International Journal of Biological Macromolecules 204 (2022) 89–102

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Review

Multimeric G-quadruplexes: A review on their biological roles and targeting

Ilaria Frasson [a,1], Valentina Pirota [b,c,1], Sara N. Richter [a,*], Filippo Doria [b,*]

[a] Department of Molecular Medicine, University of Padova, via A. Gabelli 63, 35121 Padova, Italy
[b] Department of Chemistry, University of Pavia, v. le Taramelli 10, 27100 Pavia, Italy
[c] G4-INTERACT, USERN, v. le Taramelli 10, 27100 Pavia, Italy

ARTICLE INFO

Keywords:
G-quadruplex
DNA/RNA secondary structures
Multimers
Biological role
Drug discovery

ABSTRACT

In human cells, nucleic acids adopt several non-canonical structures that regulate key cellular processes. Among them, G-quadruplexes (G4s) are stable structures that form in guanine-rich regions in vitro and in cells. G4 folded/unfolded state shapes numerous cellular processes, including genome replication, transcription, and translation. Moreover, G4 folding is involved in genomic instability. G4s have been described to multimerize, forming high-order structures in both DNA and/or RNA strands. Multimeric G4s can be formed by adjacent intramolecular G4s joined by stacking interactions or connected by short loops. Multimeric G4s can also originate from the assembly of guanines embedded on independent DNA or RNA strands. Notably, crucial regions of the human genome, such as the 3′-terminal overhang of the telomeric DNA as well as the open reading frame of genes involved in the preservation of neuron viability in the human central and peripheral nervous system are prone to form multimeric G4s. The biological importance of such structures has been recently described, with multimeric G4s playing potentially protective or deleterious effects in the pathogenic cascade of various diseases. Here, we portray the multifaceted scenario of multimeric G4s, in terms of structural properties, biological roles, and targeting strategies.

1. Introduction

More than forty years before Watson and Crick proposed the double helix DNA model [1] and Wilkins and Franklin gathered their X-ray diffraction data and asserted that the DNA must be prone to form secondary strucures to fulfill its multifaceted biological roles [2–4], the German chemist Ivar Bang noted that guanylic acid (now known as guanosine monophosphate) tended to aggregate in solution forming jelly-like structures [5], Bang's study puzzled the expert in the field for almost five decades until Gellert and colleagues gathered fiber X-ray diffraction data on guanylic acid [6], revealing the assembly of tetrameric elements into large helical structures, thus explaining the aggregates observed by Bang. Subsequent studies showed that four molecules of guanylic acid arrange into a planar tetrad in which each of the four guanines is the donor and acceptor of hydrogen bonds. Bang and Gellert focused on a structure that is now referred to as G-quartet, which can be adopted by guanosine residues in both ribose and deoxyribose backbones, in vitro, under physiological conditions [5,7–9]. Subsequent studies proved that the guanine residues embedded in the human genomic DNA, or in the genome of important human pathogens, tend to form G-quartets that stack on top of each other, stabilized by positive physiological cations (mostly Na^+ and K^+), to form non-canonical secondary structures, typically referred to as G-quadruplexes (G4s) [10–15]. G4s can be unimolecular or intermolecular and can adopt multiple topologies depending on strand arrangements, as well as loop length and nucleotide composition [16,17]. For years, little consideration was paid to G4s as their possible presence and role in biological systems were underestimated. The description that the human telomeric DNA could fold into G4 structures both in vitro and in the nucleus of human cells [8,9,18–20] revealed that G4s can compete with duplex DNA formation in vivo, thus prompting elucidation of their biological significance. To date, G4s have been associated with key cellular processes such as oncogene promoter regulation [21–27], cellular transcriptome shaping [28–31], DNA replication [32], DNA damage and genome instability [33–37], as well as with RNA transcription, translation, and processing [38–40]. Indeed, G4s are strategically embedded within the human genome, where they have been mostly considered as single structures, with examples of genomic regions, mostly promoters, that can fold in

* Corresponding authors.
E-mail addresses: sara.richter@unipd.it (S.N. Richter), filippo.doria@unipv.it (F. Doria).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ijbiomac.2022.01.197
Received 3 December 2021; Received in revised form 20 January 2022; Accepted 31 January 2022
Available online 4 February 2022

diverse but mutually exclusive G4s [21,41–45].

Nucleic acids have the propensity to multimerize, via interactions between complementary strands and consequent duplex formation [46]. Such interactions shape chromosome structure and govern many biological processes such as RNA silencing or the cellular response to exogenous nucleic acids [47,48]. It has recently become evident that nucleic acids multimerize also independently from base-pairing mediated mechanisms. Non-canonical nucleic acid structures, such as G4s, can form a wide variety of multimeric structures [49–52]. In vitro, G4 sequences were reported to assembly as dimers, tetramers, and other highly ordered structures named G-wires [49,53–56]. The presence and folding propensity of multimeric G4s in human cells have been predominantly depicted for the telomeric regions, where G4 multimers have been associated with numerous cancer processes and proposed as innovative antineoplastic marks targetable by G4 ligands [57,58]. Much recently multimeric G4s have been associated with critical neurological disorders [59]. To date, G4 ligands specifically interacting with G4 multimers at the neuronal level are still lacking, nevertheless, targeting of multimeric structures may represent an innovative treatment option for these neglected pathologies. This review describes the current state of the art of biologically relevant multimeric G4s in terms of structural properties, genomic location, and role at the cellular level. The potential role of G4-ligands in multimeric G4 targeting is also fully presented.

2. Monomeric vs multimeric G-quadruplex structures

G4 assembly does not entirely depend on the Watson and Crick base pairing but it also relies on the Hoogsteen hydrogen-bonding pattern. The Hoogsteen base pairing between guanines enables the formation of the G-tetrad or G-quartet, the building block of G4s. To form a G-tetrad, four guanines associate via eight hydrogen bonds, from both the Watson and Crick and Hoogsteen faces of adjacent nucleobases (Fig. 1A). The stacking of multiple planar G-tetrads on top of one another enables the folding of the G4 structure. A large variety of DNA and RNA sequences that can assembly into G4 have been described [16,38]: nucleotide sequences that can fold in G4 contain at least four islands of at least two guanines separated by random nucleotides. The nucleotides between adjacent guanine islands are referred to as loops [16]. Loop nucleotide composition and length can vary and determine G4 conformation and stability [60]. Unimolecular or intramolecular G4s, i.e. single G4 structures originating from a unique DNA or RNA strand, have been extensively studied [10,61] . The tetrads of a unimolecular G4 can fold into parallel, antiparallel or hybrid structures, with the loops adopting three different conformations: strand-reversal (or propeller, connecting parallel contiguous strands), lateral (or edgewise, connecting antiparallel contiguous strands), and diagonal (connecting antiparallel diagonal strands) (Fig. 1B) [60].

The formation of multimeric G4 structures relies on various features of nucleic acids. Multimeric G4s can assembly from G-tetrads that accommodate guanine residues coming from separate nucleic acid strands, and are named intermolecular. Intermolecular G4s can be dimeric, trimeric, and tetrameric and the strands taking part in the G4 structure can come exclusively from DNA strands or from both DNA and RNA molecules (namely DNA:RNA hybrid) [51,62]. Moreover, multimeric G4s can originate from subsequent intramolecular G4s on the same nucleic acid strand that establish highly-ordered structures, via stacking of monomeric subunits [60]. The stacking of monomeric subunits is influenced by G4 topology and loop orientation. In parallel G4s, strands are leaning in the same direction as loops on the sides of the tetrads, thus stacking of multiple structures is favorable [63,64]. The hybrid topology is also considered favorable for multimer folding, considering loop distribution [65]. Whereas in the antiparallel topology, the strands are in the opposite orientation and the loops lean out of the G4 core, which can impede stacking interactions of terminal G-tetrads or hinder strand bending to consent G4 folding of subsequent unimolecular structures [66]. G4 stacking in multimerization plays multiple roles: stacking shapes the tridimensional structure topology responsible for protein recognition at the cellular level but also determines the possible levels of ligand interactions. G4 multimers can accommodate ligands by end or lateral stacking but also by intercalation between adjacent G4s [67–71]. To accomplish their complex biological roles, both monomeric and multimeric G4s need to fold/unfold following precise thermodynamic processes. In the past years, many groups tried to shed light on the kinetic mechanisms underlying the folding of G4s, using the human telomere sequence([GGGTTA]n where $n \geq 4$), and relative variations, as model [72]. Indeed, the human telomeric sequence is a single-stranded multimeric G4-DNA sequence that can fold in multiple topologies, form multimers, rely on additional G-tracts possibly acting as spare tires, and it is involved in the front-line investigation for its biological functions [73–76]. More recently, the folding pattern of different G4 sequences embedded in the double-stranded DNA environment, corresponding to replication origins and promoter regions, was reported [77–79]. The folding/unfolding dynamics of G4 structures have been studied by circular dichroism (CD), UV absorption spectrum, fluorescence resonance energy transfer (FRET), optical or magnetic tweezers, NMR, and have also been simulated by computational and theoretical approaches [77,80]. Taken together, these studies attested that each G4 sequence has a peculiar polymorphic structure that folds/unfolds via multiple folding intermediates, with the human telomeric sequences showing the weakest and briefest stability and the more variable folding dynamics, and promoter/replication origin-embedded G4 sequences showing short folding time, long persistence and the strength to fold also when embedded in the double-stranded DNA [73–75,77–80].

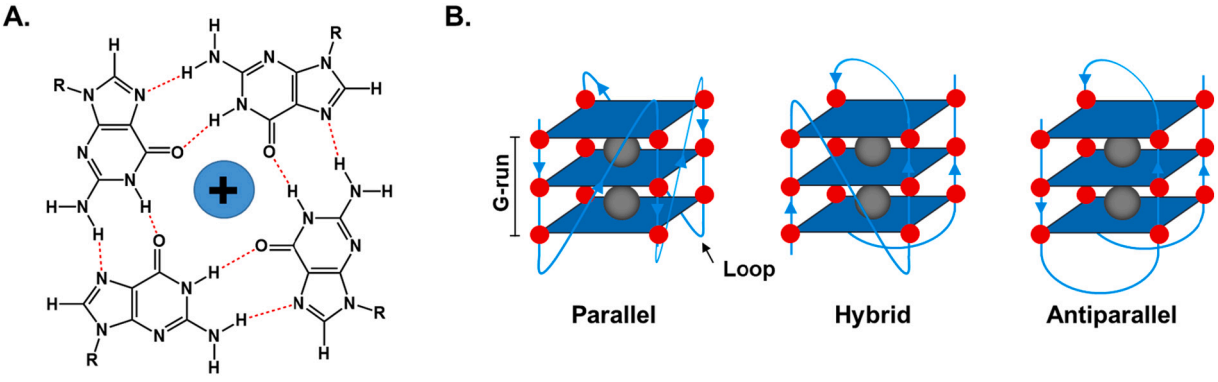

Fig. 1. Chemical structure of the G4 tetrad and different G4 topologies. A. Hoogsteen H-bonds among four guanines which form a G-tetrad; B. Different topologies of intramolecular G4 structures.

3. Multimeric G4 presence and function at the human genome level

The presence of multimeric G4s in the human genome has been widely predicted [18,81], but for many years their concrete folding at the nuclear level, as well as their biological significance have remained concealed. The first predicted multimeric G4 was embedded in the human telomeric region, with its single-stranded DNA (ssDNA) 3′-overhang composed of numerous repeated 5′-TTAGGG-3′ motifs [17]. The structure of the telomeric intramolecular G4 has been widely debated, as it was first studied in sodium solution [60] and later characterized in the more physiological potassium solution to fold into a parallel G4 when in the crystalline state [82], whereas into an equilibrium of two hybrid structures when in solution [83,84]. Telomeric DNA and embedded G4s have been associated with cellular senescence regulation and telomeres aberrant processing by the human telomerase complex, as well as with cancer onset and progression [26,85]. Thus, compounds interacting with the telomeric intramolecular G4s have been proposed as promising anticancer molecules [60,68,86]. The presence of multiple intramolecular G4s implies that telomeric ssDNA repeats could form multimeric structures, consisting of adjacent monomeric G4s that could fold as independent structures, appearing like "beads on a string" on ssDNA (Fig. 2A) [87–90]. On the other hand, telomeric G4s could fold also into macro-structures where the single G4 interacts with the neighboring ones via stacking interfaces (Fig. 2B) [91–93]. Early in vitro assays showed that both multimeric conformations can potentially be assumed at the telomere level, with the structure composed of stacking and interacting G4s being more likely [94]. More recently, studies merging data from CD spectra, molecular and hydrodynamic analyses, and small-angle X-ray scattering assays integrated with available high-resolution NMR, showed that the human telomeric sequence, repeated up to 96 nucleotides, maximizes stacking G4 folding, thus sparing no free G-tracts between G4 subunits (Fig. 2 C and D) [91,92,95]. In eukaryotic cells, the telomeric DNA has been reported to be transcribed into telomeric repeat-containing long non-coding RNAs of heterogeneous length, referred to as TERRA [96]. TERRA expression is variable: it is regulated by cell stress levels, DNA damage processes, and the cell cycle state [97,98]. TERRA has been shown to modulate telomerase activity, to hinder the histone methyltransferase LSD1, to compete with single-stranded DNA-binding proteins, and participate in telomere maintenance [97–101]. Both NMR and mass spectrometry studies attested that TERRA sequences, containing from two to eight G-tracts, are extremely prone to assembly into multimeric G4s via stacking interactions [83,102,103]. TERRA G4 assembly beneficiates from crowding conditions and displays a high polymorphic state, with multimers being one of the possible folded states [102]. The presence of folded G4s at the telomeric level in the nuclei of human cells has been evidenced using anti-G4 antibodies or TERRA RNA G4 -specific pyrene excimer probe [18,96,104,105]. The biological role of multimeric structures at the telomere level has not been clearly indicated but, analogously to monomeric G4s, multimeric G4s have been associated with protein recognition and telomerase processing, thus their stabilization by small-molecule ligands can alter both telomere structure stability and aberrant elongation in neoplastic processes, leading to DNA damage response, telomeric dysfunction and induction of tumor cell senescence and apoptosis (Table 1). Again, the development of highly specific telomeric G4 ligands as new anticancer agents captured extensive attention, with the prospect that compounds targeting the multimeric G4s may be more specific and display lower side effects [86].

More recently, multimeric G4s have been highlighted in human coding sequences of genes related to neuronal disorders and sequence expansion. Neurological disorders, comprising Huntington's disease, amyotrophic lateral sclerosis (ALS), fragile X syndrome, frontotemporal dementia (FTD), myotonic dystrophy, and innumerable ataxias, can be triggered by expansions of short nucleic acid repeats in specific genomic locations. Notably, the short nucleotide repeats involved in the expansion are G-rich sequences (i.e. 5'-GGGGCC-3′ in ALS and FTD) able to fold into intramolecular G4s and, due to the expansion, also into multimeric G4s [59,106]. Depending on the nucleotide repeat expansion location, the G4 related pathological mechanism may be at the DNA

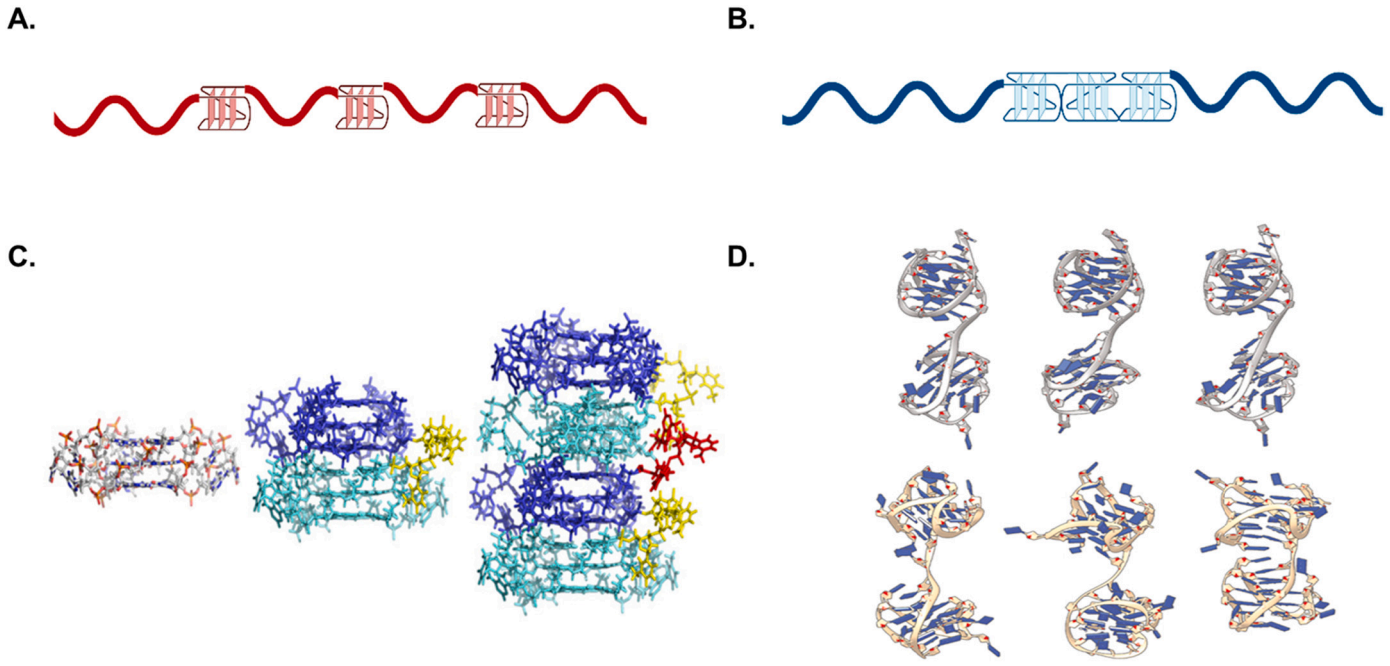

Fig. 2. Possible multimeric folding of the telomeric G4 motif. A. "Beads on a string" folding on the DNA template; B. Multimeric G4 folding via stacking interactions of intramolecular G4s; C. Stick representations of the construction of the multimeric 45-mer telomeric G-quadruplex from two 21-mer units (cyan and blue), connected together by a TTA loop (yellow). On the right, two 45-mer units were joined by a TTA loop (red) to form a 93-mer [91]; D. Best fit model (48-mer, top left model) and best fit ensemble of conformers. Models are oriented with their 5′ ends at the top [92]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Summary of roles and predicted effects of multimeric G4s at the human genome level.

DNA locus or gene/protein G4 motif	Related disease	Role of multimeric G4s
Telomere TTAGGG motif	Aberrant cell growth and cancer	Protein recognition and telomerase complex binding [92]
TERRA UUAGGG motif	Not reported	Telomere maintainance and protein recognition [98,115,116]
C9orf72 GGGGCC hexanucleotide repeat expansion	Amyotrophic Lateral Sclerosis/ Frontal-temporal Dementia (ALS/ FTD)	Negative transcription regulation [113] C9orf72 mRNA induced toxicity [59]. Generation of DNA: RNA hybrids [113] Sequestration of cellular proteins [113]
FMR1/FMRP CGG trinucleotide repeat expansion	Fragile-X syndrome (FXS) and Fragile X-related tremor/ ataxia syndrome (FXTAS)	FMRP mRNA inefficient translation [117] Interaction with cellular proteins and inclusion in cellular inclusion bodies [118] Generation of aberrant polypeptides from non-ATG-starting sites [118]
DNA:RNA hybrid at transcription bubble Variable motif	Not reported	Transcription enhancement [114]
Mitochondrial DNA transcription stop site GGGGGAGGGGGGGUUUGGUGG sequence	Not reported	Mitochondrial DNA transcription termination [119,120,120–123]

level (i.e. abnormal transcription), at the RNA level (i.e. atypical RNA processing, translation regulation, and subcellular localization), or at the protein level (i.e. aberrant translation, protein misfolding), with these possibilities being not mutually exclusive [107].

ALS is a neurological disorder characterized by progressive degeneration of motor neurons within the brain and spinal cord. FTD is similar to ALS, and it is characterized by permanent neuronal loss in the frontal and temporal cortices of the brain. Besides the neurological pathological spectrum, ALS and FTD share also genetic causes. The most common cause of both diseases is the hexanucleotide repeat expansion (HRE), (GGGGCC)n, in the open reading frame 72 of the non-coding region of chromosome 9 (*C9orf72*) [108,109]. ALS/FTD positive patients hide thousands of HRE repeats in their genome, in contrast to control patients [109]. The proposed mechanisms of pathogenesis in ALS/FTD included negative *C9orf72* transcription regulation, with RNA polymerase stalling and generation of truncated RNAs, cellular toxicity of the RNA transcripts containing the expansion motifs, sequestration of important cellular proteins and splicing factors, and translation of an abnormal polypeptide from non-ATG-starting sites (repeat-associated non-ATG (RAN) translation) [59,110]. Transcripts originating from *C9orf72* repeat expansions were reported to fold into G4 structures as well as to adopt an equilibrium of two folded states, hairpin and G4 [111,112]. The formation of RAN peptides was associated with the formation of hairpin structures, as hairping-binding but not G4-binding small molecules were able to hamper RAN translation in vitro [111]. In contrast, the *C9orf72* HRE was reported to form multiple stable G4 structures both at the DNA and RNA level and also to form DNA:RNA hybrid G4s (Table 1) [59,107,113,114]. These multimeric G4s are concomitant causes of neuronal death at the basis of ALS and FTD.

Fragile X syndrome (FXS) is the most common inherited genetic disease, characterized by intellectual disability and autistic behavior. FXS is induced by the aberrant function of the RNA binding protein known as fragile X mental retardation 1 protein (FMRP), encoded by the *FMR1* gene [124,125]. FMRP is an mRNA-binding protein that is essential for normal neurological metabolism, as it is primarily involved in dendritic mRNA transport and postsynaptic translation [126]. Expansion and hypermethylation of the trinucleotide (CGG)n repeats within the 5′-untranslated region (UTR) of *FMR1* mRNA, along with aberrant CpG island hypermethylation preceding the open reading frame of *FMR1*, trigger FMRP dysfunction, and neuronal damage. The same trinucleotide expansion has been linked to an FXS-related neurological disease, the Fragile X-related tremor/ataxia syndrome (FXTAS) [127]. In normal humans, 4–55 repeats of the (CGG) trinucleotide are present, whereas dynamic mutations expand the trinucleotide sequence to more than 200–2000 repeats in FXS and FTAXS patients [117]. The trinucleotide expansion present at the 5'-UTR of FMRP coding RNA, which is common to both neurological disabilities, has been shown to fold into parallel G4 structures, putatively able to form multimeric G4s that in FXS and FTAXS patients impede FMRP efficient translation, drastically diminishing protein synthesis and, thus, triggering neuronal dysfunction [117]. More recently, other pathogenic mechanisms have been characterized: (i) the expanded CGG repeat and related G4 structures interact with RNA binding proteins (RBPs), such as the heterogeneous nuclear ribonucleoprotein (hnRNP) A2B1, Di George syndrome critical region 8 (DGCR8), src-associated in mitosis (Sam68) and TAR DNA binding protein (TDP-43), which accumulate into toxic inclusion bodies and (ii) *FMR1* mRNA containing expanded CGG repeats initiates non-AUG-related translation, rising large amounts of polypeptides that are toxic to the neuronal cell (Table 1) [118]. As for *C9orf72* expansion transcript, the *FMR1* mRNA was reported to form, besides G4s, also hairpin structures that do not stimulate the interferon-inducible protein kinase (PKR), and are inefficiently processed by the human enzyme Dicer [128]. The study of the presence, structural topology, and expected pathological role of multimeric G4s in neurological diseases is a recent topic in the G4 field if compared to G4-mediated cancer biology [26]. Even more so is the development of G4 ligands for neurological multimeric G4s. The G4-stabilizing ligands (BRACO-19 and PDS) have been reported to cause neuronal damage in mice [129]. In contrast, administration of small molecules that specifically stabilize the RNA-G4 originating from the C9orf72 expansion showed promising improvement of the FTD/ALS phenotype both in vitro and in vivo [130]. The possible effect of G4-destabilizing compounds [57,131] in the context of the aforementioned neurological disorders has not been reported yet.

The latest reports presenting sequencing data on the human genome using G4-ligands or anti-G4 antibodies highlighted the presence of clusters of G4-forming regions in specific loci of the human DNA: CpG islands, transcription and replication regulators, helicases hypersensitive regions, oncogenes, and regions recognized by BMI1, a member of the Polycomb Repressive Complex 1 (PRC1) [132–134]. The multimeric assembly of the G4 sequences embedded in those regions has not been reported yet, nonetheless analysis of the public sequencing data using a recently published multimeric G4-tailored algorithm may reveal the presence and the localization of putative multimeric G4-forming sequences [135]. Forthcoming studies may disclose novel biological roles of consecutive G4-forming sequences and disclose innovative pharmacological targets.

In addition to the multimeric G4s described thus far, multimeric G4s can arise from arrangements of DNA and RNA strands, termed DNA:RNA hybrids [136,137]. These multimers form during transcription when guanines in the non-template strand interact with guanines in the nascent mRNA molecules. This particular structure, formed at the transcription bubble, has recently been associated with an enhancement in transcription, rather than an RNA polymerase stalling (Table 1) [114].

A second important DNA:RNA multimeric G4 has been characterized at the mitochondrial DNA level: termination of the mitochondrial RNA polymerase (POLRMT) transcription depends on the formation of stable DNA:RNA hybrid G4s at the mitochondrial DNA replication priming site

(Table 1) [119–122]. In detail, the RNA priming of the first strand of mitochondrial DNA is thought to depend on POLRMT. The nascent RNA can either elongate to generate full transcripts or terminate at the three conserved sequence blocks (CSB I, II, and III). Recently, transcription termination at the human CSBII site has been shown to depend on the formation of DNA:RNA hybrid G4 structures [120,123].

4. Design of effective G4-ligands for multimeric G4 systems

Monomeric G4 sequences have been widely exploited as biophysical models to screen for new potential stabilizing G4-ligands (G4Ls), also when the target G4s were thought to exist primarily as multimers in nature. Nevertheless, the increasing demonstration of the existence of highly organized repeated (dimeric or multimeric) G4 structures [94,102] as well as of their biological relevance [51], prompted the scientific community to identify and synthesize new small molecules capable of preferentially binding multimeric G4 structures. The intent was both to increase selectivity towards the multimeric G4s versus the large number of monomeric G4s present at the biological level, thus possibly limiting the side effects, and to improve the compounds' biological activity by reaching effective stabilization of the G4 consecutive structures by cooperative binding [51].

Considering the biological need to effectively interact with multimeric G4s, G4Ls that had shown high binding capabilities on monomeric G4s were firstly tested on multimeric G4 structures, both the telomeric ones and those involved in neurodegenerative diseases [138,139]. For the most promising G4Ls, the subsequent development pathways to increase their specificity were essentially two: (i) modify the side pendants to make them more selective towards a multimeric G4 system over a monomeric one [140]; (ii) covalently linking G4L-binding units together to increase affinity and specificity for two or more consecutive G4s of a multimeric system through a cooperative binding mechanism [141–143].

Based on the different considered structural geometry of the G4 multimerization, the design and the development of new effective ligands can be rationally divided into three major classes: (i) a one-to-one interaction (1:1), where each G4-ligand unit binds one monomer of the multimeric G4 structure; (ii) a one-to-two interaction (1:2), where the ligand intercalates itself between two consecutive G4-monomeric units stabilizing both at the same time; (iii) a two-to-one+one interaction (2:1+1), where the G4-ligand consists of two units one of which placed itself in the pocket between two consecutive G4s, interacting with both through π-stacking interactions, while the second G4L-unit, binds a single G4 structure, by exploiting different binding sites (Fig. 3). In all these cases, the proposed G4Ls take principally advantage of π-stacking interactions with terminal G-tetrads of each G4 unit, considering that this type of binding in general guarantees a strong stabilization of the target [144].

The presence of consecutive G4 units determines a binding possibility clearly different from a single G4 structure, which becomes increasingly obsolete for studying such systems. In fact, the existence of a G4-G4 interface, also called pocket task, is a highly characterizing element of multimeric G4s and provides an enormously advantageous preferential binding site for the design of new specific ligands, offering the possibility of improving their affinity than intrinsic selectivity.

So, in this chapter, we will discuss only the most recently developed G4-ligands, whose specific selectivity towards a multimeric G4-model with respect to a monomeric one has been demonstrated by biophysical or computational studies. Although G4Ls that bind multimeric G4 structures are increasingly being developed, in-depth studies on their binding mode and biological data are mainly available for multimeric telomeric G4s. In addition, studies on compounds that bind G4 multimers involved in neurodegenerative diseases are still in their early stages and principally involve molecules already known to bind monomeric G4s [130,138,140,145,146]. For these reasons, in the following chapters only compounds binding multimeric telomeric G4s will be presented.

4.1. One-to-one interaction

One-to-one interaction is based on the possibility that each binding unit can coordinate with a single G4 structure. This is mainly possible for large compounds that are unable to fit into the interface between two consecutive G4s. Although the geometric element characterizing these multimeric G4 structures is not fruitful, covalently linking several binding units, one after the other, has allowed achieving interesting results both from the point of view of specific selectivity and as a function of biological readouts.

This is the case, for example, of the Zinc (II) chiral-supramolecular complex **Ni-M** (Fig. 4), which exhibited an impressive selectivity towards high-ordered dimeric G4 over monomeric one [147]. Indeed, the enantiopure isomer **Ni-M** recognizes the dimeric G4 model G2T1 (sequence $AGGG(TTAGGG)_7$) with a 200-fold higher affinity ($Ka = 4.6 \pm 0.8 \times 10^7\ M^{-1}$ by fluorescence titration method and $Ka = 2.7 \pm 0.3 \times 10^7\ M^{-1}$ by ITC method) than the monomeric one, despite the authors demonstrated that it binds two consecutive G4 units, following a 1:1 stoichiometry, instead of inserting itself into the interface between two consecutive G4s. This binding geometry is probably due to the large size of this triple helicate structure, too big for the multimeric G4 pocket, as in the case of the dimeric dinickel(II)-salphen complex **1** (Fig. 4) [148].

As for **Ni-M**, fluorescent titration assays using a G2T1 sequence with fluorescent adenine isomers located on the four different exposed G-quartets (adenine residues position 7, 13, 31, and 37), highlighted the ability of **1** to bind G2T1 exploiting π-stacking interactions according to the one-to-one model here proposed [8]. In particular, to synthesize a

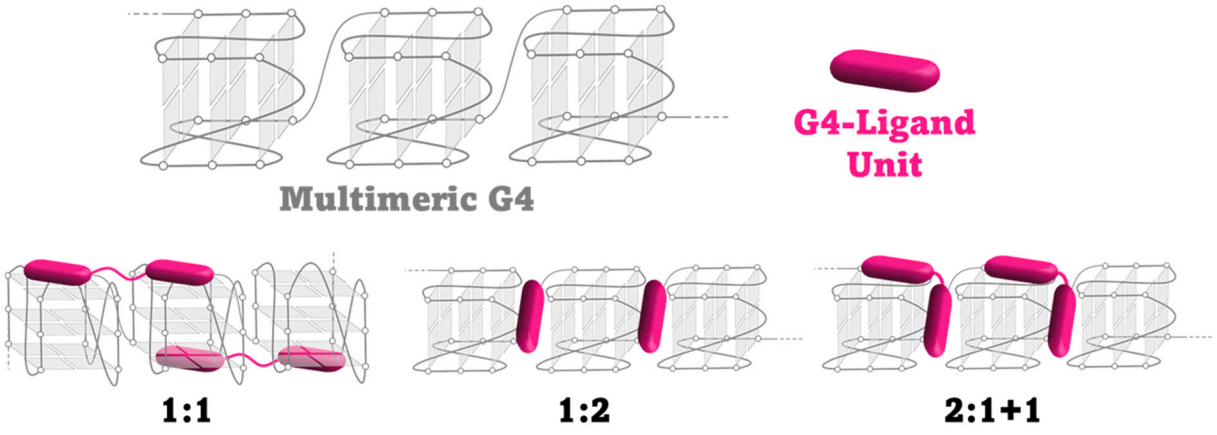

Fig. 3. Schematic representation of proposed binding interaction of G4Ls on multimeric G4s.

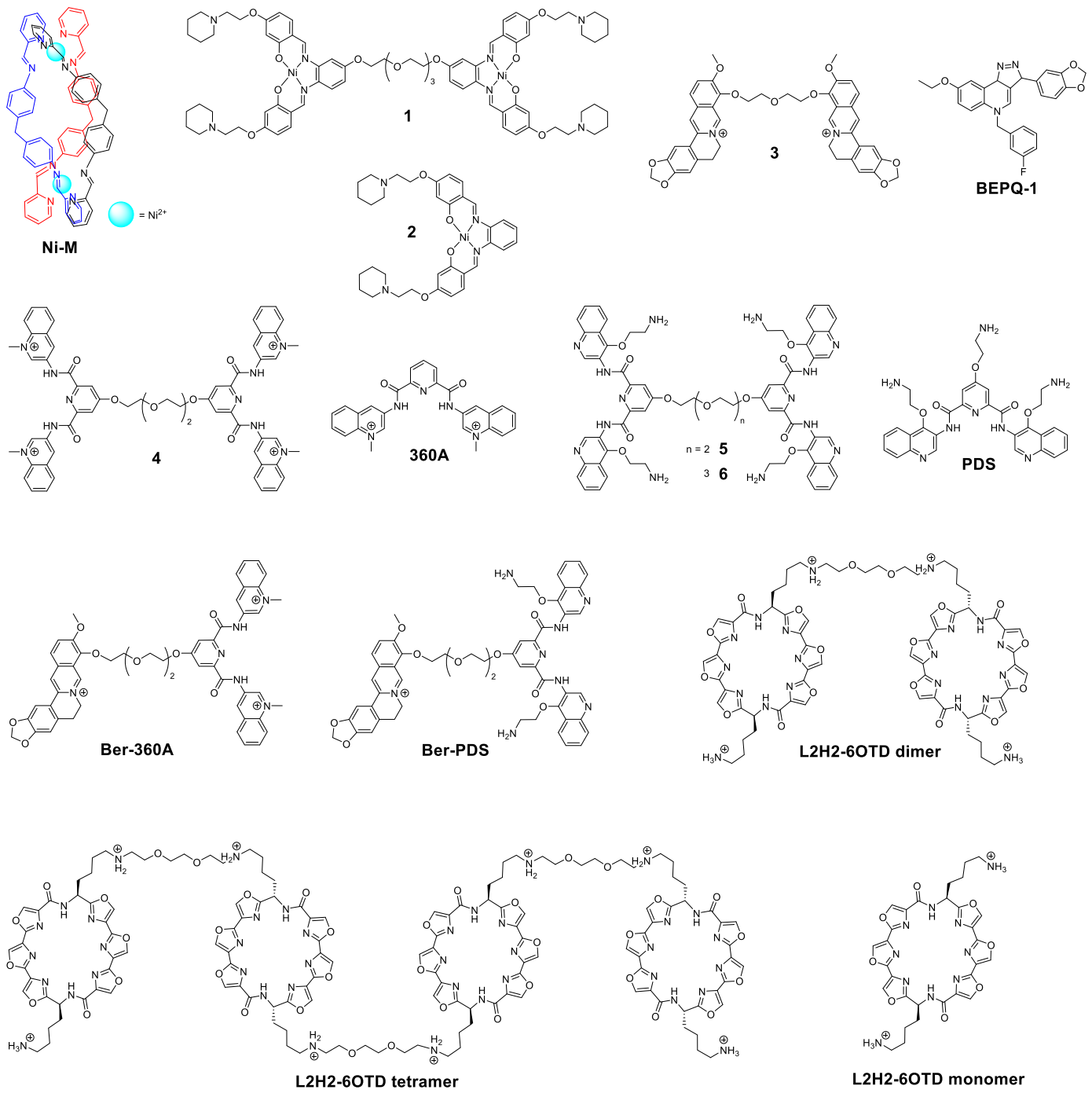

Fig. 4. Chemical structures of G4-ligands able to interact with G4 multimeric systems following a one-to-one binding mode.

molecule capable of interacting with the G2T1 dimeric model, the authors covalently linked two nickel(II)-salphen units, already widely known as good ligands for monomeric G4 [149], with polyethylene glycol (PEG) linkers of different lengths. Although CD-melting data highlighted that mono nickel(II)-salphen complex **2** (Fig. 4) higher stabilize both monomeric (G1) and dimeric (G2T1) G4 models than dinickel(II)-complex **1** (stabilization of 20 °C for **2** versus 14.1 °C for **1** in [G4L]:[G2T1] stoichiometry of 4:1 and 2:1 respectively), the latter showed a higher selectivity towards the dimeric G4 structure. Indeed, **1** showed a selectivity of 30-fold higher for antiparallel G2T1 (Ka = 3.2 ± 0.4 × 10^7 M^{-1} by UV/vis spectroscopy) and 6-fold higher for mixed-type G2T1 (Ka = 1.4 ± 0.3 × 10^7 M^{-1} by UV/vis spectroscopy), over the respective monomeric G1 (selectivity of **2** for (i) antiparallel G2T1 vs. G1 = 5; (ii) mixed-type G2T1 vs. G1 = 1) [148]. In addition, compound **1** shown encouraging micromolar cytotoxicity against HeLa and MCF-7 cancer cells with IC_{50} values by MTT assay of 5.7 ± 0.7 μM and 5.4 ± 0.6 μM respectively [150].

Extremely efficient in recognizing mixed-typed G2T1 (G2T1M) over antiparallel G2T1 (G2T1A) and both monomeric G1 (mixed-type and antiparallel) resulted to be the berberine dimer **3** (Fig. 4) [151]. Its shorter PEG linker allows it to have an impressive selectivity of 76-fold higher for G2T1M (Ka = 7.5 ± 0.1 × 10^8 M^{-1} by UV/vis spectroscopy) versus G2T1A and, even, 508-folded higher when compared to the monomeric system G1M. Despite the promising selectivity, **3** stabilizes G2T1M by only 11 °C and G2T1A by 8.3 °C ([**3**]:[G2T1] stoichiometry of 4:1) [151]. This is also reflected in lower levels of cytotoxicity against

HeLa and MCF-7 cancer cells with IC_{50} values by MTT assay of 38 ± 2 μM and > 50 μM respectively [150]. More interesting, however, are its fluorescent properties. Indeed, the intrinsic fluorescence of **3**, absent without the biological target, is 11-fold enhanced towards G2T1M than G1M, suggesting a promising application of this dimer as a highly selective fluorescent sensor for multimeric G4 systems [151].

Even more efficient as a switch-on sensor of multimeric G4 structures at telomeric level has proved to be the commercially available quinoline derivative **BEPQ-1** (Fig. 4), with one of the lowest detection limits reached to date, equal to 0.11 μM [152]. Its fluorescence is enhanced more than 20-fold in presence of htg45 (sequence $GGG(TTAGGG)_7$), with weak cytotoxicity in A549 cancer cells (75% cell viability after 12 h incubation at 10 μM concentration). Negligible fluorescence response was demonstrated with the addition of monomeric G4s, duplex DNA, and single-stranded DNA [152].

Covalently merging well-known G4-binding units to create a ligand more congenial for multimeric G4 systems, is the design that has also supported the development of the bisquinolinium dimer **4** (Fig. 4) [150].

Based on intriguing results obtained for **1** and **3**, in which two effective G4-binding units are linked by PEG spacer, the authors demostrated that three ethylene glycol (EG) repeats properly space the two bisquinolinium monomer **360A** (Fig. 4), exhibiting 60-fold higher binding selectivity towards G2T1A than the antiparallel G1 ($Ka = 4.3 \pm 0.1 \times 10^7\ M^{-1}$ by UV/vis spectroscopy). This result turned out to be very promising, considering that the corresponding **360A** monomer had a significantly lower degree of selectivity versus the multimeric structure (only 3-fold higher towards G2T1 versus G1) despite a comparable binding constant value ($Ka = 2.3 \pm 0.3 \times 10^7\ M^{-1}$ by UV/vis spectroscopy). In addition, dimer **4** reached a high stabilization of G2T1A, with a ΔT_m value higher than 28.1 °C in a molar ratio of [**4**]:[G2T1A] = 4:1, versus a negligible stabilization towards G1 and null towards duplex DNA. Stabilization comparison further emphasized the specificity of **4** versus multimeric G4s: indeed, **360A** efficiently stabilized both G1 and G2T1 structures, resulting in a 1.5-fold higher stabilization of G2T1 versus G1. Notably, the biological activity of compound **4** was also promising. It induced concentration-dependent inhibition of telomerase activity with an IC_{50} value by TRAP-LIG assay of 5.0 ± 0.2 μM, compared to IC_{50} of 9.0 ± 0.6 μM obtained with **360A**. Nonetheless, its activity in cells (cytotoxicity) evaluated by MTT assay against two different cancer cell lines was less (IC_{50} of 9.5 ± 2.0 μM and 23.9 ± 1.5 μM in HeLa and MCF-7 cancer cells, respectively) than that obtained with the corresponding monomer **360A** (IC_{50} of 6.4 ± 1.6 μM and 8.8 ± 2.0 μM in HeLa and MCF-7 cancer cells, respectively) [150]. The authors suggested that this difference may derive from the greater size and charge effect of the bisquinolinium dimer **4** compared to the **360A** monomer, which can impair transport within the cells.

Very recently, the same group demonstrated that the reasonable regulation of the PEG-linker length among the two binding units of the G4-ligand can modulate a better selectivity between G1T2A and G1T2M [153]. Indeed, dimeric Pyridostatine (PDS) compound **5** (Fig. 4), with a spacer of three EG units, showed higher binding affinity ($Ka = 4.0 \pm 0.5 \times 10^7\ M^{-1}$ by UV/vis spectroscopy and $Ka = 1.7 \pm 0.5 \times 10^7\ M^{-1}$ by ITC) towards G2T1A with 50-fold selectivity over G1A. On the contrary, PDS-dimer **6** (Fig. 4), spaced by four EG units, greatly prefers the mixed-type dimer G2T1M ($Ka\ of\ 2.3 \pm 0.4 \times 10^7\ M^{-1}$ by UV/vis spectroscopy and $Ka = 1.2 \pm 0.3 \times 10^7\ M^{-1}$ by ITC) with 39-fold selectivity over G1M. Comparing these data with the monomeric counterpart **PDS** (Fig.4), the higher binding selectivity of the PDS-dimers versus dimeric G4s over monomeric ones is tangible. **PDS** preferentially binds antiparallel G2T1 over G1 with a selectivity index of only 1-fold higher, while it has greater affinity towards the monomeric mixed G1 over G2T1M (G2T1M/G1M selectivity of 0.2). In a molar ratio of [PDS-dimers]:[G2T1] = 6:1, both these PDS-dimers can stabilize G1T2A with a 17- (for **5**) and 13-fold (for **6**) higher stabilization over G1, while only **6** can efficiently stabilize G1T2M (ΔT_m of 15.3 °C by CD-melting) slightly destabilizing G1M by −4.8 °C. Despite the monomeric **PDS** efficiently stabilized both G2T1A (ΔT_m = 30.2 °C) and G2T1M (ΔT_m = 11.3 °C), it also showed high stabilization of the monomeric G1A (ΔT_m = 34.7 °C) and G1M (ΔT_m > 30 °C), stressing once again no preference for multimeric G4 structures [153]. These PDS-dimers showed weaker telomerase inhibition than monomer **PDS** [154], though concentration-dependent inhibition of telomerase activity in a micromolar range was identified for both, with an estimation of IC_{50} by TRAP-LIG assay of 8.7 ± 0.6 μM and 3.6 ± 0.7 μM for **5** and **6** respectively (IC_{50} for **PDS** equal to 0.4 ± 0.05 μM) [153]. However, more in-depth studies on such dimers in cancer cells are still lacking.

Very recently, the same group developed two new promising G4L-dimers composed of a berberine unit (**Ber**) covalently linked to **360A** (**Ber-360A**, Fig.4) or **PDS** (**Ber-PDS**, Fig.4) moieties to simultaneously improve stabilization (by **360A** or **PDS**) and detection sensitivity (by **Ber**) of dimeric/multimeric G4s in vitro as well as in cells [155]. The one-to-one binding mode towards two adjacent G4 of the G2T1structures (antiparallel and mixed-type) was confirmed by spectrophotometric titrations, fluorescent titrations with Ap-labelled G4s, as well as by in silico studies for both **Ber-360A** and **Ber-PDS**. Following the previously demonstrated data for the monomers **360A** [150] and **PDS** [153], **Ber-360A** showed a higher stabilization for antiparallel G2T1 (ΔT_m = 26.2 °C by CD-melting with $Ka = 19.5 \pm 5.8\ \mu M^{-1}$ by fluorescent titrations) than mixed-type G2T1 ($\Delta T_m \sim 11$ °C by CD-melting with $Ka = 16.9 \pm 4.7\ \mu M^{-1}$ by fluorescent titrations), as well as **Ber-PDS** revealed a comparable stabilization of G2T1A ($\Delta T_m \sim 20$ °C by CD-melting with $Ka = 21.8 \pm 4.0\ \mu M^{-1}$ by fluorescent titrations) and G1T2M (ΔT_m = 15.8 °C by CD-melting with $Ka = 11.1 \pm 1.0\ \mu M^{-1}$ by fluorescent titrations). In addition, both the G4L-dimers proved higher selectivity for G2T1 structures compared to different monomeric G4s (G1A, G1M, ckit-1, ckit-2, cmyc) as well as dsDNA [155]. These results become even more promising by analyzing the quantum yield of these dimeric ligands, able to significantly ignite their fluorescence response only in the presence of dimeric G4 structures G2T1, which is kept constant even after adding an excess of 6 equivalents of mixed-type or antiparallel G1 as a competitor. Between the two dimeric ligands, **Ber-PDS** evinced higher quantum yield values for both dimeric G4 structures, i.e. Φx = 0.54 for G2T1A (2.1-fold higher than **Ber-360A**) and Φx = 0.26 for G2T1M (2.4-fold higher than **Ber-360A**), with an extraordinary subnanomolar detection limit for G2T1A (0.44 nM) and a nanomolar one for G2T1M [155]. Even more interesting, while **Ber-360A** appears to remain mainly in the cytoplasm, probably binding RNA structures, **Ber-PDS** induced a strong emission response localized in the nucleoli of lived and fixed HeLa cells. This fluorescence response is quenced only upon addition of 8 equivalents of the well-known G4 ligand **BRACO-19** (80 μM) [144], which competes for the binding to the G4 target. In addition to being promising probes, both these dimeric G4Ls have shown concentration-dependent telomerase inhibition with IC_{50} values by TRAP-LIG assay of 1.2 ± 0.2 μM and 0.8 ± 0.2 μM, respectively, for **Ber-360A** and **Ber-PDS**, values comparable with those of **360A** ($IC_{50} = 3.2 \pm 0.6$ μM) and **PDS** ($IC_{50} = 0.4 \pm 0.1$ μM) monomers (Fig.4). Both **Ber-360A** and **Ber-PDS** also proved higher anticancer activities against HeLa (IC_{50} of 10.9 ± 1.2 μM and 9.2 ± 0.8 μM, respectively) and HepG2 (IC_{50} of 12.9 ± 2.2 μM and 10.1 ± 0.3 μM respectively) cancerous cells than the normal L02 cell line (IC_{50} of 117.8 ± 5.2 μM and 22.9 ± 1.2 μM, respectively) by MTT assay, although only **Ber-360A** has an effective therapeutic window towards cancerous cell lines A549 (IC_{50} = 36.3 ± 2.7 μM), MDA-MB-231 (IC_{50} = 27.3 ± 2.8 μM), and MCF-7 (IC_{50} = 24.1 ± 2.1 μM) [155]. Taken together, these results lay interesting bases for the use of these two conjugates for theranostic purposes.

Among all compounds presented here, the most extended G4L system is certainly represented by the tetrameric Telomestatin derivative **L2H2-6OTD tetramer** (Fig. 4) [156]. While **L2H2-6OTD monomer** and **L2H2-6OTD dimer** (Fig.4) efficiently bind and stabilize monomeric telomeric G4 (sequence $(TTAGGG)_4TTA$), with comparable bound fraction (respectively of 56.4% and 57.4%) and stabilization

(respectively of 11.0 °C and 14.7 °C by UV-melting), the tetrameric derivative **L2H2-6OTD tetramer** is completely inefficient in binding monomeric G4 (bound fraction of 9.7%), so much so that UV-melting data show no variation in stabilization of such structure. On the contrary, about 50% of **L2H2-6OTD tetramer** is occupied in binding with the multimeric G4 target if we consider a hexameric G4 model, highlighting its ability in binding multimeric G4 system 40-fold more efficiently concerning shorter G4 sequences. This trend was also confirmed by CD-melting results, from which it is shown the stabilization induced by **L2H2-6OTD tetramer** of 18.2 °C for a dimeric G4 model until 30.1 °C for a tetrameric G4 one [156]. In addition, while **L2H2-6OTD monomer** and **L2H2-6OTD dimer** showed similar affinities for monomeric or hexameric G4 systems (K_d of 14.2 ± 1.2 nM and 20.6 ± 1.8 nM, respectively, for **L2H2-6OTD monomer**, and K_d values of 13.8 ± 1.4 nM and 17.9 ± 2.1 nM, respectively, for **L2H2-6OTD dimer**), **L2H2-6OTD tetramer** showed more than 43-fold higher affinity for the hexameric G4 system (K_d = 16.2 ± 2.8 nM), than for the monomeric G4 (K_d = ~700 ± 50 nM). The selectivity that this multimeric binder system guarantees is even more interesting. The authors showed that, unlike the monomeric and dimeric derivatives, the tetrameric derivative is not able to efficiently bind the monomeric G4 of the Bcl2 promoter sequence, further emphasizing that it is possible to recognize only multimeric G4 systems compared to monomeric systems of different nature by appropriately managing the concentration of **L2H2-6OTD tetramer** used [156]. These results can be well explained by considering a polyvalent binding mechanism of **L2H2-6OTD tetramer**, where the one-to-one interaction mode G4 vs G4L is cooperative for subsequent interactions, increasing the overall stabilization of the target the more the number of consecutive G4s present increases. Nevertheless, its high molar mass does not make the tetramer in its current form ideally functional for cellular applications, despite the cellular uptake could be addressed by exploiting additive specific delivery techniques.

4.2. One-to-two interaction

As anticipated, the distinct structural element of multimeric G4s is the interface between two consecutive G4s, also known as "binding pocket", which become an intriguing and unique site for the development of selective G4-ligands for these particular repeated secondary structures. Therefore, many research groups focused their attention on molecules with a large planar aromatic portion, known as capable of interacting with G4s through π-π stacking to force this type of interaction, and among all, Sugiyama et al. were the first to validate the feasibility of this strategy exploiting a new molecular entity. Cyclic helicene **M1** (Fig. 5), indeed, arose highly selective towards G2T1 dimeric G4 structure without particularly interacting with monomeric ones. This result was highlighted by 70% decreased CD intensity and 80% fluorescence quenching of **M1** in the presence of G2T1 [157]. Nevertheless, comparable dissociation constants (K_D) between **M1** and G2T1 or G1 were estimated by surface plasmon resonance (433 ± 13 nM and 541 ± 11 nM, respectively). Molecular modeling studies underline that the ability to fit perfectly into the binding pocket between two G4s lies in the orientation of the left-handed helicene and on the specific shape of **M1**. This strong interaction revealed potent dose-dependent telomerase inhibition, by TRAP assay, which was complete at 0.5 μM compound [157].

Considering that the porphyrin core is almost the same size as a G-quartet, several derivatives of it have been developed, including ***p-***

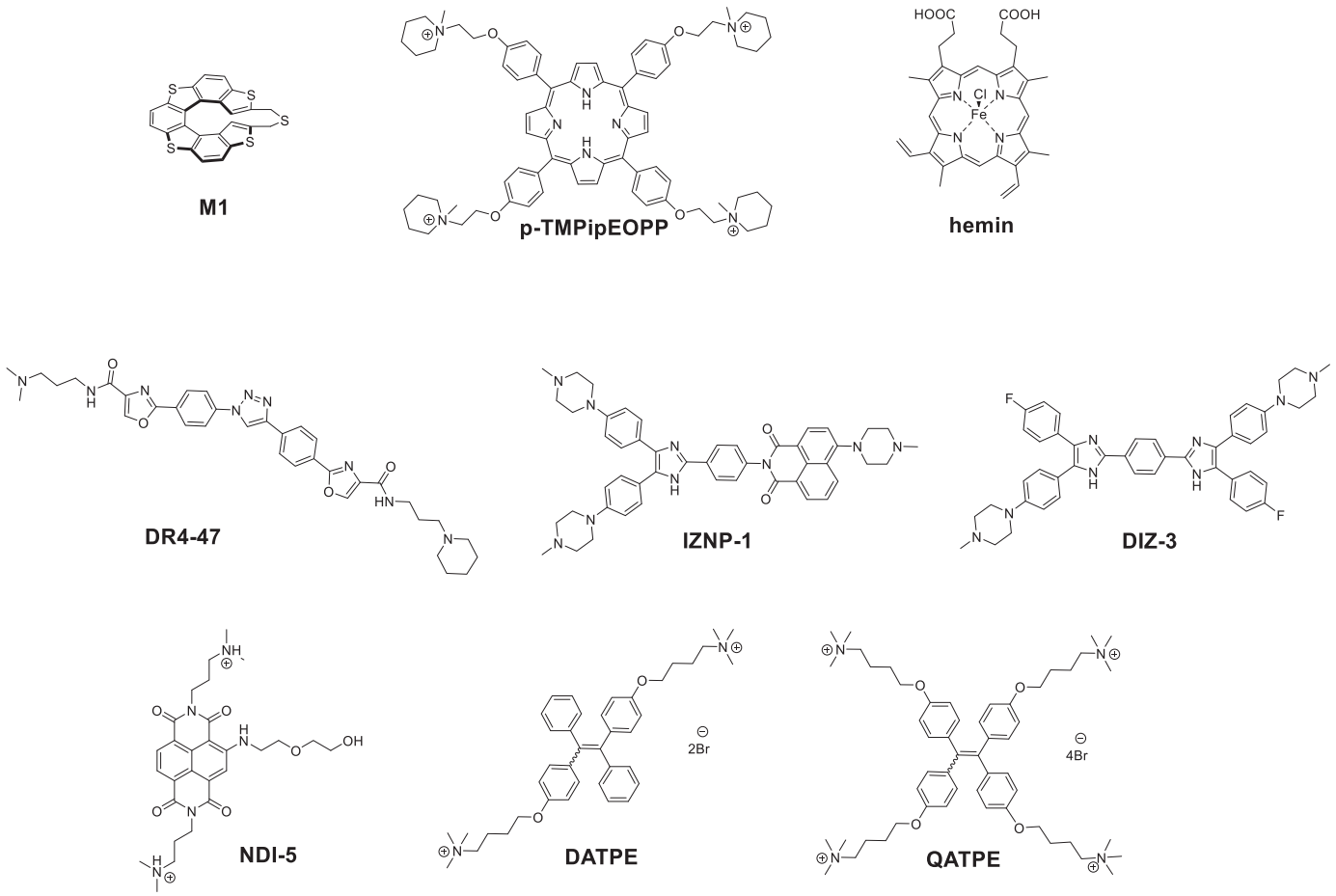

Fig. 5. Chemical structures of G4-ligands able to interact with G4 multimeric systems following a one-to-two binding mode.

TMPipEOPP (Fig. 5), which showed high selectivity towards G4s over duplex, single-stranded, and triplex DNAs [158]. ***p*-TMPipEOPP** interacts with telomeric multimeric G4s adopting two different binding modes as a function of the dimension of the loop that connects two adjacent G4s: sandwich-like end-stacking mode between two different G4 structures, not covalently linked together, and a pocket-intercalating mode (here indicated as one-to-two interaction) [158]. While this ligand can bind monomeric G4s following a sandwich-like end-stacking mode with a [***p*-TMPipEOPP**]:[monomeric G4s] stoichiometry of 1:2, a 1:1 stoichiometry with the single one-to-two binding mode was found only with the Hum59 sequence ($TAGGG(TTAGGG)_9$), in which the two G4 units are spaced by a large loop of 15 nucleotides ($Ka = 0.62 \times 10^6\ M^{-1}$ by UV/vis titration spectra). Despite ***p*-TMPipEOPP** interacts differently with monomeric and dimeric G4s, it induces an almost identical stabilization, thus in the end being not particularly selective for multimeric structures [158].

Physiological **hemin** (Fig. 5) also prefers the interface between two consecutive G4s as a preferential binding site on the multimeric G4 systems, as suggested by higher **hemin**-related DNAzyme activity which develops in this region [159]. Indeed, a strong difference in catalytic behavior of ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) oxidation was found depending on the monomeric (22AG), dimeric (46AG), or trimeric (70AG) model of the considered telomeric G4 in the presence of **hemin** and hydrogen peroxide. This is principally due to the higher hydrophobic environment of the "binding pocket" and to its ability to better protect **hemin** against H_2O_2-degradation, being an "internal" interaction site. Considering a 1:1 stoichiometry between **hemin** (1 μM) and DNA (strand concentration of 1 μM), kinetic experiments were compared identifying the initial rates of the catalytic process which turned out as 5.6- and 3.4-fold higher in the presence of 70AG ($v_0 = 1.51\ \mu M min^{-1}$) and 46AG ($v_0 = 0.93\ \mu M min^{-1}$) respectively than in the presence of 22AG ($v_0 = 0.27\ \mu M min^{-1}$) [159].

Contextually, inspired by the important role that triazole moieties have exhibited on G4s stabilization [160], telomerase inhibition, and anticancer activity [161,162], Moses et al. developed a hybrid oxazole-triazole ligand **DR4–47** to achieve the preferential stabilization of the ligand-induced parallel folded structure of telomeric multimeric G-quadruplexes (Fig. 5) [163]. Using molecular dynamics simulations and CD assays, the authors suggested that **DR4-47** is capable to interact between adjacent G4 units in telomeric multimeric G4s through π-π stacking, and due to extra electrostatic interaction between the protonable side chains and the groove. These structural peculiarities contributed to the specific binding of **DR4-47** to telomeric multimeric G-quadruplexes ($\Delta T_m > 33.5$ °C in the presence of G2T1 by CD-melting experiments) making it an interesting starting point for future developments [163].

A more close-fitting complex between the binding pocket and a new G4-ligand entity was obtained by Huang et al. with the development of the triaryl-substituted imidazole derivative **IZNP-1** (Fig. 5) [86]. **IZNP-1** resulted in a particular selectivity towards multimeric over monomeric G4 models thanks to its ability to intercalate into the pocket between two consecutive G4s. Indeed, while K_D in the presence of monomeric G4 was not determinable due to the weak enhancement of fluorescence attributable to negligible interaction, high dissociation constant values were identified in the presence of multimeric htg45 (7.8 μM), htg51 (11.0 μM), and htg57 (13.3 μM). This selectivity is also reflected in its ability to stabilize the multimeric structures (ΔT_m values from 20 °C to 33 °C by CD-melting) over monomeric htg21 ($\Delta T_m = 6$ °C by CD-melting) [86]. Interesting, **IZNP-1** was slightly more active in cancerous cells (IC_{50} of 5.4 and 6.5 μM on Siha and A549 cancer cells after 24 h) than in healthy ones (IC_{50} of 13.3 μM on normal BJ fibroblasts after 24 h), inducing cell cycle arrest and cellular apoptosis in a dose-dependent manner (apoptotic cell populations equal to 26.6% and 63.0% respectively in the early and late stage of Siha cells treatment with 10 μM of ligand for 12 h). In addition, **IZNP-1** also induced telomere shortening and cell senescence. Strictly related to telomeric DNA damage and telomere dysfunction, a significant increase of phosphorylated H2AX (γH2AX) foci, of which 62% co-localized with TRF2 protein, was proven, highlighting the high selectivity of this ligand damage at the telomere level (for comparison, γH2AX foci induced by **BRACO-19** co-localize with TRF2 protein for only the 39%) [86]. Enthralling, **IZNP-1** impressive selectivity towards multimeric G4s was also confirmed by its inability in downregulating the transcription of different genes that present monomeric G4s in their promoter region [86].

Inspired by the above excellent results, Hu et al. synthesized another dimeric aryl-substituted imidazole derivative **DIZ-3** (Fig. 5) [164], which exhibited high binding ability ($K_D = 4.6$ μM) and stabilizing effect ($\Delta T_m = 12.7$ °C by CD-melting) towards dimeric htg45 over monomeric G4 ($\Delta T_m = 1.7$ °C). The reported binding study of **DIZ-3** confirms the selective interaction with the binding site located at the pocket interface. Furthermore, **DIZ-3** showed dose-dependent inhibitory activity on U2OS cancer cells (IC_{50} of 2.1 μM) with a cytotoxic effect of an order of magnitude higher than that obtained on normal BJ fibroblasts (IC_{50} of 29.3 μM). The accumulation of cells in the S phase (from 24.0% to 32.2%) of U2OS cancer cells after 24 h treatment with **DIZ-3**, as well as the increase of apoptotic cells (from 10.1% to 24.9%) pointed out how **DIZ-3** is a good candidate for inducing telomeric DNA damage in cancer cells. As **IZNP-1**, also **DIZ-3** did not affect transcription of other genes that contain monomeric G4 in their promoter, consolidating the high selectivity of this structure for multimeric G4 systems [164].

More recently, a novel monomeric naphthalene diimide, **NDI-5** has been investigated as a binder of multimeric G4s [165]. **NDI-5** showed the capacity to bind the pocket between the two consecutive G4 units of Tel46 both in biophysical assays and docking simulations with very good affinity ($Ka_1 = 1.6 \pm 0.9 \times 10^7\ M^{-1}$ by ITC method). This affinity has important repercussions in terms of stabilization (ΔT_m of 12 °C by CD-melting) and from the biological point of view. An increase of γH2AX (of around 3-fold with 1 μM **NDI-5**) on transformed human fibroblasts (BJ-EHLT), completely absent in normal fibroblasts, as well as an elective and high cytotoxic effect in cancer cells (IC_{50} of 79 nM on HeLa cells), highlighted the significant specificity and selectivity of this ligand against cancer cells [166].

In addition, different research groups focused their attention on the development of G4 selective fluorescent sensors. Among all, sensors with unique aggregation-induced emission properties seem to be able to be exploited as fluorescent probes for label-free detection of G4 formation. Their switch-on mechanism makes them suitable for the targeting of the multimeric binding pocket. In this field, Kong et al. developed two tetraphenylethene (TPE) derivatives **DATPE** and **QATPE** (Fig. 5), analyzing their recognition properties and the abilities to selective turn on their emission upon binding [167]. **DATPE**, with two positively charged side arms, exhibited a very selective fluorescence response towards telomeric multimeric G4s than **QATPE**. In addition, **DATPE** showed a very low level of cytotoxicity and no effect on multimeric G4 stability, emphasizing its possible use as an efficient fluorescent probe to detect multimeric G4s in vivo.

4.3. *Two-to-one+one interaction*

The main advantage of this binding mode lies in the possibility of taking advantage of a second additive interaction (e.g. grooves intercalation) to the characteristic π-stacking binding pinpointed in the pocket interface between two consecutive G4s.

This is the behavior adopted by the recently proposed naphthalene diimide (NDIs) dimers **7–8** (Fig. 6) with a long alkyl spacer [68]. While one NDI unit (preferentially the tri-substituted ones) perfectly fits the pocket task between two consecutive G4s, the second unit facilitates additional interactions with the regions flanking the pocket highlighting a 1:1 complex with htg45 model. In particular, the positive charged lateral pendant of **8** further increase htg45 stabilization through electrostatic and stacking interaction between the ammonium group and the phosphate backbone or the nucleotide basis respectively [68]. This

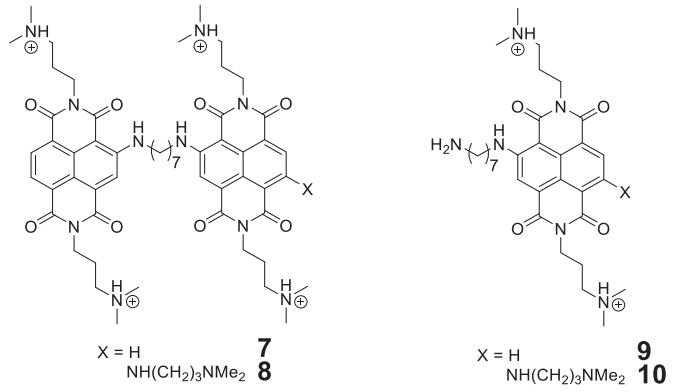

Fig. 6. Chemical structures of dimeric NDIs able to interact with G4 multimeric systems following a two-to-one+one binding mode.

additive binding mode gives rise to impressive cytotoxicity against tumor cell lines of different histological origins, exhibiting nanomolar IC_{50} values in the case of **7** (IC_{50} ranging from 8.6 nM against U2OS cells to 1.31 nM against MDA-MB231 cells) and, even, subnanomolar IC_{50} values in the case of **8** (IC_{50} ranging from 0.85 nM against HT29 cells to 0.085 nM against MDA-MB231 cells) [168]. This one-order magnitude difference may lie in the greater selectivity of **8** for htg45 (binding constant calculated per site of interaction equal to LogKa = 7.11) over the monomeric model hTel22 (LogKa = 5.82) compared to **7** (LogKa equal to 6.62 and 6.44 respectively for hTel45 and hTel22) [68]. Two-to-one+one interaction mode resulted to be fundamental for obtaining such cytotoxic effects against cancer cells: indeed the corresponding monomeric NDIs **9** and **10** (Fig. 6) exhibited considerably inferior IC_{50} values, ranging from <10 nM against MDA-MB231 cells in the presence of **9** until 200 nM against HT29 in the presence of **10**.

In addition, both these two NDI-dimers resulted to be powerful inducers of DNA damage response as well as telomere dysfunction-induced foci, revealing certain selectivity for cancer versus normal cells [168].

5. Conclusions

G4s can fold into numerous multimeric structures, ranging in size and spatial distribution on the nucleic acid strand. The multimerization process creates highly ordered structures, whose structural requirements have been recently elucidated in vitro. At the human cellular level, pivotal studies have associated the presence of multimeric G4s with remarkable biological roles, such as the regulation of cellular growth and senescence. Multimeric G4s have also been characterized as causative factors in crucial neurological disorders. A deeper understanding of their biological role will be crucial for unveiling how multimeric G4s are involved in the pathogenesis of diseases such as neurodegeneration and cancer. Given their unique structural properties, multimeric G4s have become a feasible target for the design of innovative drug-like compounds. In-depth studies are looked-for to uncover the role of G4s and the cellular players involved in disease establishment. These approaches will also show previously unknown players in the intricate etiology of cancer processes and neurodegenerative disorders, while prompting the development of novel therapeutic strategies.

Funding

This work was supported by the Italian Foundation for Cancer Research (AIRC; 21850 to S.N.R.)

Declaration of competing interest

none declared.

References

[1] J.D. Watson, F.H. Crick, Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid, Nature 171 (1953) 737–738, https://doi.org/10.1038/171737a0.
[2] M.H.F. Wilkins, H.R. Wilson, L.D. Hamilton, Secondary structures of DNA*, Proc. Natl. Acad. Sci. U. S. A. 65 (1970) 761–762 (accessed December 29, 2021), https://www.ncbi.nlm.nih.gov/pmc/articles/PMC282971/.
[3] M.H. Wilkins, S. Arnott, D.A. Marvin, L.D. Hamilton, Some misconceptions on Fourier analysis and Watson-Crick base pairing, Science 167 (1970) 1693–1694, https://doi.org/10.1126/science.167.3926.1693.
[4] D.A. Marvin, M. Spencer, M.H.F. Wilkins, L.D. Hamilton, A new configuration of deoxyribonucleic acid, Nature 182 (1958) 387–388, https://doi.org/10.1038/182387b0.
[5] R.K. Ralph, W.J. Connors, H.G. Khorana, Secondary structure and aggregation in deoxyguanosine oligonucleotides, J. Am. Chem. Soc. 84 (1962) 2265–2266, https://doi.org/10.1021/ja00870a055.
[6] M. Gellert, M.N. Lipsett, D.R. Davies, Helix formation by guanylic acid, Proc. Natl. Acad. Sci. U. S. A. 48 (1962) 2013–2018, https://doi.org/10.1073/pnas.48.12.2013.
[7] T.M. Bryan, P. Baumann, G-quadruplexes: from guanine gels to chemotherapeutics, Mol. Biotechnol. 49 (2011) 198–208, https://doi.org/10.1007/s12033-011-9395-5.
[8] D. Sen, W. Gilbert, Formation of parallel four-stranded complexes by guanine-rich motifs in DNA and its implications for meiosis, Nature 334 (1988) 364–366, https://doi.org/10.1038/334364a0.
[9] W.I. Sundquist, A. Klug, Telomeric DNA dimerizes by formation of guanine tetrads between hairpin loops, Nature 342 (1989) 825–829, https://doi.org/10.1038/342825a0.
[10] S. Burge, G.N. Parkinson, P. Hazel, A.K. Todd, S. Neidle, Quadruplex DNA: sequence, topology and structure, Nucleic Acids Res. 34 (2006) 5402–5415, https://doi.org/10.1093/nar/gkl655.
[11] K. Li, L. Yatsunyk, S. Neidle, Water spines and networks in G-quadruplex structures, Nucleic Acids Res. 49 (2021) 519–528, https://doi.org/10.1093/nar/gkaa1177.
[12] R. Perrone, M. Nadai, I. Frasson, J.A. Poe, E. Butovskaya, T.E. Smithgall, M. Palumbo, G. Palù, S.N. Richter, A dynamic G-quadruplex region regulates the HIV-1 long terminal repeat promoter, J. Med. Chem. 56 (2013) 6521–6530, https://doi.org/10.1021/jm400914r.
[13] E. Lavezzo, M. Berselli, I. Frasson, R. Perrone, G. Palù, A.R. Brazzale, S.N. Richter, S. Toppo, G-quadruplex forming sequences in the genome of all known human viruses: a comprehensive guide, PLoS Comput. Biol. 14 (2018), e1006675, https://doi.org/10.1371/journal.pcbi.1006675.
[14] I. Frasson, M. Nadai, S.N. Richter, Conserved G-quadruplexes regulate the immediate early promoters of human alphaherpesviruses, Mol. Basel Switz. 24 (2019), https://doi.org/10.3390/molecules24132375.
[15] E. Ruggiero, I. Zanin, M. Terreri, S.N. Richter, G-quadruplex targeting in the fight against viruses: an update, Int. J. Mol. Sci. 22 (2021) 10984, https://doi.org/10.3390/ijms222010984.
[16] J. Spiegel, S. Adhikari, S. Balasubramanian, The structure and function of DNA G-quadruplexes, Trends Chem. 2 (2020) 123–136, https://doi.org/10.1016/j.trechm.2019.07.002.
[17] M.L. Bochman, K. Paeschke, V.A. Zakian, DNA secondary structures: stability and function of G-quadruplex structures, Nat. Rev. Genet. 13 (2012) 770–780, https://doi.org/10.1038/nrg3296.
[18] G. Biffi, D. Tannahill, J. McCafferty, S. Balasubramanian, Quantitative visualization of DNA G-quadruplex structures in human cells, Nat. Chem. 5 (2013) 182–186, https://doi.org/10.1038/nchem.1548.
[19] J.R. Williamson, M.K. Raghuraman, T.R. Cech, Monovalent cation-induced structure of telomeric DNA: the G-quartet model, Cell 59 (1989) 871–880, https://doi.org/10.1016/0092-8674(89)90610-7.
[20] E. Henderson, C.C. Hardin, S.K. Walk, I. Tinoco, E.H. Blackburn, Telomeric DNA oligonucleotides form novel intramolecular structures containing guanine-guanine base pairs, Cell 51 (1987) 899–908, https://doi.org/10.1016/0092-8674(87)90577-0.
[21] T.A. Brooks, L.H. Hurley, Targeting MYC expression through G-quadruplexes, GenesCancer 1 (2010) 641–649, https://doi.org/10.1177/1947601910377493.
[22] J. Dickerhoff, J. Dai, D. Yang, Structural recognition of the MYC promoter G-quadruplex by a quinoline derivative: insights into molecular targeting of parallel G-quadruplexes, Nucleic Acids Res. 49 (2021) 5905–5915, https://doi.org/10.1093/nar/gkab330.
[23] E. Buglione, D. Salerno, C.A. Marrano, V. Cassina, G. Vesco, L. Nardo, M. Dacasto, R. Rigo, C. Sissi, F. Mantegazza, Nanomechanics of G-quadruplexes within the promoter of the KIT oncogene, Nucleic Acids Res. 49 (2021) 4564–4573, https://doi.org/10.1093/nar/gkab079.
[24] Y.-J. Shin, V. Kumarasamy, D. Camacho, D. Sun, Involvement of G-quadruplex structures in regulation of human RET gene expression by small molecules in human medullary thyroid carcinoma TT cells, Oncogene 34 (2015) 1292–1299, https://doi.org/10.1038/onc.2014.65.
[25] X. Cui, H. Chen, Q. Zhang, M. Xu, G. Yuan, J. Zhou, Exploration of the structure and recognition of a G-quadruplex in the her2 proto-oncogene promoter and its transcriptional regulation, Sci. Rep. 9 (2019) 3966, https://doi.org/10.1038/s41598-019-39941-5.
[26] N. Kosiol, S. Juranek, P. Brossart, A. Heine, K. Paeschke, G-quadruplexes: a promising target for cancer therapy, Mol. Cancer 20 (2021) 40, https://doi.org/10.1186/s12943-021-01328-4.

[27] S. Cogoi, A. Ferino, G. Miglietta, E.B. Pedersen, L.E. Xodo, The regulatory G4 motif of the Kirsten ras (KRAS) gene is sensitive to guanine oxidation: implications on transcription, Nucleic Acids Res. 46 (2018) 661–676, https://doi.org/10.1093/nar/gkx1142.
[28] S. Lago, F.M. Cernilogar, M. Kazerani, H.D. Moreno, M. Nadai, G. Schotta, S. N. Richter, Promoter G-quadruplexes and Transcription Factors Cooperate to Shape the Cell Type-specific Transcriptome, BioRxiv, 2020, https://doi.org/10.1101/2020.08.27.236778, 2020.08.27.236778.
[29] S. Lago, M. Nadai, E. Ruggiero, M. Tassinari, M. Marušič, B. Tosoni, I. Frasson, F. M. Cernilogar, V. Pirota, F. Doria, J. Plavec, G. Schotta, S.N. Richter, The MDM2 inducible promoter folds into four-tetrad antiparallel G-quadruplexes targetable to fight malignant liposarcoma, Nucleic Acids Res. 49 (2021) 847–863, https://doi.org/10.1093/nar/gkaa1273.
[30] J. Spiegel, S.M. Cuesta, S. Adhikari, R. Hänsel-Hertsch, D. Tannahill, S. Balasubramanian, G-quadruplexes are transcription factor binding hubs in human chromatin, Genome Biol. 22 (2021) 117, https://doi.org/10.1186/s13059-021-02324-z.
[31] K.G. Zyner, D.S. Mulhearn, S. Adhikari, S. Martínez Cuesta, M. Di Antonio, N. Erard, G.J. Hannon, D. Tannahill, S. Balasubramanian, Genetic interactions of G-quadruplexes in humans, elife 8 (2019), e46793, https://doi.org/10.7554/eLife.46793.
[32] P. Prorok, M. Artufel, A. Aze, P. Coulombe, I. Peiffer, L. Lacroix, A. Guédin, J.-L. Mergny, J. Damaschke, A. Schepers, C. Cayrou, M.-P. Teulade-Fichou, B. Ballester, M. Méchali, Involvement of G-quadruplex regions in mammalian replication origin activity, Nat. Commun. 10 (2019) 3274, https://doi.org/10.1038/s41467-019-11104-0.
[33] A.V. Pavlova, E.A. Kubareva, M.V. Monakhova, M.I. Zvereva, N.G. Dolinnaya, Impact of G-quadruplexes on the regulation of genome integrityDNA Damage and Repair, Biomolecules 11 (2021) 1284, https://doi.org/10.3390/biom11091284.
[34] A. De Magis, S.G. Manzo, M. Russo, J. Marinello, R. Morigi, O. Sordet, G. Capranico, DNA damage and genome instability by G-quadruplex ligands are mediated by R loops in human cancer cells, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 816–825, https://doi.org/10.1073/pnas.1810409116.
[35] T.M. Bryan, Mechanisms of DNA replication and repair: insights from the study of G-quadruplexes, Molecules 24 (2019) 3439, https://doi.org/10.3390/molecules24193439.
[36] J. Zell, F.R. Sperti, S. Britton, D. Monchaud, DNA folds threaten genetic stability and can be leveraged for chemotherapy, RSC Chem. Biol. 2 (2021) 47–76, https://doi.org/10.1039/D0CB00151A.
[37] A.N. Khristich, S.M. Mirkin, On the wrong DNA track: molecular mechanisms of repeat-mediated genome instability, J. Biol. Chem. 295 (2020) 4134–4170, https://doi.org/10.1074/jbc.REV119.007678.
[38] C.K. Kwok, A.B. Sahakyan, S. Balasubramanian, Structural analysis using SHALiPE to reveal RNA G-quadruplex formation in human precursor MicroRNA, Angew. Chem. Int. Ed Engl. 55 (2016) 8958–8961, https://doi.org/10.1002/anie.201603562.
[39] A.B. Sahakyan, P. Murat, C. Mayer, S. Balasubramanian, G-quadruplex structures within the 3' UTR of LINE-1 elements stimulate retrotransposition, Nat. Struct. Mol. Biol. 24 (2017) 243–247, https://doi.org/10.1038/nsmb.3367.
[40] P. Murat, G. Marsico, B. Herdy, A.T. Ghanbarian, G. Portella, S. Balasubramanian, RNA G-quadruplexes at upstream open reading frames cause DHX36- and DHX9-dependent translation of human mRNAs, Genome Biol. 19 (2018) 229, https://doi.org/10.1186/s13059-018-1602-2.
[41] C.E. Kaiser, N.A. Van Ert, P. Agrawal, R. Chawla, D. Yang, L.H. Hurley, Insight into the complexity of the i-motif and G-quadruplex DNA structures formed in the KRAS promoter and subsequent drug-induced gene repression, J. Am. Chem. Soc. 139 (2017) 8522–8536, https://doi.org/10.1021/jacs.7b02046.
[42] A.M. Fleming, J. Zhou, S.S. Wallace, C.J. Burrows, A role for the fifth G-track in G-quadruplex forming oncogene promoter sequences during oxidative stress: do these "spare tires" have an evolved function? ACS Cent. Sci. 1 (2015) 226–233, https://doi.org/10.1021/acscentsci.5b00202.
[43] J.T. Grün, A. Blümler, I. Burkhart, J. Wirmer-Bartoschek, A. Heckel, H. Schwalbe, Unraveling the kinetics of spare-tire DNA G-quadruplex folding, J. Am. Chem. Soc. 143 (2021) 6185–6193, https://doi.org/10.1021/jacs.1c01089.
[44] Y. Cui, D. Kong, C. Ghimire, C. Xu, H. Mao, Mutually exclusive formation of G-quadruplex and i-motif is a general phenomenon governed by steric hindrance in duplex DNA, Biochemistry 55 (2016) 2291–2299, https://doi.org/10.1021/acs.biochem.6b00016.
[45] S. Dhakal, Z. Yu, R. Konik, Y. Cui, D. Koirala, H. Mao, G-quadruplex and i-motif are mutually exclusive in ILPR double-stranded DNA, Biophys. J. 102 (2012) 2575–2584, https://doi.org/10.1016/j.bpj.2012.04.024.
[46] D.H. Turner, Thermodynamics of base pairing, Curr. Opin. Struct. Biol. 6 (1996) 299–304, https://doi.org/10.1016/s0959-440x(96)80047-9.
[47] D.P. Bartel, MicroRNAs: target recognition and regulatory functions, Cell 136 (2009) 215–233, https://doi.org/10.1016/j.cell.2009.01.002.
[48] O. Igoucheva, V. Alexeev, K. Yoon, Differential cellular responses to exogenous DNA in mammalian cells and its effect on oligonucleotide-directed gene modification, Gene Ther. 13 (2006) 266–275, https://doi.org/10.1038/sj.gt.3302643.
[49] T.C. Marsh, E. Henderson, G-wires: self-assembly of a telomeric oligonucleotide, d (GGGGTTGGGG), into large superstructures, Biochemistry 33 (1994) 10718–10724, https://doi.org/10.1021/bi00201a020.
[50] D. Sen, W. Gilbert, Novel DNA superstructures formed by telomere-like oligomers, Biochemistry 31 (1992) 65–70, https://doi.org/10.1021/bi00116a011.
[51] S. Kolesnikova, E.A. Curtis, Structure and function of multimeric G-quadruplexes, Mol. Basel Switz. 24 (2019), E3074, https://doi.org/10.3390/molecules24173074.
[52] S. Kolesnikova, M. Hubálek, L. Bednárová, J. Cvacka, E.A. Curtis, Multimerization rules for G-quadruplexes, Nucleic Acids Res. 45 (2017) 8684–8696, https://doi.org/10.1093/nar/gkx637.
[53] J.-L. Mergny, A. De Cian, S. Amrane, M.W. da Silva, Kinetics of double-chain reversals bridging contiguous quartets in tetramolecular quadruplexes, Nucleic Acids Res. 34 (2006) 2386–2397, https://doi.org/10.1093/nar/gkl098.
[54] F. Rosu, V. Gabelica, H. Poncelet, E. De Pauw, Tetramolecular G-quadruplex formation pathways studied by electrospray mass spectrometry, Nucleic Acids Res. 38 (2010) 5217–5225, https://doi.org/10.1093/nar/gkq208.
[55] Y. Krishnan-Ghosh, D. Liu, S. Balasubramanian, Formation of an interlocked quadruplex dimer by d(GGGT), J. Am. Chem. Soc. 126 (2004) 11009–11016, https://doi.org/10.1021/ja049259y.
[56] Y. Kato, T. Ohyama, H. Mita, Y. Yamamoto, Dynamics and thermodynamics of dimerization of parallel G-quadruplexed DNA formed from d(TTAGn) (n = 3–5), J. Am. Chem. Soc. 127 (2005) 9980–9981, https://doi.org/10.1021/ja050191b.
[57] I.M.A. del Mundo, K.M. Vasquez, G. Wang, Modulation of DNA structure formation using small molecules, Biochim. Biophys. Acta BBA - Mol. Cell Res. 1866 (2019), 118539, https://doi.org/10.1016/j.bbamcr.2019.118539.
[58] N. Maizels, G4-associated human diseases, EMBO Rep. 16 (2015) 910–922, https://doi.org/10.15252/embr.201540607.
[59] E. Wang, R. Thombre, Y. Shah, R. Latanich, J. Wang, G-quadruplexes as pathogenic drivers in neurodegenerative disorders, Nucleic Acids Res. 49 (2021) 4816–4830, https://doi.org/10.1093/nar/gkab164.
[60] Y. Chen, D. Yang, Sequence, stability, structure of G-quadruplexes and their drug interactions, Al. CHAPTER, in: Serge Beaucage (Ed.), Curr. Protoc. Nucleic Acid Chem, 2012, https://doi.org/10.1002/0471142700.nc1705s50. Unit17.5.
[61] K. Lyu, E.Y.-C. Chow, X. Mou, T.-F. Chan, C.K. Kwok, RNA G-quadruplexes (rG4s): genomics and biological functions, Nucleic Acids Res. 49 (2021) 5426–5450, https://doi.org/10.1093/nar/gkab187.
[62] D. Yang, G-quadruplex DNA and RNA, in: D. Yang, C. Lin (Eds.), G-Quadruplex Nucleic Acids Methods Protoc, Springer, New York, NY, 2019, pp. 1–24, https://doi.org/10.1007/978-1-4939-9666-7_1.
[63] C.J. Lech, B. Heddi, A.T. Phan, Guanine base stacking in G-quadruplex nucleic acids, Nucleic Acids Res. 41 (2013) 2034–2046, https://doi.org/10.1093/nar/gks1110.
[64] N.Q. Do, K.W. Lim, M.H. Teo, B. Heddi, A.T. Phan, Stacking of G-quadruplexes: NMR structure of a G-rich oligonucleotide with potential anti-HIV and anticancer activity, Nucleic Acids Res. 39 (2011) 9448–9457, https://doi.org/10.1093/nar/gkr539.
[65] C. Gao, Z. Liu, H. Hou, J. Ding, X. Chen, C. Xie, Z. Song, Z. Hu, M. Feng, H. I. Mohamed, S. Xu, G.N. Parkinson, S. Haider, D. Wei, BMPQ-1 binds selectively to (3+1) hybrid topologies in human telomeric G-quadruplex multimers, Nucleic Acids Res. 48 (2020) 11259–11269, https://doi.org/10.1093/nar/gkaa870.
[66] N. Smargiasso, F. Rosu, W. Hsia, P. Colson, E.S. Baker, M.T. Bowers, E. De Pauw, V. Gabelica, G-quadruplex DNA assemblies: loop length, cation identity, and multimer formation, J. Am. Chem. Soc. 130 (2008) 10208–10216, https://doi.org/10.1021/ja801535e.
[67] F.R. Sperti, T. Charbonnier, P. Lejault, J. Zell, C. Bernhard, I.E. Valverde, D. Monchaud, Biomimetic, smart, and multivalent ligands for G-quadruplex isolation and bioorthogonal imaging, ACS Chem. Biol. 16 (2021) 905–914, https://doi.org/10.1021/acschembio.1c00111.
[68] F. Manoli, F. Doria, G. Colombo, B. Zambelli, M. Freccero, I. Manet, The binding pocket at the interface of multimeric telomere G-quadruplexes: myth or reality? Chem. Weinh. Bergstr. Ger. 27 (2021) 11707–11720, https://doi.org/10.1002/chem.202101486.
[69] S.D. Fonzo, J. Amato, F. D'Aria, M. Caterino, F. D'Amico, A. Gessini, J.W. Brady, A. Cesàro, B. Pagano, C. Giancola, Ligand binding to G-quadruplex DNA: new insights from ultraviolet resonance Raman spectroscopy, Phys. Chem. Chem. Phys. 22 (2020) 8128–8140, https://doi.org/10.1039/D0CP01022G.
[70] Z.-Y. Sun, X.-N. Wang, S.-Q. Cheng, X.-X. Su, T.-M. Ou, Developing novel G-quadruplex ligands: from interaction with nucleic acids to interfering with nucleic acid-protein interaction, Molecules 24 (2019) 396, https://doi.org/10.3390/molecules24030396.
[71] D. Monchaud, M.-P. Teulade-Fichou, A hitchhiker's guide to G-quadruplex ligands, Org. Biomol. Chem. 6 (2008) 627–636, https://doi.org/10.1039/b714772b.
[72] J. Šponer, G. Bussi, P. Stadlbauer, P. Kührová, P. Banáš, B. Islam, S. Haider, S. Neidle, M. Otyepka, Folding of guanine quadruplex molecules-funnel-like mechanism or kinetic partitioning? An overview from MD simulation studies, Biochim. Biophys. Acta Gen. Subj. 2017 (1861) 1246–1263, https://doi.org/10.1016/j.bbagen.2016.12.008.
[73] R. Rocca, F. Palazzesi, J. Amato, G. Costa, F. Ortuso, B. Pagano, A. Randazzo, E. Novellino, S. Alcaro, F. Moraca, A. Artese, Folding intermediate states of the parallel human telomeric G-quadruplex DNA explored using well-tempered metadynamics, Sci. Rep. 10 (2020) 3176, https://doi.org/10.1038/s41598-020-59774-x.
[74] J.T. Grün, A. Blümler, I. Burkhart, J. Wirmer-Bartoschek, A. Heckel, H. Schwalbe, Unraveling the kinetics of spare-tire DNA G-quadruplex folding, J. Am. Chem. Soc. 143 (2021) 6185–6193, https://doi.org/10.1021/jacs.1c01089.
[75] J.T. Grün H. Schwalbe Folding dynamics of polymorphic G-quadruplex structures, Biopolymers. n/a (n.d.) e23477. doi:10.1002/bip.23477.

[76] R.D. Gray, J.O. Trent, S. Arumugam, J.B. Chaires, Folding landscape of a parallel G-quadruplex, J. Phys. Chem. Lett. 10 (2019) 1146–1151, https://doi.org/10.1021/acs.jpclett.9b00227.
[77] P.L.T. Tran, M. Rieu, S. Hodeib, A. Joubert, J. Ouellet, P. Alberti, A. Bugaut, J.-F. Allemand, J.-B. Boulé, V. Croquette, Folding and persistence times of intramolecular G-quadruplexes transiently embedded in a DNA duplex, Nucleic Acids Res. 49 (2021) 5189–5201, https://doi.org/10.1093/nar/gkab306.
[78] H. You, J. Wu, F. Shao, J. Yan, Stability and kinetics of c-MYC promoter G-quadruplexes studied by single-molecule manipulation, J. Am. Chem. Soc. 137 (2015) 2424–2427, https://doi.org/10.1021/ja511680u.
[79] Y. Cheng, Q. Tang, Y. Li, Y. Zhang, C. Zhao, J. Yan, H. You, Folding/unfolding kinetics of G-quadruplexes upstream of the P1 promoter of the human BCL-2 oncogene, J. Biol. Chem. 294 (2019) 5890–5895, https://doi.org/10.1074/jbc.RA119.007516.
[80] Y. Cheng, Y. Zhang, H. You, Characterization of G-quadruplexes folding/unfolding dynamics and interactions with proteins from single-molecule force spectroscopy, Biomolecules 11 (2021) 1579, https://doi.org/10.3390/biom11111579.
[81] R. Hänsel-Hertsch, M. Di Antonio, S. Balasubramanian, DNA G-quadruplexes in the human genome: detection, functions and therapeutic potential, Nat. Rev. Mol. Cell Biol. 18 (2017) 279–284, https://doi.org/10.1038/nrm.2017.3.
[82] G.N. Parkinson, M.P.H. Lee, S. Neidle, Crystal structure of parallel quadruplexes from human telomeric DNA, Nature 417 (2002) 876–880, https://doi.org/10.1038/nature755.
[83] A.T. Phan, Human telomeric G-quadruplex: structures of DNA and RNA sequences, FEBS J. 277 (2010) 1107–1117, https://doi.org/10.1111/j.1742-4658.2009.07464.x.
[84] J. Dai, C. Punchihewa, A. Ambrus, D. Chen, R.A. Jones, D. Yang, Structure of the intramolecular human telomeric G-quadruplex in potassium solution: a novel adenine triple formation, Nucleic Acids Res. 35 (2007) 2440–2450, https://doi.org/10.1093/nar/gkm009.
[85] D. Varshney, J. Spiegel, K. Zyner, D. Tannahill, S. Balasubramanian, The regulation and functions of DNA and RNA G-quadruplexes, Nat. Rev. Mol. Cell Biol. 21 (2020) 459–474, https://doi.org/10.1038/s41580-020-0236-x.
[86] M.-H. Hu, S.-B. Chen, B. Wang, T.-M. Ou, L.-Q. Gu, J.-H. Tan, Z.-S. Huang, Specific targeting of telomeric multimeric G-quadruplexes by a new triaryl-substituted imidazole, Nucleic Acids Res. 45 (2017) 1606–1618, https://doi.org/10.1093/nar/gkw1195.
[87] H. Yu, X. Gu, S. Nakano, D. Miyoshi, N. Sugimoto, Beads-on-a-string structure of long telomeric DNAs under molecular crowding conditions, J. Am. Chem. Soc. 134 (2012) 20060–20069, https://doi.org/10.1021/ja305384c.
[88] L. Petraccone, C. Spink, J.O. Trent, N.C. Garbett, C.S. Mekmaysy, C. Giancola, J. B. Chaires, Structure and stability of higher-order human telomeric quadruplexes, J. Am. Chem. Soc. 133 (2011) 20951–20961, https://doi.org/10.1021/ja209192a.
[89] J. Abraham Punnoose, Y. Ma, M.E. Hoque, Y. Cui, S. Sasaki, A.H. Guo, K. Nagasawa, H. Mao, Random formation of G-quadruplexes in the full-length human telomere overhangs leads to a kinetic folding pattern with targetable vacant G-tracts, Biochemistry 57 (2018) 6946–6955, https://doi.org/10.1021/acs.biochem.8b00957.
[90] A. Kar, N. Jones, N.Ö. Arat, R. Fishel, J.D. Griffith, Long repeating (TTAGGG) n single-stranded DNA self-condenses into compact beaded filaments stabilized by G-quadruplex formation, J. Biol. Chem. 293 (2018) 9473–9485, https://doi.org/10.1074/jbc.RA118.002158.
[91] S. Haider, G.N. Parkinson, S. Neidle, Molecular dynamics and principal components analysis of human telomeric quadruplex multimers, Biophys. J. 95 (2008) 296–311, https://doi.org/10.1529/biophysj.107.120501.
[92] R.C. Monsen, S. Chakravarthy, W.L. Dean, J.B. Chaires, J.O. Trent, The solution structures of higher-order human telomere G-quadruplex multimers, Nucleic Acids Res. 49 (2021) 1749–1768, https://doi.org/10.1093/nar/gkaa1285.
[93] Y. Xu, T. Ishizuka, K. Kurabayashi, M. Komiyama, Consecutive formation of G-quadruplexes in human telomeric-overhang DNA: a protective capping structure for telomere ends, Angew. Chem. Int. Ed. 48 (2009) 7833–7836, https://doi.org/10.1002/anie.200903858.
[94] L. Petraccone, Higher-order quadruplex structures, Top. Curr. Chem. 330 (2013) 23–46, https://doi.org/10.1007/128_2012_350.
[95] R. Buscaglia, R.D. Gray, J.B. Chaires, Thermodynamic characterization of human telomere quadruplex unfolding, Biopolymers 99 (2013) 1006–1018, https://doi.org/10.1002/bip.22247.
[96] Y. Xu, Y. Suzuki, K. Ito, M. Komiyama, Telomeric repeat-containing RNA structure in living cells, Proc. Natl. Acad. Sci. 107 (2010) 14579–14584, https://doi.org/10.1073/pnas.1001177107.
[97] C.M. Azzalin, P. Reichenbach, L. Khoriauli, E. Giulotto, J. Lingner, Telomeric repeat containing RNA and RNA surveillance factors at mammalian chromosome ends, Science 318 (2007) 798–801, https://doi.org/10.1126/science.1147182.
[98] Y. Mei, Z. Deng, O. Vladimirova, N. Gulve, F.B. Johnson, W.C. Drosopoulos, C. L. Schildkraut, P.M. Lieberman, TERRA G-quadruplex RNA interaction with TRF2 GAR domain is required for telomere integrity, Sci. Rep. 11 (2021) 3509, https://doi.org/10.1038/s41598-021-82406-x.
[99] E. Cusanelli, P. Chartrand, Telomeric repeat-containing RNA TERRA: a noncoding RNA connecting telomere biology to genome integrity, Front. Genet. 6 (2015) (accessed January 13, 2022), https://www.frontiersin.org/article/10.3389/fgene.2015.00143.
[100] K. Rippe, B. Luke, TERRA and the state of the telomere, Nat. Struct. Mol. Biol. 22 (2015) 853–858, https://doi.org/10.1038/nsmb.3078.
[101] S. Redon, P. Reichenbach, J. Lingner, The non-coding RNA TERRA is a natural ligand and direct inhibitor of human telomerase, Nucleic Acids Res. 38 (2010) 5797–5806, https://doi.org/10.1093/nar/gkq296.
[102] G.W. Collie, G.N. Parkinson, S. Neidle, F. Rosu, E. De Pauw, V. Gabelica, Electrospray mass spectrometry of telomeric RNA (TERRA) reveals the formation of stable multimeric G-quadruplex structures, J. Am. Chem. Soc. 132 (2010) 9328–9334, https://doi.org/10.1021/ja100345z.
[103] H. Martadinata, A.T. Phan, Structure of propeller-type parallel-stranded RNA G-quadruplexes, formed by human telomeric RNA sequences in K+ solution, J. Am. Chem. Soc. 131 (2009) 2570–2578, https://doi.org/10.1021/ja806592z.
[104] A. Henderson, Y. Wu, Y.C. Huang, E.A. Chavez, J. Platt, F.B. Johnson, R.M. Brosh, D. Sen, P.M. Lansdorp, Detection of G-quadruplex DNA in mammalian cells, Nucleic Acids Res. 42 (2014) 860–869, https://doi.org/10.1093/nar/gkt957.
[105] E.Y.N. Lam, D. Beraldi, D. Tannahill, S. Balasubramanian, G-quadruplex structures are stable and detectable in human genomic DNA, Nat. Commun. 4 (2013) 1796, https://doi.org/10.1038/ncomms2792.
[106] R. Simone, P. Fratta, S. Neidle, G.N. Parkinson, A.M. Isaacs, G-quadruplexes: emerging roles in neurodegenerative diseases and the non-coding transcriptome, FEBS Lett. 589 (2015) 1653–1668, https://doi.org/10.1016/j.febslet.2015.05.003.
[107] E.G. Conlon, J.L. Manley, RNA-binding proteins in neurodegeneration: mechanisms in aggregate, Genes Dev. 31 (2017) 1509–1528, https://doi.org/10.1101/gad.304055.117.
[108] A.E. Renton, E. Majounie, A. Waite, J. Simón-Sánchez, S. Rollinson, J.R. Gibbs, J. C. Schymick, H. Laaksovirta, J.C. van Swieten, L. Myllykangas, H. Kalimo, A. Paetau, Y. Abramzon, A.M. Remes, A. Kaganovich, S.W. Scholz, J. Duckworth, J. Ding, D.W. Harmer, D.G. Hernandez, J.O. Johnson, K. Mok, M. Ryten, D. Trabzuni, R.J. Guerreiro, R.W. Orrell, J. Neal, A. Murray, J. Pearson, I. E. Jansen, D. Sondervan, H. Seelaar, D. Blake, K. Young, N. Halliwell, J. B. Callister, G. Toulson, A. Richardson, A. Gerhard, J. Snowden, D. Mann, D. Neary, M.A. Nalls, T. Peuralinna, L. Jansson, V.-M. Isoviita, A.-L. Kaivorinne, M. Hölttä-Vuori, E. Ikonen, R. Sulkava, M. Benatar, J. Wuu, A. Chiò, G. Restagno, G. Borghero, M. Sabatelli, , ITALSGEN Consortium, D. Heckerman, E. Rogaeva, L. Zinman, J.D. Rothstein, M. Sendtner, C. Drepper, E.E. Eichler, C. Alkan, Z. Abdullaev, S.D. Pack, A. Dutra, E. Pak, J. Hardy, A. Singleton, N.M. Williams, P. Heutink, S. Pickering-Brown, H.R. Morris, P.J. Tienari, B.J. Traynor, A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD, Neuron 72 (2011) 257–268, https://doi.org/10.1016/j.neuron.2011.09.010.
[109] M. DeJesus-Hernandez, I.R. Mackenzie, B.F. Boeve, A.L. Boxer, M. Baker, N. J. Rutherford, A.M. Nicholson, N.A. Finch, H. Flynn, J. Adamson, N. Kouri, A. Wojtas, P. Sengdy, G.-Y.R. Hsiung, A. Karydas, W.W. Seeley, K.A. Josephs, G. Coppola, D.H. Geschwind, Z.K. Wszolek, H. Feldman, D.S. Knopman, R. C. Petersen, B.L. Miller, D.W. Dickson, K.B. Boylan, N.R. Graff-Radford, R. Rademakers, Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS, Neuron 72 (2011) 245–256, https://doi.org/10.1016/j.neuron.2011.09.011.
[110] A.D. Gitler, H. Tsuiji, There has been an awakening: emerging mechanisms of C9orf72 mutations in FTD/ALS, Brain Res. 1647 (2016) 19–29, https://doi.org/10.1016/j.brainres.2016.04.004.
[111] Z.-F. Wang, A. Ursu, J.L. Childs-Disney, R. Guertler, W.-Y. Yang, V. Bernat, S. G. Rzuczek, R. Fuerst, Y.-J. Zhang, T.F. Gendron, I. Yildirim, B.G. Dwyer, J. E. Rice, L. Petrucelli, M.D. Disney, The hairpin form of r(G4C2)exp in c9ALS/FTD is repeat-associated non-ATG translated and a target for bioactive small molecules, cellChem. Biol. 26 (2019) 179–190.e12, https://doi.org/10.1016/j.chembiol.2018.10.018.
[112] J.P. Taylor, Neurodegenerative diseases: G-quadruplex poses quadruple threat, Nature 507 (2014) 175–177, https://doi.org/10.1038/nature13067.
[113] A.R. Haeusler, C.J. Donnelly, G. Periz, E.A.J. Simko, P.G. Shaw, M.-S. Kim, N. J. Maragakis, J.C. Troncoso, A. Pandey, R. Sattler, J.D. Rothstein, J. Wang, C9orf72 nucleotide repeat structures initiate molecular cascades of disease, Nature 507 (2014) 195–200, https://doi.org/10.1038/nature13124.
[114] C.-Y. Lee, C. McNerney, K. Ma, W. Zhao, A. Wang, S. Myong, R-loop induced G-quadruplex in non-template promotes transcription by successive R-loop formation, Nat. Commun. 11 (2020) 3392, https://doi.org/10.1038/s41467-020-17176-7.
[115] J. Song, J.-P. Perreault, I. Topisirovic, S. Richard, RNA G-quadruplexes and their potential regulatory roles in translation, Translation. 4 (2016), e1244031, https://doi.org/10.1080/21690731.2016.1244031.
[116] T. Tian, Y.-Q. Chen, S.-R. Wang, X. Zhou, G-quadruplex: a regulator of gene expression and its chemical targeting, Chem. 4 (2018) 1314–1344, https://doi.org/10.1016/j.chempr.2018.02.014.
[117] N. Ofer, P. Weisman-Shomer, J. Shklover, M. Fry, The quadruplex r(CGG)n destabilizing cationic porphyrin TMPyP4 cooperates with hnRNPs to increase the translation efficiency of fragile X premutation mRNA, Nucleic Acids Res. 37 (2009) 2712–2722, https://doi.org/10.1093/nar/gkp130.
[118] S. Asamitsu, Y. Yabuki, S. Ikenoshita, K. Kawakubo, M. Kawasaki, S. Usuki, Y. Nakayama, K. Adachi, H. Kugoh, K. Ishii, T. Matsuura, E. Nanba, H. Sugiyama, K. Fukunaga, N. Shioda, CGG repeat RNA G-quadruplexes interact with FMRpolyG to cause neuronal dysfunction in fragile X-related tremor/ataxia syndrome, Sci. Adv. 7 (2021), eabd9440, https://doi.org/10.1126/sciadv.abd9440.
[119] K. Zheng, R. Wu, Y. He, S. Xiao, J. Zhang, J. Liu, Y. Hao, Z. Tan, A competitive formation of DNA:RNA hybrid G-quadruplex is responsible to the mitochondrial transcription termination at the DNA replication priming site, Nucleic Acids Res. 42 (2014) 10832–10844, https://doi.org/10.1093/nar/gku764.

[120] P.H. Wanrooij, J.P. Uhler, Y. Shi, F. Westerlund, M. Falkenberg, C.M. Gustafsson, A hybrid G-quadruplex structure formed between RNA and DNA explains the extraordinary stability of the mitochondrial R-loop, Nucleic Acids Res. 40 (2012) 10334–10344, https://doi.org/10.1093/nar/gks802.
[121] P.H. Wanrooij, J.P. Uhler, T. Simonsson, M. Falkenberg, C.M. Gustafsson, G-quadruplex structures in RNA stimulate mitochondrial transcription termination and primer formation, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 16072–16077, https://doi.org/10.1073/pnas.1006026107.
[122] M. Falabella, R.J. Fernandez, F.B. Johnson, B.A. Kaufman, Potential roles for G-quadruplexes in mitochondria, Curr. Med. Chem. 26 (2019) 2918–2932, https://doi.org/10.2174/0929867325666180228165527.
[123] M. Falabella, J.E. Kolesar, C. Wallace, D. de Jesus, L. Sun, Y.V. Taguchi, C. Wang, T. Wang, I.M. Xiang, J.K. Alder, R. Maheshan, W. Horne, J. Turek-Herman, P. J. Pagano, C.M. St, N. Croix, L.A. Sondheimer, F.B. Yatsunyk, B.A. Kaufman Johnson, G-quadruplex dynamics contribute to regulation of mitochondrial gene expression, Sci. Rep. 9 (2019) 5605, https://doi.org/10.1038/s41598-019-41464-y.
[124] J.C. Darnell, C.E. Fraser, O. Mostovetsky, R.B. Darnell, Discrimination of common and unique RNA-binding activities among fragile X mental retardation protein paralogs, Hum. Mol. Genet. 18 (2009) 3164–3177, https://doi.org/10.1093/hmg/ddp255.
[125] J.C. Darnell, E. Klann, The translation of translational control by FMRP: therapeutic targets for fragile X syndrome, Nat. Neurosci. 16 (2013) 1530–1536, https://doi.org/10.1038/nn.3379.
[126] H. Siomi, M.C. Siomi, R.L. Nussbaum, G. Dreyfuss, The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein, Cell 74 (1993) 291–298, https://doi.org/10.1016/0092-8674(93)90420-u.
[127] R.J. Hagerman, M. Leehey, W. Heinrichs, F. Tassone, R. Wilson, J. Hills, J. Grigsby, B. Gage, P.J. Hagerman, Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X, Neurology 57 (2001) 127–130, https://doi.org/10.1212/wnl.57.1.127.
[128] V. Handa, T. Saha, K. Usdin, The fragile X syndrome repeats form RNA hairpins that do not activate the interferon-inducible protein kinase, PKR, but are cut by dicer, Nucleic Acids Res. 31 (2003) 6243–6248, https://doi.org/10.1093/nar/gkg818.
[129] J.F. Moruno-Manchon, P. Lejault, Y. Wang, B. McCauley, P. Honarpisheh, D. A. Morales Scheihing, S. Singh, W. Dang, N. Kim, A. Urayama, L. Zhu, D. Monchaud, L.D. McCullough, A.S. Tsvetkov, Small-molecule G-quadruplex stabilizers reveal a novel pathway of autophagy regulation in neurons, elife 9 (2020), e52283, https://doi.org/10.7554/eLife.52283.
[130] R. Simone, R. Balendra, T.G. Moens, E. Preza, K.M. Wilson, A. Heslegrave, N. S. Woodling, T. Niccoli, J. Gilbert-Jaramillo, S. Abdelkarim, E.L. Clayton, M. Clarke, M.-T. Konrad, A.J. Nicoll, J.S. Mitchell, A. Calvo, A. Chio, H. Houlden, J.M. Polke, M.A. Ismail, C.E. Stephens, T. Vo, A.A. Farahat, W.D. Wilson, D. W. Boykin, H. Zetterberg, L. Partridge, S. Wray, G. Parkinson, S. Neidle, R. Patani, P. Fratta, A.M. Isaacs, G-quadruplex-binding small molecules ameliorate C9orf72 FTD/ALS pathology in vitro and in vivo, EMBO Mol. Med. 10 (2018) 22–31, https://doi.org/10.15252/emmm.201707850.
[131] J. Mitteaux, P. Lejault, F. Wojciechowski, A. Joubert, J. Boudon, N. Desbois, C. P. Gros, R.H.E. Hudson, J.-B. Boulé, A. Granzhan, D. Monchaud, Identifying G-quadruplex-DNA-disrupting small molecules, J. Am. Chem. Soc. 143 (2021) 12567–12577, https://doi.org/10.1021/jacs.1c04426.
[132] W. Yoshida, H. Saikyo, K. Nakabayashi, H. Yoshioka, D.H. Bay, K. Iida, T. Kawai, K. Hata, K. Ikebukuro, K. Nagasawa, I. Karube, Identification of G-quadruplex clusters by high-throughput sequencing of whole-genome amplified products with a G-quadruplex ligand, Sci. Rep. 8 (2018) 3116, https://doi.org/10.1038/s41598-018-21514-7.
[133] R. Rodriguez, K.M. Miller, J.V. Forment, C.R. Bradshaw, M. Nikan, S. Britton, T. Oelschlaegel, B. Xhemalce, S. Balasubramanian, S.P. Jackson, Small-molecule-induced DNA damage identifies alternative DNA structures in human genes, Nat. Chem. Biol. 8 (2012) 301–310, https://doi.org/10.1038/nchembio.780.
[134] R. Hanna, A. Flamier, A. Barabino, G. Bernier, G-quadruplexes originating from evolutionary conserved L1 elements interfere with neuronal gene expression in Alzheimer's disease, Nat. Commun. 12 (2021) 1828, https://doi.org/10.1038/s41467-021-22129-9.
[135] M. Berselli, E. Lavezzo, S. Toppo, QPARSE: searching for long-looped or multimeric G-quadruplexes potentially distinctive and druggable, Bioinforma. Oxf. Engl. 36 (2020) 393–399, https://doi.org/10.1093/bioinformatics/btz569.
[136] Y. Xu, T. Kimura, M. Komiyama, Human telomere RNA and DNA form an intermolecular G-quadruplex, Nucleic Acids Symp. Ser. 2008 (2004) 169–170, https://doi.org/10.1093/nass/nrn086.
[137] M.P. Crossley, M. Bocek, K.A. Cimprich, R-loops as cellular regulators and genomic threats, Mol. Cell 73 (2019) 398–411, https://doi.org/10.1016/j.molcel.2019.01.024.
[138] J. Zhao, Q. Zhai, Recent advances in the development of ligands specifically targeting telomeric multimeric G-quadruplexes, Bioorg. Chem. 103 (2020), 104229, https://doi.org/10.1016/j.bioorg.2020.104229.
[139] K. Mori, S. Gotoh, T. Yamashita, R. Uozumi, Y. Kawabe, S. Tagami, F. Kamp, B. Nuscher, D. Edbauer, C. Haass, Y. Nagai, M. Ikeda, The porphyrin TMPyP4 inhibits elongation during the noncanonical translation of the FTLD/ALS-associated GGGGCC repeat in the C9orf72 gene, J. Biol. Chem. 297 (2021), 101120, https://doi.org/10.1016/j.jbc.2021.101120.
[140] X.-X. Huang, L.-N. Zhu, B. Wu, Y.-F. Huo, N.-N. Duan, D.-M. Kong, Two cationic porphyrin isomers showing different multimeric G-quadruplex recognition specificity against monomeric G-quadruplexes, Nucleic Acids Res. 42 (2014) 8719–8731, https://doi.org/10.1093/nar/gku526.
[141] C. Saintomé, P. Alberti, N. Guinot, P. Lejault, J. Chatain, P. Mailliet, J.-F. Riou, A. Bugaut, Binding properties of mono- and dimeric pyridine dicarboxamide ligands to human telomeric higher-order G-quadruplex structures, Chem. Commun. Camb. Engl. 54 (2018) 1897–1900, https://doi.org/10.1039/c7cc07048a.
[142] K. Iida, S. Majima, T. Nakamura, H. Seimiya, K. Nagasawa, Evaluation of the interaction between long telomeric DNA and macrocyclic hexaoxazole (6OTD) dimer of a G-quadruplex ligand, Mol. Basel Switz. 18 (2013) 4328–4341, https://doi.org/10.3390/molecules18044328.
[143] C.-Q. Zhou, J.-W. Yang, C. Dong, Y.-M. Wang, B. Sun, J.-X. Chen, Y.-S. Xu, W.-H. Chen, Highly selective, sensitive and fluorescent sensing of dimeric G-quadruplexes by a dimeric berberine, Org. Biomol. Chem. 14 (2016) 191–197, https://doi.org/10.1039/c5ob01723h.
[144] V. Pirota, M. Stasi, A. Benassi, F. Doria, Chapter six - an overview of quadruplex ligands: their common features and chemotype diversity, in: S. Neidle (Ed.), Annu. Rep. Med. Chem, Academic Press, 2020, pp. 163–196, https://doi.org/10.1016/bs.armc.2020.04.008.
[145] H. Alniss, B. Zamiri, M. Khalaj, C.E. Pearson, R.B. Macgregor, Thermodynamic and spectroscopic investigations of TMPyP4 association with guanine- and cytosine-rich DNA and RNA repeats of C9orf72, Biochem. Biophys. Res. Commun. 495 (2018) 2410–2417, https://doi.org/10.1016/j.bbrc.2017.12.108.
[146] J.C. Grigg, N. Shumayrikh, D. Sen, G-quadruplex structures formed by expanded hexanucleotide repeat RNA and DNA from the neurodegenerative disease-linked C9orf72 gene efficiently sequester and activate heme, PLOS ONE 9 (2014), e106449, https://doi.org/10.1371/journal.pone.0106449.
[147] C. Zhao, L. Wu, J. Ren, Y. Xu, X. Qu, Targeting human telomeric higher-order DNA: dimeric G-quadruplex units serve as preferred binding site, J. Am. Chem. Soc. 135 (2013) 18786–18789, https://doi.org/10.1021/ja410723r.
[148] C.-Q. Zhou, T.-C. Liao, Z.-Q. Li, J. Gonzalez-Garcia, M. Reynolds, M. Zou, R. Vilar, Dinickel-salphen complexes as binders of human telomeric dimeric G-quadruplexes, Chem. Weinh. Bergstr. Ger. 23 (2017) 4713–4722, https://doi.org/10.1002/chem.201700276.
[149] A. Łęczkowska, J. Gonzalez-Garcia, C. Perez-Arnaiz, B. Garcia, A.J.P. White, R. Vilar, Binding studies of metal-salphen and metal-bipyridine complexes towards G-quadruplex DNA, Chem. Weinh. Bergstr. Ger. 24 (2018) 11785–11794, https://doi.org/10.1002/chem.201802248.
[150] T.-C. Liao, T.-Z. Ma, Z. Liang, X.-T. Zhang, C.-Y. Luo, L. Liu, C.-Q. Zhou, A comparative study on high selectivities of human telomeric dimeric G-quadruplexes by dimeric G-quadruplex binders, Chem. Weinh. Bergstr. Ger. 24 (2018) 15840–15851, https://doi.org/10.1002/chem.201802796.
[151] Z.-Q. Li, T.-C. Liao, C. Dong, J.-W. Yang, X.-J. Chen, L. Liu, Y. Luo, Y.-Y. Liang, W.-H. Chen, C.-Q. Zhou, Specifically targeting mixed-type dimeric G-quadruplexes using berberine dimers, Org. Biomol. Chem. 15 (2017) 10221–10229, https://doi.org/10.1039/c7ob02326j.
[152] J. Zhao, Z. Yang, Q. Zhai, D. Wei, Specific recognition of telomeric multimeric G-quadruplexes by a simple-structure quinoline derivative, Anal. Chim. Acta 1132 (2020) 93–100, https://doi.org/10.1016/j.aca.2020.07.017.
[153] T.-Z. Ma, M.-J. Zhang, T.-C. Liao, J.-H. Li, M. Zou, Z.-M. Wang, C.-Q. Zhou, Dimers formed with the mixed-type G-quadruplex binder pyridostatin specifically recognize human telomere G-quadruplex dimers, Org. Biomol. Chem. 18 (2020) 920–930, https://doi.org/10.1039/c9ob02470k.
[154] S. Müller, S. Kumari, R. Rodriguez, S. Balasubramanian, Small-molecule-mediated G-quadruplex isolation from human cells, Nat. Chem. 2 (2010) 1095–1098, https://doi.org/10.1038/nchem.842.
[155] T.-C. Liao, T.-Z. Ma, S.-B. Chen, A. Cilibrizzi, M.-J. Zhang, J.-H. Li, C.-Q. Zhou, Human telomere double G-quadruplex recognition by berberine-bisquinolinium imaging conjugates in vitro and in cells, Int. J. Biol. Macromol. 158 (2020) 1299–1309, https://doi.org/10.1016/j.ijbiomac.2020.04.171.
[156] J. Abraham Punnoose, Y. Ma, Y. Li, M. Sakuma, S. Mandal, K. Nagasawa, H. Mao, Adaptive and specific recognition of telomeric G-quadruplexes via polyvalency induced unstacking of binding units, J. Am. Chem. Soc. 139 (2017) 7476–7484, https://doi.org/10.1021/jacs.7b00607.
[157] K. Shinohara, Y. Sannohe, S. Kaieda, K. Tanaka, H. Osuga, H. Tahara, Y. Xu, T. Kawase, T. Bando, H. Sugiyama, A chiral wedge molecule inhibits telomerase activity, J. Am. Chem. Soc. 132 (2010) 3778–3782, https://doi.org/10.1021/ja908897j.
[158] L.-N. Zhu, B. Wu, D.-M. Kong, Specific recognition and stabilization of monomeric and multimeric G-quadruplexes by cationic porphyrin TMPipEOPP under molecular crowding conditions, Nucleic Acids Res. 41 (2013) 4324–4335, https://doi.org/10.1093/nar/gkt103.
[159] L. Stefan, F. Denat, D. Monchaud, Deciphering the DNAzyme activity of multimeric quadruplexes: insights into their actual role in the telomerase activity evaluation assay, J. Am. Chem. Soc. 133 (2011) 20405–20415, https://doi.org/10.1021/ja208145d.
[160] M. Tassinari, A. Lena, E. Butovskaya, V. Pirota, M. Nadai, M. Freccero, F. Doria, S. N. Richter, A fragment-based approach for the development of G-quadruplex ligands: role of the amidoxime moiety, Mol. Basel Switz. 23 (2018) E1874, https://doi.org/10.3390/molecules23081874.
[161] J. Dash, Z.A.E. Waller, G.D. Pantoş, S. Balasubramanian, Synthesis and binding studies of novel diethynyl-pyridine amides with genomic promoter DNA G-quadruplexes, Chem. Weinh. Bergstr. Ger. 17 (2011) 4571–4581, https://doi.org/10.1002/chem.201003157.
[162] S. Sparapani, S.M. Haider, F. Doria, M. Gunaratnam, S. Neidle, Rational design of acridine-based ligands with selectivity for human telomeric quadruplexes, J. Am. Chem. Soc. 132 (2010) 12263–12272, https://doi.org/10.1021/ja1003944.

[163] A.R.O. Cousins, D. Ritson, P. Sharma, M.F.G. Stevens, J.E. Moses, M.S. Searle, Ligand selectivity in stabilising tandem parallel folded G-quadruplex motifs in human telomeric DNA sequences, Chem. Commun. Camb. Engl. 50 (2014) 15202–15205, https://doi.org/10.1039/c4cc07487d.
[164] M.-H. Hu, X.-T. Lin, B. Liu, J.-H. Tan, Dimeric aryl-substituted imidazoles may inhibit ALT cancer by targeting the multimeric G-quadruplex in telomere, Eur. J. Med. Chem. 186 (2020), 111891, https://doi.org/10.1016/j.ejmech.2019.111891.
[165] V. Pirota, C. Platella, D. Musumeci, A. Benassi, J. Amato, B. Pagano, G. Colombo, M. Freccero, F. Doria, D. Montesarchio, On the binding of naphthalene diimides to a human telomeric G-quadruplex multimer model, Int. J. Biol. Macromol. 166 (2021) 1320–1334, https://doi.org/10.1016/j.ijbiomac.2020.11.013.
[166] C. Platella, V. Pirota, D. Musumeci, F. Rizzi, S. Iachettini, P. Zizza, A. Biroccio, M. Freccero, D. Montesarchio, F. Doria, Trifunctionalized naphthalene diimides and dimeric analogues as G-quadruplex-targeting anticancer agents selected by affinity chromatography, Int. J. Mol. Sci. 21 (2020) E1964, https://doi.org/10.3390/ijms21061964.
[167] Q. Zhang, Y.-C. Liu, D.-M. Kong, D.-S. Guo, Tetraphenylethene derivatives with different numbers of positively charged side arms have different multimeric G-quadruplex recognition specificity, Chem. Weinh. Bergstr. Ger. 21 (2015) 13253–13260, https://doi.org/10.1002/chem.201501847.
[168] F. Doria, E. Salvati, L. Pompili, V. Pirota, C. D'Angelo, F. Manoli, M. Nadai, S. N. Richter, A. Biroccio, I. Manet, M. Freccero, Dyads of G-quadruplex ligands triggering DNA damage response and tumour cell growth inhibition at subnanomolar concentration, Chem. Weinh. Bergstr. Ger. 25 (2019) 11085–11097, https://doi.org/10.1002/chem.201900766.