Bioorganic & Medicinal Chemistry Letters 19 (2009) 3903–3908

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Selective G-quadruplex ligands: The significant role of side chain charge density in a series of perylene derivatives

Emanuela Micheli [a,b], Caterina Maria Lombardo [c], Danilo D'Ambrosio [b], Marco Franceschin [a], Stephen Neidle [c], Maria Savino [b,d,*]

[a] Dipartimento di Chimica, Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185 Roma, Italy
[b] Dipartimento di Genetica e Biologia Molecolare, Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185 Roma, Italy
[c] Cancer Research UK Biomolecular Structure Group, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom
[d] Istituto di Biologia e Patologia Molecolare del CNR, Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185 Roma, Italy

ARTICLE INFO

Article history:
Received 9 February 2009
Revised 20 March 2009
Accepted 23 March 2009
Available online 26 March 2009

Keywords:
G-quadruplex DNA
Perylene derivatives
Polyamines
Telomerase inhibitors
FRET
TRAP assay
CD spectroscopy
UV/vis absorption
Competition experiments

ABSTRACT

The human telomeric G-quadruplex structure is a promising target for the design of cancer drugs. The selectivity of G-quadruplex ligands with respect to duplex genomic DNA is of especial importance. The high selectivity of polyamine conjugated perylene derivatives appears to be regulated by side-chain charge density, as indicated by data from a FRET melting assay and induced CD spectroscopy.

Telomeres are specific nucleoprotein complexes which provide a protective cap at the end of linear eukaryotic chromosomes.[1] Telomeric DNA is characterized by short G-rich tandem repeat sequences, whose length varies in different organisms. It is guanine-rich and the extreme 3′-end extends as a single-strand overhang. This is the substrate of telomerase, a reverse transcriptase enzyme, which is involved in the maintenance of telomere length.[2] Telomerase is physiologically active in germinal, hematopoietic and epithelial cells; in addition the enzyme is reactivated in almost all human tumor cells,[3] where it is responsible for their excessive proliferation and immortality. For this reason telomerase is a suitable target for anticancer therapy and in the last few years considerable efforts have been made to design telomerase inhibitors as possible anti-cancer drugs.[4–6]

One approach to telomerase inhibition is to modify its substrate, for example by induction of G-quadruplex structures at the 3′ telomeric end.[7] Induction of a G-quadruplex conformation can be achieved by small molecules, characterized by an extended aromatic core, that favors stacking interactions with terminal G-quartets and basic side-chains (positively charged in physiological conditions) which interact with the four grooves of the G-quadruplex.[8,9] Perylene diimides,with positively-charged side chains present suitable features to interact with the G-quadruplex and have the ability to induce different G-quadruplex structures and to inhibit telomerase, depending on side-chain basicity and length.[10]

We have recently synthesized eight polyamine perylene diimide compounds, termed POL ligands (Fig. 1),[11] in an attempt to conjugate the perylene moiety (which stacks on terminal G-quartets) to stabilize G-quadruplex structures with strongly charged polyamines. We have investigated the ability of these compounds to induce inter- and intramolecular G-quadruplex structures using native polyacrylamide gel electrophoresis (PAGE), together with their ability to inhibit telomerase using a modified TRAP assay. The two properties appear to be significantly correlated, showing that the number and distances apart of positive charges in the side chains dramatically influence both intramolecular G-quadruplex induction and telomerase inhibition.[11] In common with most anticancer drugs, their ability to selectively recognize their target is of major importance. In the case of G-quadruplex ligands, this is re-

* Corresponding author.
E-mail addresses: stephen.neidle@pharmacy.ac.uk (S. Neidle), maria.savino@uniroma1.it (M. Savino).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.03.106

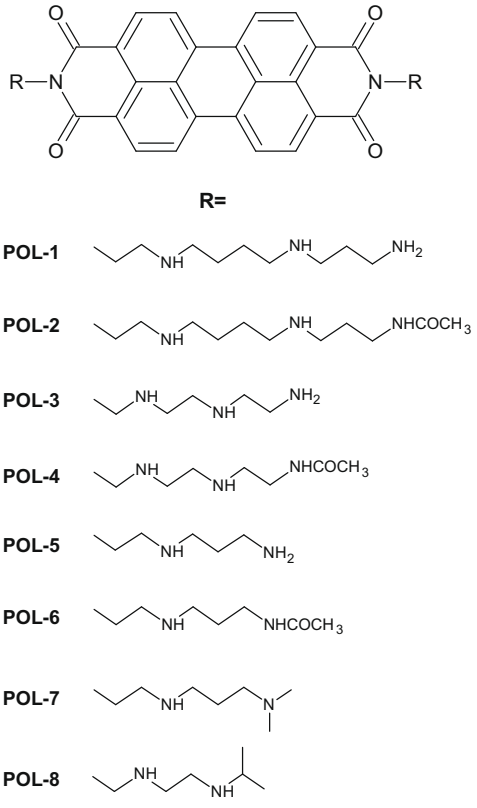

Figure 1. Structures of the eight POL perylene derivatives with different polyamine side chains.

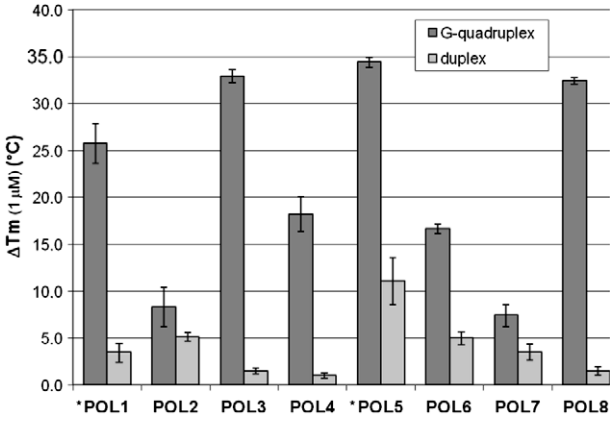

Figure 2. Histogram of the ΔT_m values obtained from the FRET melting studies at 1.0 μM drug concentration. For the compounds labeled with *(POL-1 and POL-5) the reported values correspond to 1.5 μM drug concentration. Melting temperatures are estimated as the midpoints of the melting curves for the human telomeric G-quadruplex and t-loop duplex DNA. Reported values are the means of three independent determinations.

lated to the ability of the molecule to selectively bind to G-quadruplex DNA compared to duplex DNA.

Our purpose in this study is to examine the selectivity of these new compounds for G-quadruplex DNA with respect to duplex DNA. Since their aromatic area is constant, these molecules enable one to investigate the specific role of the side chains, and if the efficiency of telomerase inhibition and induction of intramolecular G-quadruplex is coupled to selectivity between the two different DNA structural types. The study has been carried out using FRET melting assays, competition FRET melting assays, a new competition TRAP assay, absorption spectroscopy and induced circular dichroism (CD) spectroscopy with two different oligonucleotides (a human telomeric G-quadruplex and a self-complementary hairpin duplex DNA) and genomic duplex DNA. The information obtained on binding properties has enabled us to propose a model for the G-quadruplex/drug complex, which differs substantially from that for duplex DNA/drug complex.

The ability of the compounds to stabilize the two preformed DNA structures (quadruplex and duplex) was studied using a FRET melting assay.[12] ΔT_m values, reported in Figure 2, are given at a 1 μM ligand concentration for most of the drugs, since at this concentration there is a high level of G4-DNA stabilization (see Fig. S1 in Supplementary data). For two ligands (POL-1 and POL-5), however, data at 1.5 μM concentration are reported, since at 1.0 μM no relevant stabilization was observed. We also ran competition FRET experiments (Fig. 3), in which the perylene derivatives affinity for G4-DNA is evaluated in the presence of increasing concentrations of sonicated calf thymus duplex DNA (ct dsDNA).[13] In most cases, using ct dsDNA, we obtained results in agreement with those observed with hairpin duplex DNA (t-loop); surprisingly affinity for G-quadruplex DNA was not affected by the presence of the ct dsDNA also for molecules with high affinity for the t-loop (i.e., POL-2 and POL-5). This finding suggests that care must be taken in using synthetic oligonucleotides as model systems for genomic DNA, as also shown very recently by an ESI-mass spectrometry study on perylene derivatives selectivity.[14] It is evident that for all the studied ligands the number of positive charges in the side-chains has a major influence on G4-DNA thermal stabilization. Stabilization is strongly influenced by charge separation, perhaps rather more than the number of charges. For the pairs of compounds POL-1/POL-2, POL-3/POL-4 and POL-5/POL-6, ΔT_m values are significantly higher for those ligand having side-chains with three positive charges (POL-1, POL-3) or two (POL-5) compared to those with equal side-chains, except than the terminal group is acetylated. It is worth noting that selectivity is only for the interactions with G-quadruplex DNA, and is in large part absent for duplex DNA. The thermal stability of the t-loop dsDNA is only slightly increased by ligand interactions and surprisingly unaffected by the acetylation of side-chain terminal amino groups.

The two ligands POL-7 and POL-8 cannot be strictly compared since they have different terminal groups with a distinct hydrophobicity. Both have two positive charges, with the pairs POL-5 and POL-7, and POL-3 and POL-8 having the same charge separation. POL-8 shows both greater G-quadruplex thermal stabilization and greater selectivity with respect to POL-7. This result is to be well correlated with the general behavior of the other perylene derivatives. The features derived from the FRET melting assay are in good agreement with the results obtained from competitive FRET. The presence of ct dsDNA does not affect ligand stabilization of the G-quadruplex. Effective competition is only observed for the acetylated ligands POL-4 and POL-6, and for POL-7.

Since the most promising results in the competitive FRET assay were obtained with POL-3 and POL-8, these two molecules were selected for a further selectivity study using a competitive TRAP assay, in which telomerase inhibition is evaluated in presence of ct dsDNA.[15] This study was possible due to the high specificity of PCR primers for telomeric DNA and the internal standard DNA. The results obtained for the two drugs POL-3 and POL-8, shown in Figure 4, indicate that, for these molecules, ct dsDNA is not a competitor of human telomeric DNA up to ten times the concentration of the telomerase substrate. This issue needs further investigation, probably with an assay that does not require PCR amplification of telomerase products; however our results support the concept of selectivity of POL-3 and POL-8 for human telomeric G-quadruplex DNA.