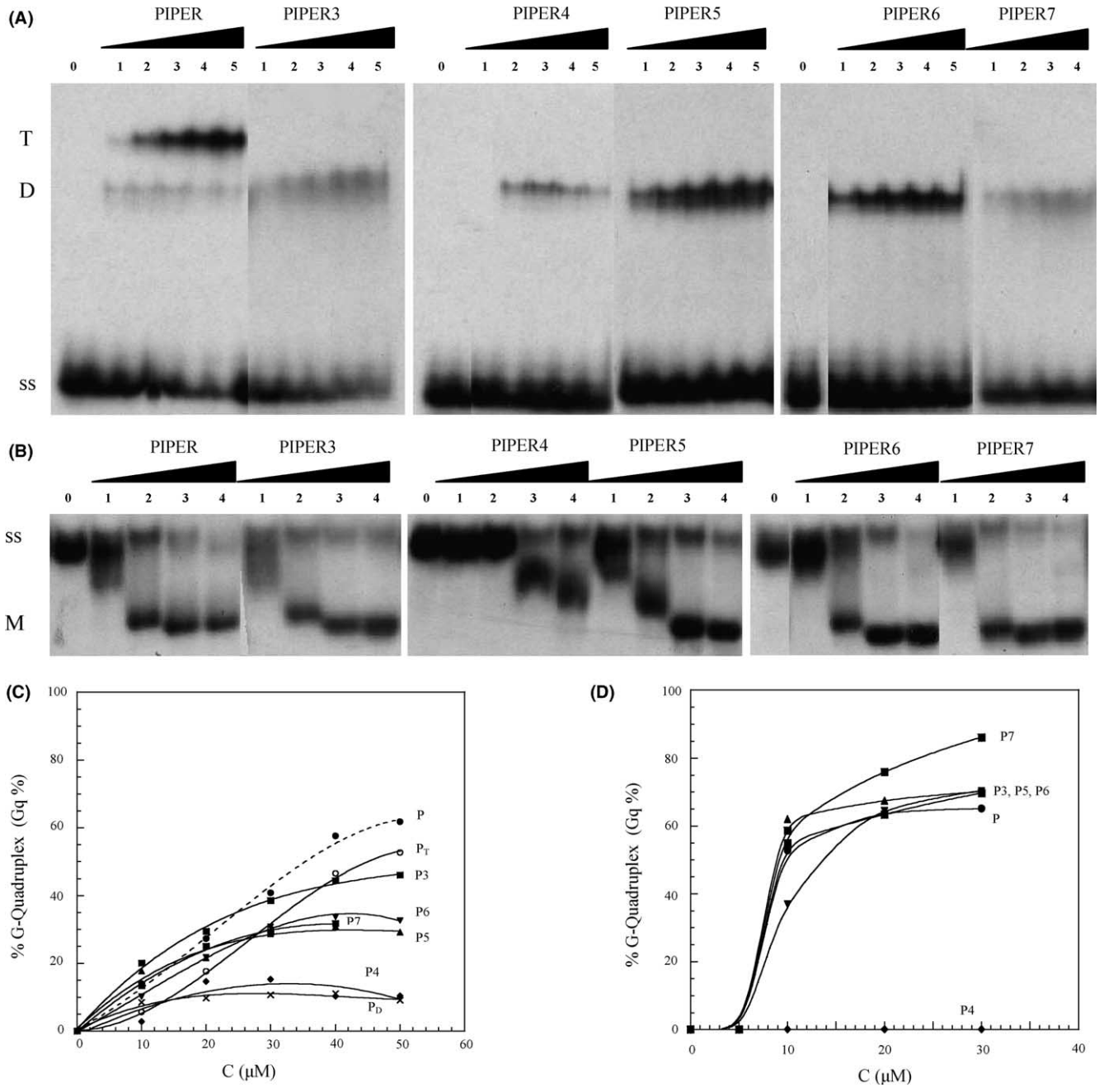


Figure 1. G-quadruplex structures formation induced by the six perylene derivatives (hydroclorides), reported in Scheme 1A, studied by native PAGE (15% polyacrylamide gel, TBE 0.5×, KCl 20 mM). (A) 2HTR (12 μM) previously heated at 95 °C for 10 min and quickly cooled in ice was incubated for 2 h at 30 °C in MES–KCl buffer (10 mM MES, pH 6.5, 50 mM KCl), in the presence of different drugs concentrations: 10 μM (lane 1), 20 μM (2), 30 μM (3), 40 μM (4), 50 μM (5) and with no drug (lane 0). (B) TSG4 (12 μM), in 10 mM MES, pH 6.5, 5 mM KCl, was incubated, as described above for 2HTR, in the presence of different drugs concentrations: 5 μM (lane 1), 10 μM (2), 20 μM (3), 30 μM (4), and with no drug (lane 0). Major bands are identified as single stranded DNA (ss), dimeric (D), tetrameric (T), and monomeric (M) G-quadruplex structures. (C–D) Percentage of G-quadruplex structures formed (G_q%) in function of drug concentration (*C* (μM)). In the case of PIPER, intermolecular dimeric (P_D) and tetrameric (P_T) structures can be distinguished (the dashed line being their sum (P)) (C). G_q% represents the ratio between the intensity of the relative band on the electrophoresis gel and the total amount of DNA, obtained by Instant Imager (Packard). Intermediate bands between ss and M in (B) were not considered when calculating values reported in (D).

ruplex structures, dimethylsulfate (DMS) methylation protection experiments were carried out (data not shown). To better compare the different behavior of the different molecules, we have reported the percentage of the induced G-quadruplex structures as a function of drugs concentration (Fig. 1C and D and Fig. 2C and D). The obtained results, using 2HTR oligomer in MES buffer pH 6.5, KCl 50 mM, show that all perylene derivatives are almost equally able to induce intermolecular G-quadruplex structures, except PIPER4, that is definitively less efficient (Fig. 1A and C). Furthermore, it is worth noting that, while PIPER2, with no charge on

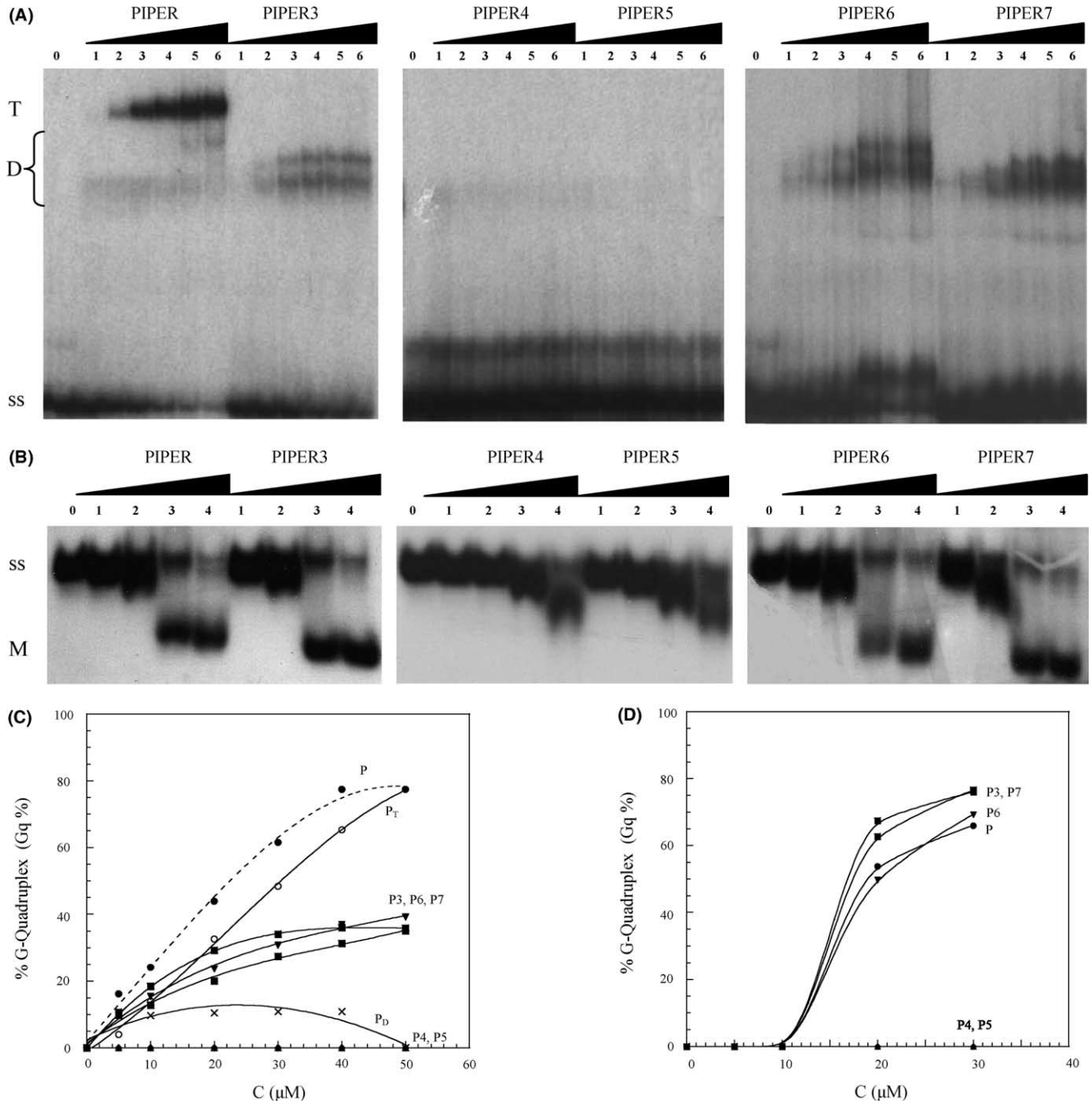


Figure 2. Samples containing 2HTR (A) or TSG4 (B), were incubated as described in the legend of Figure 1 except that they were incubated in TRAP buffer, pH 7.5 (20 mM Tris–HCl (pH 7.5), 15 mM $MgCl_2$, 10 mM EDTA, 0.5% Tween20), 50 mM KCl in the case of 2HTR and 5 mM KCl in the case of TSG4, in the presence of different drugs concentrations: 5 μM (lane 1), 10 μM (2), 20 μM (3), 30 μM (4), 40 μM (5), and 50 μM (6) and with no drug (lane 0). (C–D) Percentage of G-quadruplex structures formed (G_q%) in function of drug concentration (*C* (μM)) of the six perylene derivatives, relative to the band shift assays reported in Figure 2A (C) and in Figure 2B (D), is reported as described in the legend of Figure 1.

the side chain, is unable to form any G-quadruplex structure,[11] PIPER7, which is the same compound but methylated in correspondence of the nitrogen atom of side chains pyridine ring with the consequent acquisition of a positive charge, induces and stabilizes dimeric G-quadruplex structures, confirming the essential role of electrostatic interactions of side chains with DNA grooves. Finally, PIPER is the only perylene derivative able to induce a G-quadruplex structure that involves four strands, while it is barely efficient in inducing dimers. To study the effect of all the considered perylene derivatives in the formation of intramolecular G-quadruplex, TSG4 oligonucleotide was used. In this case, incubating the samples in the presence of KCl 5 mM (in this experimental conditions no G-quadruplex structure is formed in drug absence), all perylene derivatives are able to induce intramolecular G-quadruplex, corresponding to a high mobility electrophoretic band (Fig. 1B and D). It is worth noting that PIPER4 induces intramolecular G-quadruplex structures only at a

drug concentration higher than those considered (data not shown), since the intermediate bands, evidenced at lower concentrations, can not be considered as canonical intramolecular G-quadruplex. Performing the same experiment in the presence of KCl 50 mM, the monomeric G-quadruplex structure is formed also in absence of any drug. In these conditions, all perylene derivatives bind to the preformed intramolecular G-quadruplex structure, slightly varying its mobility (data not shown).

To take into account the different basicity of drugs side chains, which is surely important for the interactions with G-quadruplex structures, electrophoretic mobility shift assays were repeated in the same experimental conditions, except that TRAP buffer pH 7.5 was used. The obtained results are reported in Figure 2. Both PIPER4 and PIPER5 are unable to induce appreciable amount of intermolecular G-quadruplex structure (2HTR), while the behavior of the other perylene derivatives is basically unchanged (Fig. 2A and C). Considering the formation of intramolecular G-quadruplex structure (TSG4), all perylene derivatives start to induce intramolecular G-quadruplex at higher concentration (Fig. 2B and D) with respect to the experiments carried out at pH 6.5 (Fig. 1B and D); also in this case, PIPER4 and PIPER5 result the less efficient drugs, being unable to induce a canonical intramolecular G-quadruplex structure at the considered drugs concentrations.

The behavior of perylene derivatives in inducing G-quadruplex structures could be influenced also by their aggregation state, an aspect previously studied by Kerwin and co-workers.[17] To obtain more information on this topic, we measured the absorption spectra of the six perylene derivatives at decreasing concentrations, in MES buffer pH 6.5, in the wavelength range between 350 and 650 nm. The absorbance spectra of all perylene derivatives in DMSO show peaks at 462, 493, and 529 nm, whose intensities are equal for all the different compounds (data not shown), while, in the adopted conditions (MES buffer, pH 6.5), the spectra show several modifications. PIPER, PIPER3, PIPER6, and PIPER7 absorption spectra have a maximum at 500 nm with a shoulder at 540 nm, an isobestic point at 550 nm and are characterized by a strong hypochromic effect (representative PIPER and PIPER3 spectra are reported in Fig. 3A and B). The presence of one isobestic point suggests that there is an equilibrium between a monomeric and a multimeric drug form. PIPER4 and PIPER5 spectra are different considering three main features: (i) the isobestic point is absent, (ii) the hypochromic effect is larger than in the case of the other four derivatives, and (iii) the peak at 500 nm is substituted by a broad band centered at about 470 nm (representative PIPER4 spectrum is reported in Fig. 3C). All these features seem consistent with the formation of more than one type of multimeric aggregate. All these absorption spectra were measured also in TRAP buffer at pH 7.5. In these experimental conditions the absorption spectrum of PIPER (Fig. 3D) becomes very similar to those of PIPER4 and PIPER5, while the absorption spectra of the other perylene derivatives are basically unchanged (representative PIPER3 and PIPER4 spectra are reported in Fig. 3E and F).

On the basis of the obtained results and of the data previously reported,[11] it appears of interest to compare the ability of the six considered perylene derivatives to inhibit telomerase. To this aim, two different telomeric repeat amplification protocol (TRAP) assays were considered. First, the standard not telomeric TS oligonucleotide was used as telomerase substrate:[18] because of the specific feature of its sequence, TRAP only allows the detection of G-quadruplex-induced telomerase inhibition after the synthesis of at least two or four telomeric repeats. It is worth noting that after the synthesis of two telomeric repeats the DNA fragments are very similar to the oligonucleotide 2HTR, that was used in the electrophoretic mobility shift assays. Nevertheless, the concentrations of telomeric repeats having different length can not be evaluated, so that a quantitative correlation with PAGE is not possible. TRAP assays were performed using different concentrations of perylene derivatives. PIPER is able to inhibit telomerase starting from a concentration of 40 μM, as also reported in our previous work,[11] while PIPER4 and PIPER5 are unable to inhibit the enzyme even at higher concentration. In the case of PIPER3, PIPER6, and PIPER7, on the contrary, the intensity and the number of bands clearly decrease with respect to the control, at a drug concentration in the range of 5–10 μM (Fig. 4A).

TRAP assays were repeated using TSG4 oligonucleotide as telomerase substrate:[13] it is able to form intramolecular G-quadruplex also before the telomerase synthesis. In fact, the KCl concentration used in the TRAP assay (68 mM) allows the formation of G-quadruplex by TSG4. Nevertheless, a similar structure is not stable in the absence of a suitable concentration of G-quadruplex stabilizing molecules and it may be efficiently unfolded and extended by telomerase[13] (Fig. 4B, lane 0). For this reason, also in this modified TRAP assay, a quantitative correlation with PAGE results seems hazardous. The obtained data, in the presence of different perylene derivatives, are similar to those obtained using TS oligonucleotide (Fig. 4B). PIPER3, PIPER6, and PIPER7 result the most efficient perylene derivatives in inhibiting telomerase, being active at a drug concentration in the range of 5–10 μM, probably forming more stable complexes with the intramolecular G-quadruplex structure with respect to the other considered molecules.

The results, reported in this paper, allow to establish that both the efficiency in forming G-quadruplex structures, as derived by PAGE, and the telomerase inhibition, as derived by two different TRAP assays, depend on the features of perylene derivatives side chains. In all cases, a fundamental molecular feature appears to be the distance between charged nitrogen atoms in the side chains and the aromatic moiety of the drugs. This finding is consistent with the threading intercalation model proposed for this kind of molecules by Hurley and co-workers,[19] in which the drug is stacked on the terminal G-tetrad, so that the distance defined above is surely of great importance in optimizing the interactions

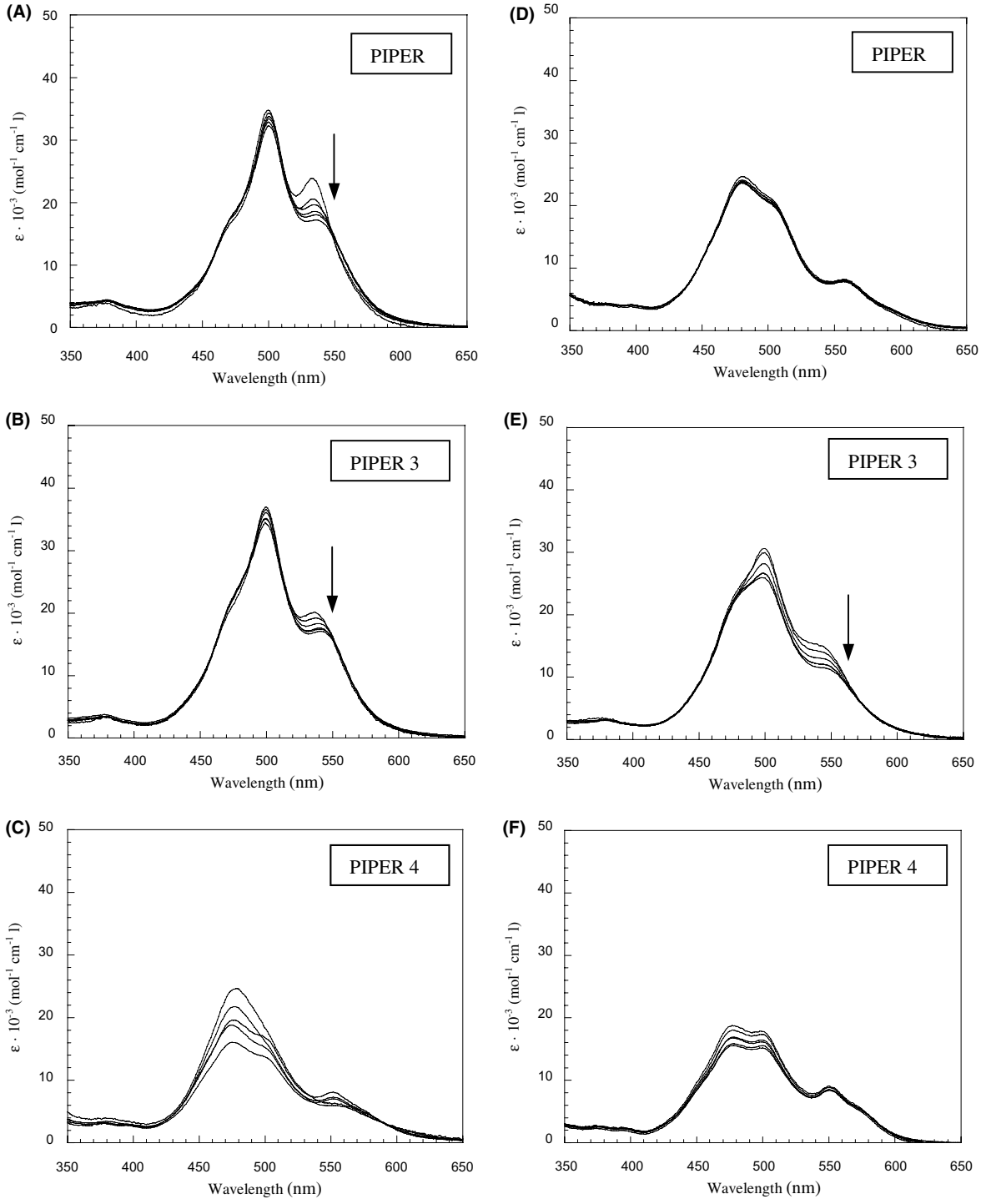


Figure 3. Visible absorption spectra of serial dilutions of three perylene derivatives, PIPER, PIPER3, and PIPER4 in 10 mM MES buffer pH 6.5 (respectively, A, B, and C) and in TRAP buffer pH 7.5 (respectively, D, E, and F). The concentrations of the drugs solutions are in the range of 5–50 μM, the higher extinction coefficients corresponding to the more dilute solutions. The arrow indicates the isobestic point.

with the phosphates in the DNA grooves. In fact, the compounds having the longest distance between the positively charged nitrogen atom on the side chains and the aromatic moiety (PIPER3, PIPER5, PIPER6, and PIPER7) are the most efficient in inducing dimeric G-quadruplex structures at pH 6.5. When the distance is shorter (PIPER and PIPER4), a different behavior is observed: PIPER4 is barely efficient in inducing dimeric G-quadruplex structures, while PIPER is the only compound able to induce tetrameric G-quadruplex. Another important aspect to be considered is the 'self-association' of the drugs in water solution, probably correlated to the pK_a values and the basicity of the side chains, as recently reported by Kerwin and co-workers.[17] The absorption spectra reported in Figure 3 put in evidence a more complex aggregation for morpholine containing

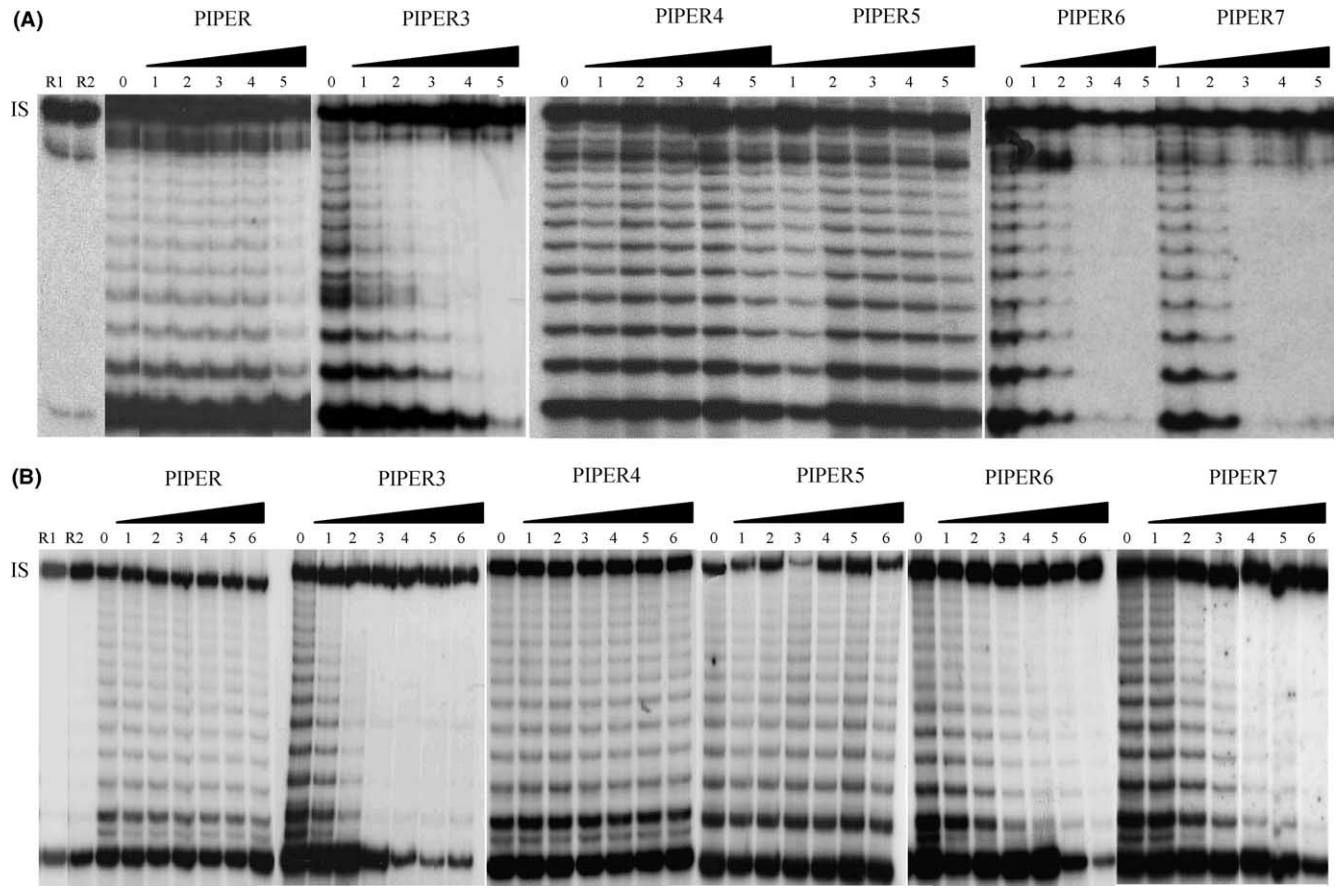


Figure 4. Inhibition of human telomerase by different perylene derivatives, by Telomerase Repeat Amplification Protocol (TRAP) assay. (A) The reaction mixture (50 μL) contains 50 μM dNTPs, 0.5 μM TS primer, 1 μL of cell extract (prepared from 10^9 cultured HeLa cells, as previously described[18]) in TRAP buffer (20 mM Tris–HCl (pH 7.5), 15 mM $MgCl_2$, 10 mM EDTA, 0.5% Tween20), 68 mM KCl. In each sample, perylene derivatives were added at different concentrations and incubated for 2 h at 30 °C, before the addition of the cell extract. After 30 min of incubation at 30 °C, the samples were purified by phenol/chloroform extraction. ^{32}P radiolabeled TS, 0.5 M ACT primer[20] and 2U Taq DNA polymerase (Eppendorf) were added and 27 PCR cycles were performed (94 °C 30″, 50 °C 30″, 72 °C 1′30″). Finally, the samples were loaded on nondenaturing 12% polyacrylamide gel. In lane 0 no drug was added, in lane R1 cell extract was not added, in lane R2 heat inactivation was performed by heating 10 μL extract at 75 °C for 10 min prior to assaying 1 μL by TRAP analysis. The considered drugs concentrations were 10 μM (lane 1), 20 μM (2), 30 μM (3), 40 μM (4), and 50 μM (5). IS is a 130 bp 'internal standard' to control the PCR amplification efficiency.[21] (B) The experimental conditions in the modified TRAP assay were the same as those described above, except for using TSG4 oligonucleotide as telomerase substrate and CXext[13] as reverse primer in PCR amplification. The considered drugs concentrations were 5 μM (lane 1), 10 μM (2), 20 μM (3), 30 μM (4), 40 μM (5), and 50 μM (6).

perylene derivatives (PIPER4 and PIPER5) with respect to the other compounds at pH 6.5. Increasing the pH to 7.5, PIPER behavior changes, becoming very similar to that of PIPER4 and PIPER5. Anyway, the complexity of these processes does not allow to find a simple correlation with PAGE results and to develop a structural model. With regard to this, it should be noted that as previously suggested by Kerwin et al.,[17a] at the moment it is not possible to establish if the binding of the drugs to the G-quadruplex DNA occurs from monomeric or aggregated ligands. In the first case, the drug multimerization should be competitive with respect to the monomeric drug binding to the G-quadruplex.

The length and the basicity of the six perylene derivatives side chains play a synergistic role in determining the activity of the drugs. In fact, PIPER4 and PIPER5, that have the same side chains basicity, result the most inefficient compounds in inducing G-quadruplex structures at pH 7.5 (Fig. 2), evidencing the important role of the drugs self aggregation. But it is worth noting that, at pH 6.5, PIPER4 is still the less efficient perylene derivative in inducing G-quadruplex structures, while PIPER5, characterized by a longer side chain, results so efficient as the other perylene derivatives (Fig. 1), pointing out an important role also for the distance between charged nitrogen atoms in the side chains and the aromatic moiety of the drugs.

The comparison between the results derived from PAGE and absorption spectroscopy in TRAP buffer and those obtained from the two different TRAP assays allows to establish that the same drugs molecular features, which determine the G-quadruplex formation strikingly influence the ability of these compounds also to inhibit telomerase. In fact PIPER3, PIPER6, and PIPER7 result the most efficient perylene derivatives, while PIPER, PIPER4, and PIPER5 appear definitively less efficient. These results suggest that a too short side chain and/or a weak basicity of side chain amines give rise to a

poor inhibitory activity, probably due to the different thermodynamic stabilization of the complexes between these drugs and G-quadruplex DNA structures. However, to firmly assess this topic, the study of the different thermodynamic stability of these complexes is surely necessary and this is now in progress in our laboratory.

Acknowledgements

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12. Perylene diimides, except PIPER7, were prepared from 3,4,9,10-perylenetetracarboxylic dianhydride (3 g) and the appropriate primary amine (10% excess), in a refluxing mixture of *N*,*N*-dimethylacetamide (20 mL) and 1,4-dioxane (20 mL). The reaction products were precipitated adding water and separated by filtration. The resulting products (80–95% yield) were purified dissolving in HCl 0.2 M and precipitating the respective hydrochlorides with acetone (75% yield). PIPER7 was prepared from the previously described PIPER2[11] (*N*,*N*′-bis[2-(2-pyridino)ethyl]-3,4,9,10-perylenetetrtacarboxylic diimide, 200 mg) by reaction with methyl-trifluoromethanesulfonate (10 equiv) in refluxing 1,2-dichloroethane (10 mL), with stirring for 2 days.[22] The reaction product was filtered, washed with chloroform, and dried under vacuum. Then it was dissolved in HCl 0.1 M and passed through a strong basic anion exchange resin Dowex1. PIPER7 was precipitated as hydrochloride with acetone in 12% total yield. Compounds were dried under vacuum and characterized by ^{1}H and ^{13}C NMR spectroscopy (CF_3CO_2D, 300 MHz, 25 °C) and elemental analysis or mass spectrometry. All perylene derivatives have been characterized and used as hydrochlorides. PIPER (*N*,*N*′-bis[2-(1-piperidino)ethyl]-3,4,9,10-perylenetetrtacarboxylic diimide): ^{1}H NMR δ: 1.7–1.9 (2H), 2.0–2.4 (10H), 3.23 (4H), 3.83 (4H), 4.10 (4H), 4.93 (4H), 7.41 (2H), 8.92 (8H); ^{13}C NMR δ: 11.6, 13.9, 26.7, 46.2, 47.5, 112.4, 115.4, 117.4, 120.3, 124.2, 127.4, 157.1; elemental analysis calcd ($C_{38}H_{38}N_4O_4Cl_2$): 66.6% C, 5.5% H, 8.2% N; found: 64.9% C, 5.9% H, 7.8% N. ESI *m*/*z*: 613 (MH^+). PIPER3 (*also known as Tel08*[17]) (*N*,*N*′-bis[2-(1-piperazino)ethyl]-3,4,9,10-perylenetetrtacarboxylic diimide): ^{1}H NMR δ: 4.1–4.5 (16H), 4.78 (4H), 5.13 (4H), 9.0–9.2 (8H); ^{13}C NMR δ: 31.2, 37.8, 45.5, 52.8, 117.5, 120.4, 122.5, 125.4, 129.3, 132.5, 162.5; elemental analysis calcd ($C_{36}H_{38}N_6O_4Cl_2$): 56.8% C, 5.0% H, 11.1% N; found: 54.9% C, 5.7% H, 10.4% N. PIPER4 (*also known as Tel10*[17]) (*N*,*N*′-bis[2-(4-morpholino)ethyl]-3,4,9,10-perylenetetracarboxylic diimide): ^{1}H NMR δ: 3.66 (4H), 4.07 (4H), 4.3–4.5 (8H), 4.57 (4H), 5.04 (4H), 8.9–9.1 (8H); ^{13}C NMR δ: 31.5, 49.4, 53.3, 60.2, 117.6, 120.6, 122.6, 125.5, 129.4, 132.6, 162.3; elemental analysis calcd ($C_{36}H_{34}N_4O_6Cl_2$): 62.7% C, 4.9% H, 8.12% N; found: 60.8% C, 5.4% H, 7.5% N. PIPER5 (*also known as Tel01*[17]) (*N*,*N*′-bis[3-(4-morpholino)propyl]-3,4,9,10-perylenetetracarboxylic diimide): ^{1}H NMR δ: 2.73 (4H), 3.62 (4H), 3.77 (4H), 4.02 (4H), 4.38 (4H), 4.59 (4H), 4.72 (4H), 8.9–9.1 (8H); ^{13}C NMR δ: 18.5, 33.9, 48.6, 52.0, 60.1, 117.7, 120.3, 122.2, 125.2, 129.0, 132.1, 161.8; elemental analysis calcd ($C_{38}H_{38}N_4O_6Cl_2$): 63.6% C, 5.3% H, 7.8% N; found: 61.8% C, 5.8% H, 7.6% N. PIPER6 (*N*,*N*′-bis[2-(2-(1-methyl)pyrrolidino)ethyl]-3,4,9,10-perylenetetracarboxylic diimide): ^{1}H NMR δ: 2.2–2.4 (8H), 2.83 (4H), 3.29 (6H), 3.47 (2H), 3.82 (2H), 4.13 (2H), 4.66 (4H), 7.79 (2H), 9.00 (8H); ^{13}C NMR δ: 17.4, 25.2, 25.4, 33.9, 36.6, 53.4, 65.2, 117.8, 120.4, 122.4, 125.3, 129.1, 132.2, 161.7; elemental analysis calcd ($C_{38}H_{38}N_4O_4Cl_2$): 66.7% C, 5.5% H, 8.2% N; found: 63.8% C, 6.1% H, 8.2% N. PIPER7 (*N*,*N*′-bis[2-(2-(1-methyl)pyridino)ethyl]-3,4,9,10-perylenetetracarboxylic diimide): ^{1}H NMR δ: 4.23 (t, J = 7.3, 4H), 5.11 (s, 6H), 5.28 (t, J = 7.3, 4H), 8.40 (t, J = 7.4, 2H), 8.57 (d, J = 7.4, 2H), 8.91 (t, J = 7.4, 2H), 9.21 (d, J = 7.4, 2H), 9.31 (d, J = 8.2, 4H), 9.37 (d, J = 8.2, 4H); ^{13}C NMR δ: 27.3, 34.5, 41.9, 117.8, 120.6, 122.6, 125.5, 125.8, 129.3, 132.6, 142.2, 142.5, 151.4, 161.8. ESI *m*/*z*: 629 [$(M-1)^+$], 315 [$(M/2)^{2+}$].
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