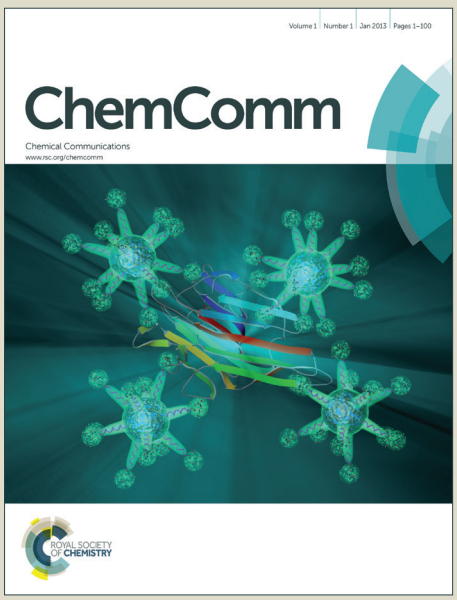
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Advances in the Molecular Design of Potential Anticancer Agents via Targeting of Human Telomeric DNA

Basudeb Maji[a] and Santanu Bhattacharya*[a,b,c]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

Telomerase is an attractive drug target to develop new generation drugs against cancer. Telomere appears from the chromosomal termini and protects it from the double-stranded DNA degradation. A short telomere promotes genomic instability like end-to-end fusion and regulates over-expression of the telomere repairing enzyme telomerase. Telomerase maintains the telomere length which may lead to genetically abnormal situations leading to cancer. Thus, the design and synthesis of efficient telomerase inhibitor is a viable strategy toward the anticancer drug development. Accordingly small molecule induced stabilization of the G-quadruplex structure formed by the human telomeric DNA is an area of contemporary scientific art. Several such compounds efficiently stabilize the G-quadruplex forms of nucleic acid which often leads to telomerase inhibition. This feature article presents the discovery and development of telomere structure, function and evolution in the telomere targeted anticancer drug design and incorporates the recent advances in this area and discuss the pros and cones in the methods and prospects for future.

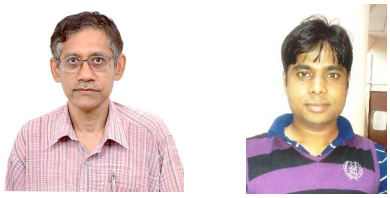
Introduction

Nucleic acid is a common drug target for the regulation of a number of genetic disorders and diseases. Negatively charged nucleic acids remain in '*super-compact*' form as complexes with positively charged histone protein. However, they open up in presence of specific enzyme to perform various biological roles. Revelation of its polymorphic nature has taken nucleic acids research beyond the sequence-specificity paradigm. Telomere, a part of the nucleic acid chromosome, demonstrates a significant propensity to polymorphism. It plays an important role in the genomic maintenance, stability and expression. One of the most deadly genetic diseases, cancer, is believed to be related to the abnormality of telomere function. The G-quadruplex DNA binding molecules are potential transcriptional regulators of oncogenes. Hence such low molecular mass ligands should in principle exert anti-proliferative properties selectively on cancer cells and could be considered as potential drugs. Here we discuss the evolution, activity and the prospects of telomere targeted drug design that may add to the new generation anticancer arsenals.

[a] *Department of Organic Chemistry, Indian Institute of Science, Bangalore-560012, India. Fax: +91-80-23600529; Tel: +91-80-22932664; E-mail: sb@orgchem.iisc.ernet.in*

[b] *Chemical Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore-560064, India.*

[c] *J. C. Bose Fellow, Department of Science and Technology, New Delhi, India.*



Santanu Bhattacharya and Basudeb Maji

Santanu Bhattacharya obtained his PhD degree from Rutgers University, New Brunswick, NJ under the supervision of Prof. Robert A. Moss. He was a NIH postdoctoral fellow at the Massachusetts Institute of Technology under the tutelage of Nobel laureate Prof. Har Gobind Khorana. He is currently full Professor and Chair of the Department of Organic Chemistry at the Indian Institute of Science, Bangalore. He is a recipient of TWAS prize in Chemistry and an elected fellow of the INSA, New Delhi and the IASc, Bangalore. His research interests include synthetic molecular design, chemical biology, molecular medicine, nanoscience and supramolecular chemistry.

Basudeb Maji obtained his B.Sc. (Hons.) in Chemistry in 2006 and MSc in Organic Chemistry in 2008 both from the University of Calcutta, WB. He obtained his PhD degree from Indian Institute of Science, Bangalore, India under the supervision of Prof. Santanu Bhattacharya and currently he is working as a research associate there. He is interested in the design of human telomeric DNA targeted anticancer drugs and their prototypes.

Discovery of telomere

From the discovery of DNA by Miescher[1] in 1869, it took more than a century to decode the tale of nucleic acid arsenal, chromosome, known as telomere (Figure 1A,B).[2] The key breakthrough in the elucidation of double-stranded DNA structure came up in 1953 by Watson and Crick.[3] This was followed by understanding of the three-dimensional packaging and storage of DNA into chromosome which was originally proposed by Boveri in 1880's.[4] The chromosomal structure contains constriction point known as the centromere which divides it into two segments, the short arms are known as the 'p' arm whereas the long arms are known as the 'q' arm. The telomeric end *telomere* (from Greek, 'telos' for end and 'meros' for part) are found in both the arms.

In 1930's McClintock first discovered the special features of chromosomal end to protect from DNA damage and 'end-to-end' fusion.[5] DNA-histone complex chromosomal structure maintains a non-coding single-stranded overhang from its 3'-end.[6] Although the telomeric overhang is crucial for the chromosomal protection it is not still clear how the overhang emerges.[7]

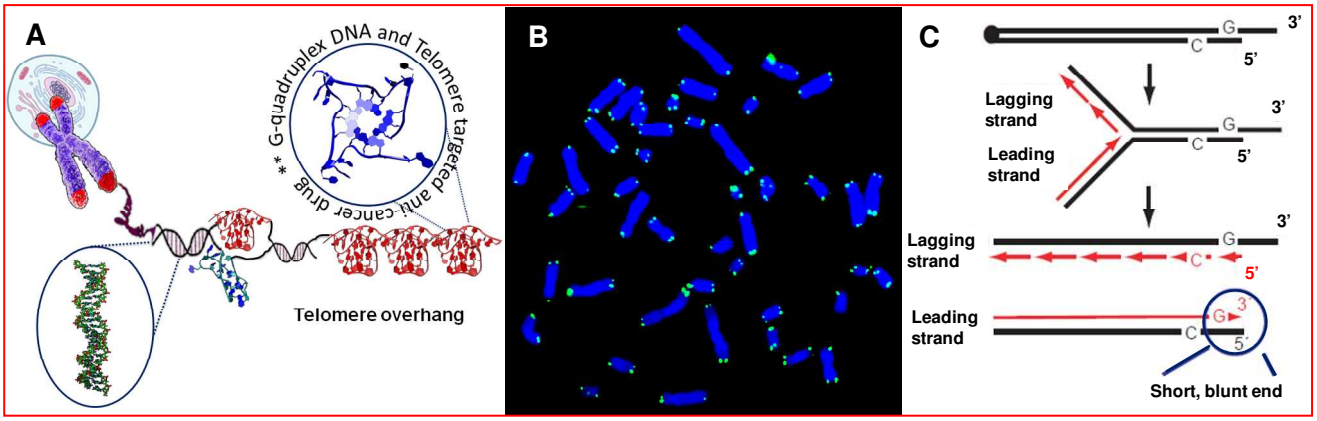


Figure 1. (A) Schematic location of telomeric G-quadruplex inside cell. (B) Image of chromosomes from an hTERT-telo1 cell stained with a telomere PNA probe. Telomeres are stained with FITC and chromosomes are stained with DAPI. Adapted with permission from reference (8), copyright 2007, BioMed Central. (C) Telomere replication and 'end-replication-problem'. Adapted with the permission from reference (2), copyright 2003, The Company of Biologists Ltd.

Role of telomere in the genomic maintenance

Till the 1960's the fundamental question that engaged the scientists was to find out why a normal cell dies? The theory of unlimited replication capacity was modified when Weismann proposed that normal cells can have only finite number of cell division capability[9] which was proved by Hayflick and Moorhead in 1961. These authors observed that the cultured normal diploid human fibroblasts could undergo only 60–80 population doublings after which they stopped dividing and suffered a growth arrest known as 'senescence'. This revolutionary concept is popularly known as 'Hayflick limit'. The cellular process was addressed at genomic level when Watson discovered that the DNA polymerase enzyme which accounts for the DNA replication cannot fully copy the 3'-end of linear DNA. This mechanism was attributed primarily to Olovnikov who termed it as the 'end-replication problem'[10] (Figure 1C). The telomere length plays an important role for the chromosomal protection and long-term viability. However, each cell doubling results in a shortage of *ca.* 150–200 bases in the telomere end which eventually causes genomic instability and the cell enters into phase III or M1 stage. When the telomere reaches a critical length, the cell enters into M2 or crisis stage where the proliferation and cell death restore a kind of balance. When the length of the telomere becomes extremely critical, the cell undergoes an apoptosis (Figure 2).[11]

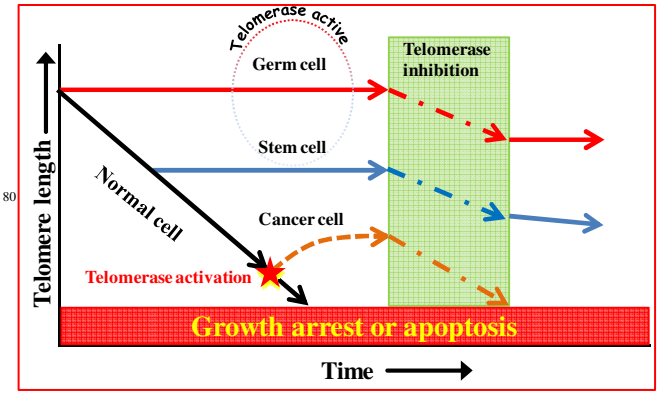


Figure 2. Effect of telomere length, telomerase activation and telomerase inhibition on normal, germ and stem cell viability.

Protection of genome and beyond

To overcome the genomic instability caused due to the telomeric DNA deletion,[12] or double-strand scission[13] or end-replication problem,[14] cell adopts certain cellular mechanism like fusion of the broken chromosomal ends together via the DNA repair machinery. On the other hand in crisis stage, it activates a telomere repairing protein which restores the short 'unstable' telomere (Figure 2). In 1982, Blackburn and Szostak discovered telomere terminal transferase or telomerase activity in Tetrahymena telomeric DNA sequences inside yeast vector.[15] The human telomerase, is a ribonucleoprotein containing the human telomerase RNA (hTR) and the human telomerase reverse

transcriptase (hTERT) which can reverse transcribe onto its own RNA template to add the nucleotides to the non-coding telomere under crisis stage.[16,17] Thus telomerase can play a great role towards the maintenance of genomic stability. Unfortunately, sometimes cells over-express telomerase which maintains the telomere length and transforms the cells into 'immortal' or cancerous state. Indeed telomerase is over-expressed in cancer cells up to 85-90% instances whereas there is hardly any evidence of their presence in normal somatic cells.[18] Thus the presence of telomerase discriminates the cancer cells from the healthy somatic cells.

Decoding of telomere

From the genomic point of view, the stability and ageing of cell depends upon the telomeric length. Critical shortening of telomere leads to cell senescence and death. However, restoration of full length of telomere results into cellular immortality, a case in point is cancer cell. Thus an understanding of the structural

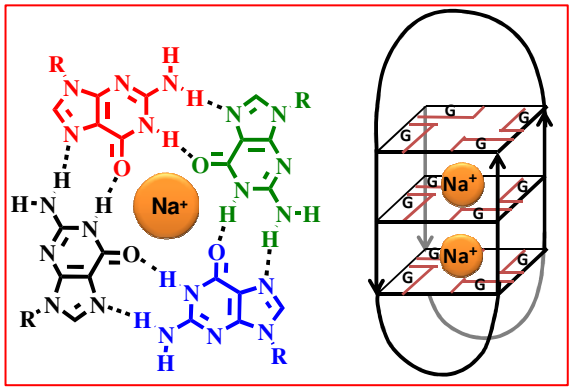


Figure 3. (A) Formation of the G-tetrad through Hoogsteen H-bonding among the guanine bases. (B) Representative intramolecular G-quadruplex DNA structure.

features of the telomere are important for the search of possible clinical applications. Interestingly the telomeric DNA contains very conserved and repetitive stretches of sequences which are G-rich in nature. The telomere repeat of vertebrates, plants, tetrahymena and oxytricha contain tandem repeats of d(TTAGGG), d(TTTAGGG), d(TTGGGG) and d(TTTTGGGG) respectively.[19] In 1987, Blackburn first proposed the possibility of a non-Watson-Crick G-G base pairing in a short stretch of telomere.[20] Williamson *et al.* in 1989 described that a long telomeric DNA containing four contiguous stretch of G-residues could fold into a more compact structure in presence of mono-valent metal ions like Na^+ and K^+. These ions get sandwiched between the four planar array of Hoogsteen-paired G-tetrads (Figure 3).[21] Thereafter the tetraplex structure containing the guanine residues has been widely known as the G-quadruplex (G4) DNA. It is believed that the single-stranded telomeric overhangs remain in equilibrium in different conformations under physiological conditions. Extensive studies have revealed that the G-quadruplex DNA structures are easily formed and these are quite stable in the physiological conditions. The discovery of the G-quadruplex structure opened a new chapter in dealing with the telomere and its possible role in oncology as well.[22]

Significance of G-quadruplex structure

Like Watson-Crick duplex DNA, the G-quadruplex DNA also has high kinetic and thermal stability. An *in vitro* study confirms that the telomerase protein does not recognize the G-quadruplex DNA even under the physiological conditions whereas it is active on single-stranded DNA rather efficiently.[6] The existence of G-quadruplex structure could not be ascertained *in vivo* until Schaffitzel *et al.* demonstrated the direct evidence of the existence of G-quadruplex DNA at the telomeric ends of macronuclei in Stylonychia lemnae using G-quadruplex DNA specific antibodies in 2001.[23] However, the basic conceptual query that remains unanswered as to how the single-stranded telomeric DNA coexists with the kinetically and thermodynamically more stable G-quadruplex structures. Cech in 2005 reported that human POT1 (protection of telomeres protein 1) protein can disrupt the telomeric G-quadruplexes allowing telomerase extension *in vitro* (Figure 4).[24,25] Thus it was established that the telomerase protein which can act only on single-stranded telomere, remains silent to the higher order structures like G-quadruplexes. It offered an opportunity for the design of a strategy towards the introduction of ligands that can stabilize the G-quadruplex DNA structure and shift the equilibrium towards it in solution. Thus the conversion of the telomere structure to a non-recognizable form like G-quadruplex DNA has become an important step towards the telomerase inhibition and its consequent therapeutic intervention.

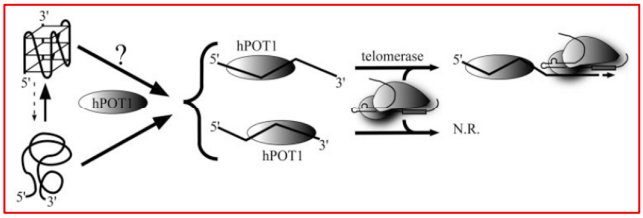


Figure 4. Model for hPOT1 disruption of intramolecular G-quadruplex DNAs, allowing their extension by telomerase. When hPOT1 binds near the 5'-end of the primer, leaving an 8-nt tail, it can be extended by telomerase. When hPOT1 binds near the 3'-end of the primer, leaving a 2-nt tail, there is no reaction (N.R.). Adapted with the permission from reference (24), copyright 2005, National Academy of Sciences, USA.

For the last two decades, various researchers have exploited the strategy of developing numerous G-quadruplex binding ligands and demonstrated their effectiveness with the help of different experimental methods. These include spectroscopic (UV-Vis, CD, Fluorescence, Raman, SPR, NMR), spectrometric (Mass), calorimetric (ITC and DSC), electrophoretic mobility shift (EMSA), enzymatic (TRAP or TRAP-LIG assay, polymerase stop assay, DNA cleavage experiments), cellular (MTT assay, cell cycle analysis), and *in vivo* studies (tumorigenesis and drug effect) along with theoretical calculations (docking and molecular dynamics simulation).

To achieve an effective and a potent drug targeting to a specific biological active site, one must consider few parameters like the target's size, electronic environment and the surrounding features of the active site. After the discovery of telomeric G-quadruplex DNA, it became essential to explore its structural characteristics. In the early 90's, the chiral property of DNA was closely examined and various predictions were made on the

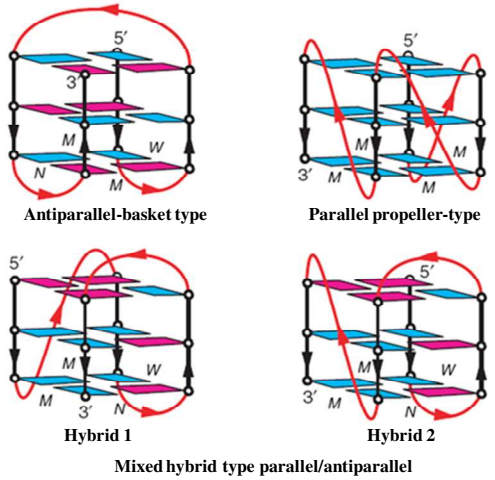


Figure 5. Schematic representation of an intramolecular human telomeric G-quadruplex DNA as obtained from NMR or X-ray crystal structural analysis. Adapted with the permission from reference (31), copyright 2006, American Chemical Society.

basis of its circular dichroism (CD) spectra. In 1990, Sen and Gilbert first reported the Na^+/K^+ switch of the G-quadruplex DNA structure.[26] The involvement of the guanine bases was confirmed using base-specific DNA cleavage experiments. Jin *et al.* correlated the results with the CD spectra and proposed a putative structure of the guanine tetrads.[27] The CD spectral measurements and NMR studies using guanine rich DNA sequences d(TG_3T) and d($TG_3T_2G_3T$) suggested that an intermolecular anti-parallel structure is formed solely by the Na^+ ions, whereas the more strongly bound K^+ ion induced a transition to the intermolecular parallel G-quadruplex DNA organization.[27]

NMR spectroscopy has been used for the prediction of the higher order DNA structures in solution. Henderson *et al.* in 1987 reported the hairpin structure formed by the tetrahymena telomeric DNA sequence (T_2G_4) stabilized by the hydrogen

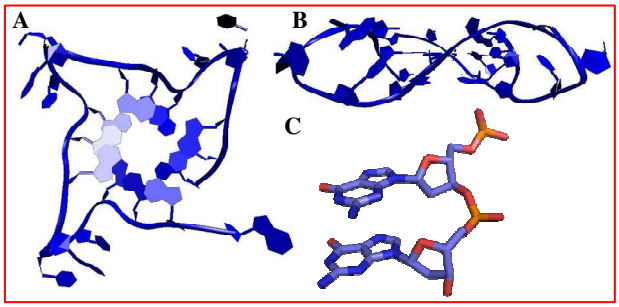


Figure 6. Top view (A) and side view (B) of the X-ray crystal structure of a parallel G-quadruplex DNA (PDB 1KF1) derived from human 22-mer telomeric DNA sequence. Orientation of the nucleobases in the parallel G-quadruplex DNA structure (C).[35]

bonds among the guanine residues in a syn-conformation.[28] Thereafter extensive NMR studies revealed the structural features of the human four-repeat 22-mer DNA sequence as well. NMR studies of the human 22-mer in solution containing Na^+ suggest a basket type G-quadruplex structure is formed comprising a mixture of both anti-parallel and parallel stranded intramolecular structure with three G-tetrads connected by one diagonal and two lateral (edgewise) TTA loops.[29] The K^+-stabilized solution showed more than one structures[30-33] but the hybrid-type G-quadruplex structure appeared to be the major one even in the presence of Na^+ ions at high concentrations.[33] The telomeric G-quadruplexes remain in dynamic equilibrium between the two conformations, i.e., the hybrid-1 and the hybrid-2 (Figure 5, Figure 7).[34] Human telomeric DNA sequences may adopt various topological geometries depending on the flanking terminal bases, stabilizing ions and molecular crowding as presented in the Figure 7. These observations suggest that K^+-stabilized hybrid G-quadruplex DNA structures exist even in presence Na^+ ions and could therefore be a putative drug target under physiological conditions.[34]

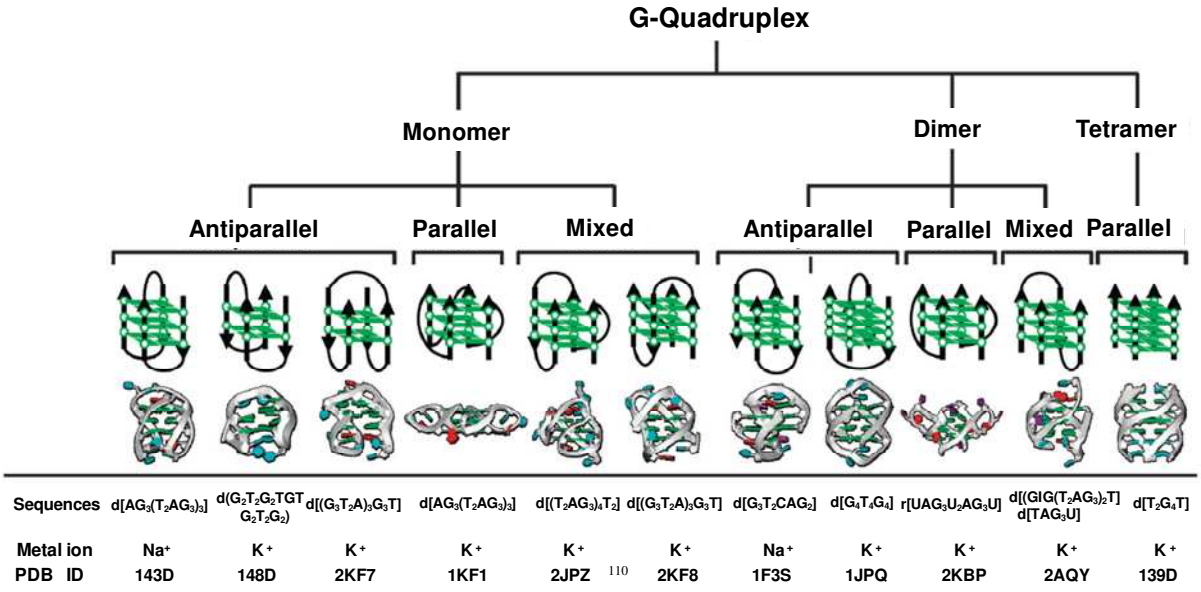


Figure 7. Polymorphic G-quadruplexes. Sequences indicated by '**I**' represents inosine. Adapted with the permission from reference (118), copyright 2012, The Royal Society of Chemistry.

It became clear when a K^+-stabilized human telomeric quadruplex DNA crystal structure was reported by Neidle in 2002. The 22-mer human DNA sequence showed orientation of all the four DNA strands in parallel direction with the three linking trinucleotide loops (TTA) positioned on the exterior of the G-quadruplex core (Figure 6). The adenine of each TTA trinucleotide link swung back to achieve a favorable intercalative interaction between the two thymine bases. All the guanine glycosidic bonds remain in an anti-conformation with C2'-endo sugar puckers to form a flattened tetrad (Figure 6).[35] Thereafter, the thermodynamic and kinetic features of the G-quadruplex DNA structure have been widely explored both by theoretical and experimental investigations.[36-38]

The G-quadruplex structure possesses tetrads of 10.9×13.6 Å side and diagonal lengths respectively held predominantly by π-π interactions along with four equivalent phosphate grooves created by the three TTA loops on the side.[39] The tetrads of both ends are potential ligand target. Among this the 5'-G-quartet surface is relatively more hydrophobic which favour the π-stacking interactions whereas the 3'-surface is more optimally poised for the electrostatic interactions. The groove and loop region may also be targeted for the design of a G-quadruplex binding ligand.[40] To stabilize the negative potential (or positive ion channel) created by the electronegative atoms (N, O) in the centre of the G-tetrad, monovalent cations like Na^+, K^+, NH_4^+ *etc* are essential. The positive ion channel in turn prefers that the ligand should be electron-deficient in nature for effectively stabilizing stacking interactions. The anionic phosphate backbone confers favorable association with a ligand carrying net positive charge.

Design of the G-quadruplex DNA binding ligands

After the discovery of telomeric G-quadruplex and their impact on genetic regulation it was considered to be an 'open' subject. "*Do telomerase antagonists represent a viable anticancer strategy?*"[23] To address this question a number of scientists started design and synthesis of organic molecules targeting G-quadruplex DNA and evaluated their effect on G-quadruplex DNA starting from biophysical to *in vitro* biochemical studies. At the early stages, the interactions of molecules such as actinomycin D, ethidium bromide, and chromomycin A3,[41] bleomycin-Nickel (III) complex,[42] carbocyanine dye[43] were investigated with the telomeric G-quadruplex DNA derived from a lower organism. By this time, a method to measure the efficiency of G-quadruplex binding ligands towards telomerase inhibition was also developed which was used as a laboratory protocol known as Telomerase Repetitive Amplification Assay (TRAP assay).[44] Subsequently this was modified to TRAP-LIG[45,46] assay in 2007 to include necessary control experiments for avoiding wrong interpretation of the biological activity involving telomerase. In 1997 Neidle and Hurley developed 2,6-diamidoanthraquinone derivatives [**1**] which showed moderate telomerase inhibition ability by standard telomerase assay (Figure 8).[47] Thereafter extensive development in the drug design was carried out especially after the elucidation of NMR and crystal structural data of a number of human telomeric G-quadruplex DNA sequences.

DNA is a negatively charged polyelectrolyte having planar aromatic bases comprising several hetero-atoms in its organization. Binding of a small molecule, with DNA occurs via (i) ionic; (ii) π-π stacking; and (iii) H-bonding interactions. Therefore an efficient G-quadruplex binding ligand should be cationic with a planar conjugated pharmacophore having hetero-atoms capable of acting as H-bond donor as well as acceptor. The highly accessible planar end tetrads have been primarily targeted for G-quadruplex recognition and accordingly planar molecules were designed first. Bhattacharya *et al.* demonstrated that not only the planarity is important but the shape of a ligand also played an important role in targeting the G-quadruplex DNA. The angular V-shaped molecules showed much better activity than the linear ones.[48] The MD simulation studies also validated the experimental findings.[48,49] Neidle and Moses independently highlighted the importance of angular shaped ligand design introducing triazole moiety via click chemistry [**15**].[50] The conformational switching of the G-quadruplex DNA by photoregulation of azene moiety [**14**] further enriched the ligand design strategy.[51] Earlier a similar strategy was also exploited with duplex DNA involving distamycin based azobenzene ligands.[52] Balasubramanian and co-workers developed bis-indole carboxamides [**4**] with benzene or pyridine moiety as the central core to govern the flexibility and rigidity through intramolecular H-bonding. However, the lack of availability of DNA interacting sites in the pyridine system rendered it a less efficient binder to the telomeric G-quadruplex DNA.[53,54]

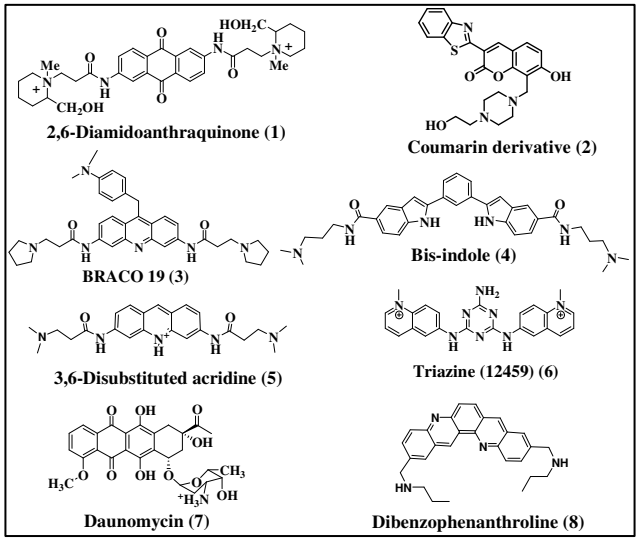


Figure 8. G-quadruplex binding ligands [I].

Cationic ligands are known to interact with the G4 DNA more strongly. However, they are not likely to be strong enough to bind with the G4 DNA in high ionic strength environments including the physiological conditions. They may also suffer from non-specific binding and poor discrimination ability over the duplex DNA. Planar ligands having flanking groove binder or a moiety carrying positive charge showed significantly higher affinity. Among these the acridine derivative BRACO 19 [**3**][55-56] and naphthalene diimide derivative BMSG-SH-3 [**13**][57,58] are the most important. These compounds are currently in their final phase of clinical viability trials. Ethidium bromide which has been extensively used as the double-stranded DNA stainer could not be used for the G4 DNA staining due to its poor binding

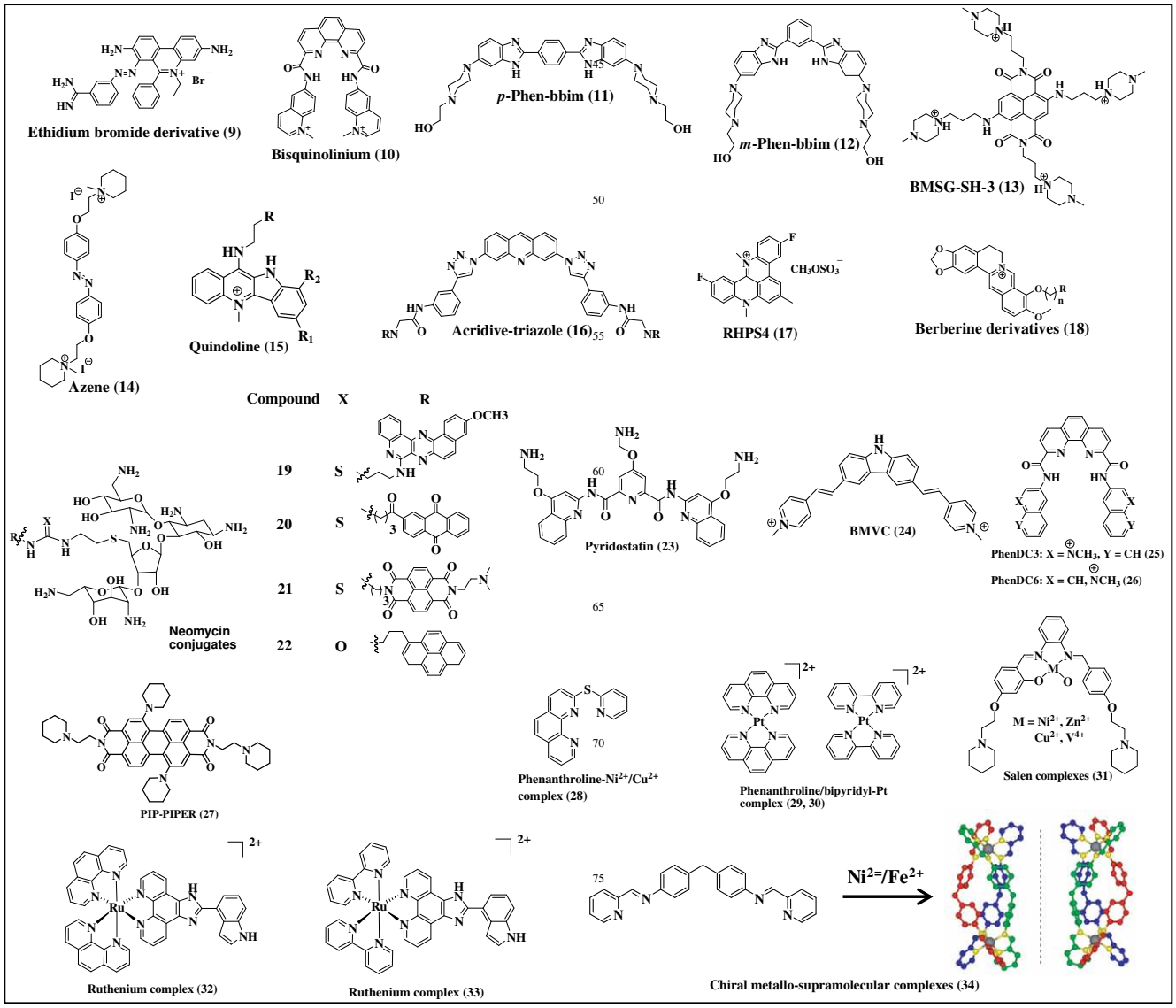


Figure 9. G-quadruplex (G4) binding ligands [II]. Structure of ligand **34** has been adapted with the permission from reference (85).

affinity towards the latter.[59,41] In 2001, Mergny derivatized ethidium [**9**] introducing an extended binding moiety to make an efficient selective G4 DNA binder, stainer and eventually a telomerase inhibitor.[60] Progressively with time, hundreds of researchers spanning across the world have contributed to the development of a large library of G4 ligands.[61,62] Many of the potent molecules have come across different generations of molecular designs from a common template. Acridine derivatives, e.g., 3,6-disubstituted acridine [**5**],[63] 3,6,9-trisubstituted acridine,[64] quinoacridinium[65] are among the most important classes of ligands. Phenanthroline ligands, like dibenzophenanthroline derivatives [**8**],[66] phenanthro-imidazole derivatives,[67] PhenDC3 [**25**], PhenDC6 [**26**],[68] 1,10-phenanthroline-2,9-carboxamide[69] *etc* are also reported for their efficient G4 binding activity. Fused ring systems comprising quindoline [**15**],[70] berberines [**18**],[71] norfloxacin,[72] levofloxacin,[72] daunomycin [**7**],[73] and RHPS4 [**17**][74] *etc* are also important. Sugar derivatives (rutin,[75] epigallocatechin,[76] neomycin-conjugates [**19-22**],[77,78]) and peptide conjugates (peptidyl-anthraquinone conjugates,[79] acridine-peptide conjugates[80]) have also been documented for their good G-quadruplex DNA binding abilities.

Organo-metallic compounds such as, schiff base complexes [**31**],[81,82] Ru (II) polypyridyl complexes [**33**],[83] 1, 10-phenanthroline Pt (II) complexes[84] [**29**]) evidenced noticeable impact on the G-quadruplex DNA binding.

The role of the molecular dimension, planarity and the nature of metal ion in such organo-metallic complexes has been extensively studied. The metal ion present in the complex may substitute the stabilizing Na^+/K^+ ions and promote the formation of the G-tetrads. Moreover, the electron-withdrawing nature of metal ions makes the co-ordinated ligand electron-deficient. This in turn increases the π-π stacking interactions among the metal complex and the G-tetrad.[81] It has been observed that with an increase in the available π-surface (from bipyridine to phenanthroline [**29**,**30**,**32**,**33**]), the affinity of a ligand with G-tetrad increases.[67,83,84] Phenanthroline moiety with an extended pyridyl co-ordination site [**28**] is reported to form 2:1 complexes with both Ni^{2+} and Cu^{2+} metal ion. Ni^{2+} providing a d^8 electronic system forms a 'tight' octahedral complex whereas Cu^{2+} being a

d^9 electronic system forms a 'loose' octahedral complex.[82] Thus, Cu^{2+}-complex gives enhanced molecular planarity than the corresponding Ni^{2+}-complex and prefers to interact with the planar G-tetrad more effectively. In contrast relatively more non-planar Ni^{2+}-complex prefers to interact with the grooves of the G-quadruplex DNA giving a prominent induced circular dichroism (ICD) band. Surprisingly, this variation in the central metal ion results in a subtle change in molecular planarity which governs their preferential affinity towards the groove vs. the G-tetrad. It also inhibits the two complexes to share a common binding site even in a co-existing situation.[82] Further the angular cylindrical metal complexes target the chiral grooves of the G4 DNA structure. For instance, the P-enantiomers of the metallo-supramolecular complexes (Ni^{2+} or Fe^{2+}) [**34**] are known for their remarkable chiral preference. On the other hand, planar metal complexes comprising an extended π-surface, similar to the G-tetrad dimension have been found to be efficient G4 DNA binder.[85]

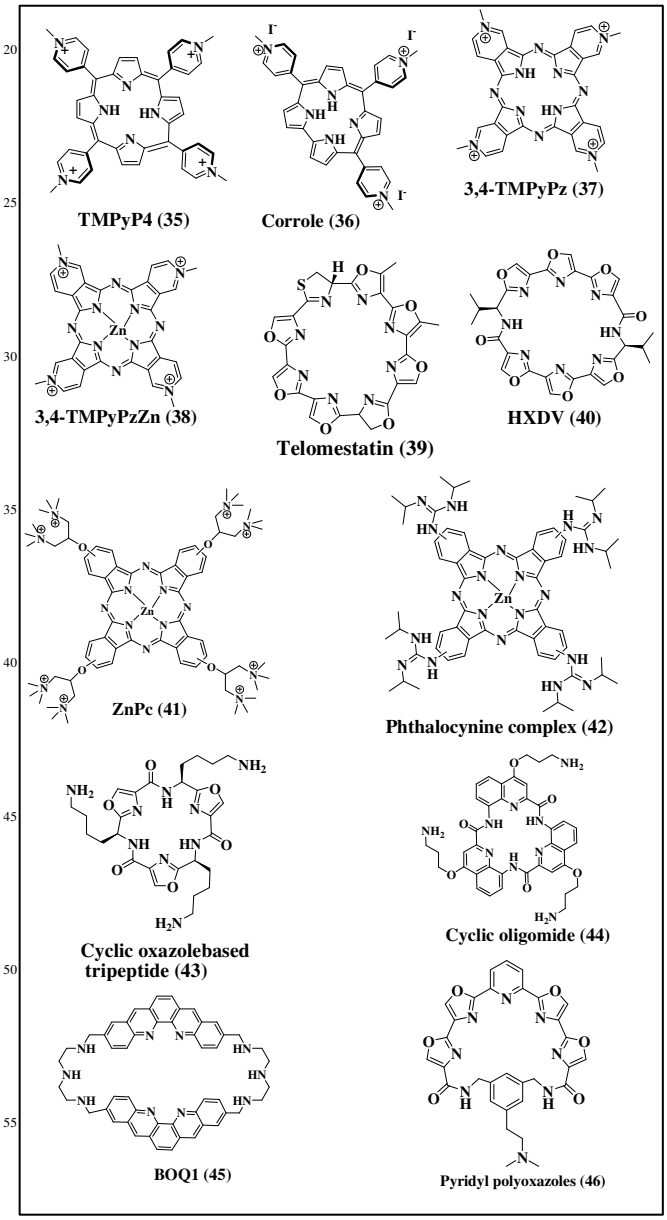


Figure 10. G-quadruplex DNA binding cyclic ligands [III].

Benzimidazole ligands which were long known for their duplex DNA binding activity[86,87] emerged as efficient and selective G-quadruplex DNA binders upon appropriate modification [**11**,**12**] (Figure 9).[48,49,88-90] Among others, triazine derivatives [**6**],[91] PIP-PIPER [**27**],[92] bis-quinolinium compounds [**10**],[39] carbazole system BMVC [**24**],[93] pyridostatin (PDS) [**23**],[94] coumarin derivatives [**2**],[95] have also been shown to possess good G-quadruplex stabilizing activity.

Another important family of G4 ligand comprises cyclic molecules (Figure 10). Among these cyclic ligands, natural product telomestatin [**39**] is distinguished for its remarkable activity.[96-100] This was isolated from the bacteria streptomyces anulatus by Shin-ya *et al.* in 2001. One interesting fact is that between its two isomers, the (*S*)-isomer showed better telomerase inhibition activity with a IC_{50} value of ~5 nM on the basis of TRAP assay.[101] The first total synthesis of telomestatin was achieved by Takahashi *et al.*[102] in 2006, which was followed by Shin,[103] Vedejs,[104] Chattopadhyay,[105] and Pattenden.[106] Many other cyclic molecules were discovered which demonstrated excellent telomerase inhibition activity among which the C_2-symmetric hexaoxazoles,[107] porphyrazine [**37**,**38**],[108] HXDV [**40**],[109] cyclo[*n*] pyrroles,[110] pyridyl polyoxazole [**46**],[111] cyclic oligoamides [**44**],[112] cyclicoxazole based tripeptide [**43**][113] deserve special mention. Porphyrin [**35**],[114,115] corrole [**36**],[116] sapphyrin[117] and phthalocynine [**41**,**42**][118] are another family of G4 ligand which made contributions in telomere chemistry and biology. Phthalocyanine [**41**] having significantly higher molecular planarity than porphyrin [**35**] with an extended π-surface matches with the G-tetrad dimension more closely and as a result the former showed almost two orders of higher magnitude of binding affinity over the later.[118] Phthalocyanine and porphyrin have excellent photophysical property which may be also used in imaging and in photo-dynamic therapy. The complexation of diamagnetic metal ions (Zn^{2+}, Al^{3+} and Ga^{3+}) enhances the photosensitivity while that with paramagnetic metal ions (Cu^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Cr^{3+}, Vo^{2+} and Pd^{2+}) eliminates the photoactivity by reducing the triplet state (T1) lifetime.[118]

To enhance the efficiency of ligand binding by minimizing entropic penalty, strategies involving development of dimeric (gemini) molecules have been adopted (Figure 11). Bhattacharya and co-workers have exploited the design of dimeric (gemini) ligands through the variation in spacer length [**48-53**].[88,89] It has been found that an oligo-oxyethylene spacer is superior to the corresponding ones based on oligo-methylene ones (unpublished results). All the gemini ligands showed better activity than their corresponding monomers. Nagasawa also reported a series of hexaoxazole dimers [**54**] with different spacer lengths, although the affinity of the dimer was found to be nearly comparable to that of the monomers in this case.[119] The other notable dimeric ligands are bis-HT [**47**],[120] BOQ_1 (bis-ortho-quinacridine) [**45**][121] and bisA (bis-acridine)[122] which formed non-planar cage like structures and showed better binding affinity than their monomeric analogues.

The discovery of thiazole orange [**63**] by Monchaud *et al.* in 2007 as a useful probe for the fluorescence sensing of G-quadruplex DNA opened up a new strategy for the G4 ligand screening.[123] Sintim and co-workers in 2011 have shown the interaction of different aromatic dyes like acriflavine [**55**],

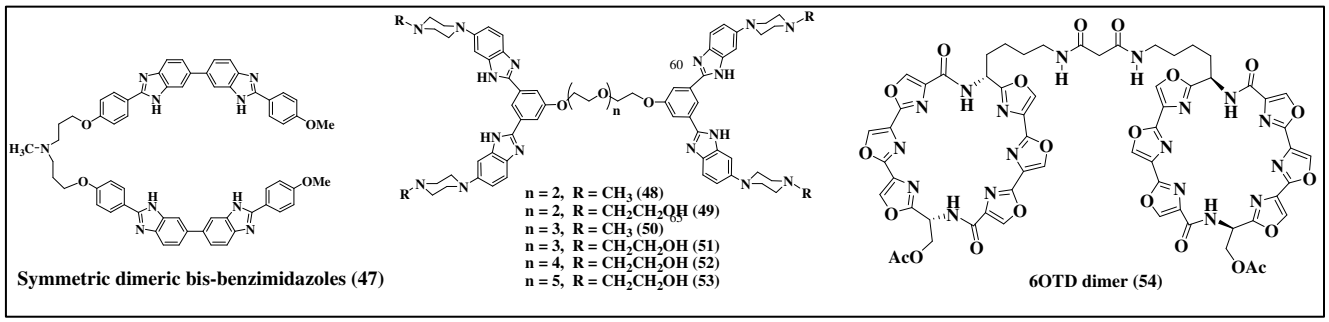


Figure 11. G-quadruplex DNA binding dimeric ligands [IV].

proflavine [**56**], pyronin [**61**], methylene blue [**62**], anthracene [**57**], malachite green [**58**], crystal violet [**59**] *etc* with the G-quadruplex DNA (Figure 12A).[124] Mergny and Fichou discovered the affinity based G4 ligand displacement assay for identifying selective G-quadruplex binders using a thiazole orange dye.[125] Later on in 2011, Mergny developed a "*fluorescent intercalator displacement assay for screening G4 ligands towards a variety of G-quadruplex structures*" (Figure 12C,D).[126] The discovery of a BODIPY-labeled macrocyclic heptaoxazole [**60**] by Nagasawa provided an impressive visual evidence of its G-quadruplex binding (Figure 12A,B).[127]

Recently, Alcaro *et al.* reported ligand and DNA structure-based virtual screening of molecular structures by means of docking experiments.[128] The authors further validated the findings with the help of various spectroscopic techniques. Chaires and coworkers have developed a competitive dialysis assay for the identification of potential G-quadruplex binding ligand and this is a convenient tool in the ligand screening process.[129] Template-assembled synthetic G-quadruplex (TASQ) has also provided a convenient method to investigate and understand the ligand-DNA interaction.[130]

Besides targeting the G4-tetrads, a parallel research activity on targeting highly accessible G-quadruplex grooves and loops have been gaining importance in medicinal chemistry as putative candidates for the development of therapeutic drugs (Figure 13).[131-133] In 2003, Maity *et al.* reported moderate affinity of duplex DNA binder Hoechst molecule [**69**] toward G-quadruplex DNA through an interaction with the AAGGT loop.[134] In the same year, Cocco *et al.* reported the inhibition of protein interactions with the G-quadruplex DNA by distamycin A [**70**] via stacking on the terminal G-quartets and contacting through the flanking bases.[135] In 2006, Neidle reported a comparative investigation of distamycin and its analogues with human telomeric G-quadruplex DNA and compared their affinity with the A-T rich duplex DNA.[136] These authors have shown that though distamycin A has rather poor selectivity towards the G-tetrads, the selectivity could be increased with the introduction of more number of pyrrole groups which allows binding with the mixed groove/G-quartet in a stacking mode. Randazzo and co-workers have carried out an extensive investigation on the interaction of distamycin A and its derivatives (by replacement of its amidinium group with an uncharged N-methyl amide moiety)

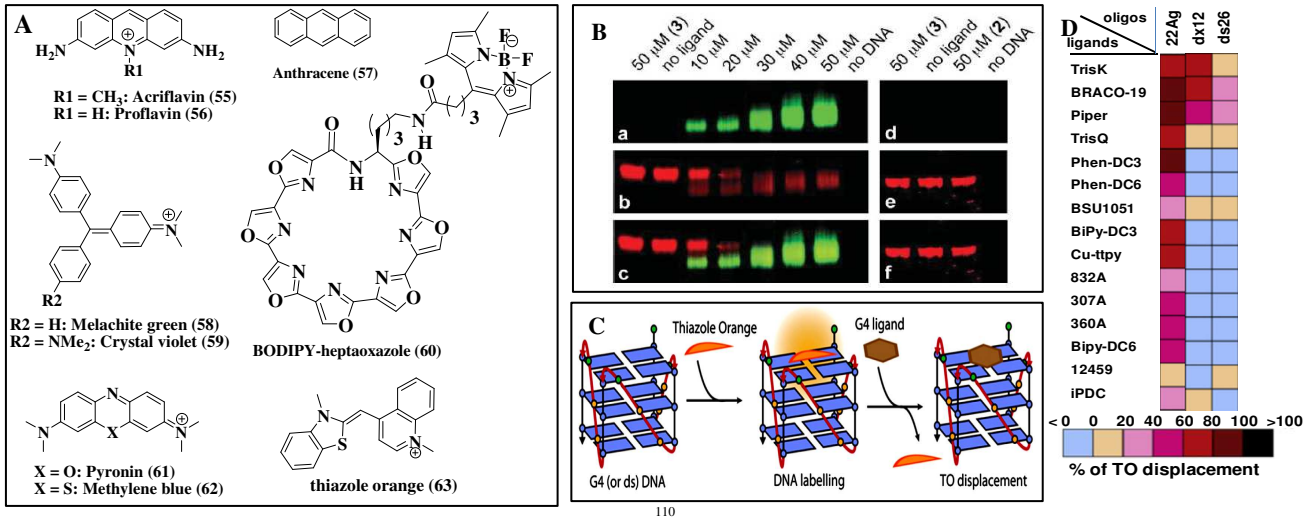


Figure 12. (A) G-quadruplex binding dye ligands [V]. (B) Visualization of the human telomeric G-quadruplex (telo24) by BODIPY compound. Gel electrophoresis (12% native polyacrylamide, in 1X TBE buffer, 4 ° C) of 10 mM oligonucleotides (a-c: telo24, d-f: ds-telo24) in the presence of various concentrations of BODIPY-heptaoxazole (no salt added). a, d) All bands were detected using the 526 nm short pass filter. The gel was stained with b) Stains-all^{R} and e) ethidium bromide and then all bands were detected using the 580–640 nm band pass filter. c, f) Merged images of a and b or d and e. Adapted with the permission from reference (127), copyright 2010, The Royal Society of Chemistry. (C) Schematic representation of fluorescence intercalator displacement assay. (D) Thiazole Orange (TO) displacement obtained with different test molecules (colour code indicated below) for human telomeric G-quadruplex (22Ag) and two duplexes (dx12 and ds26). Adapted with the permission from reference (126), copyright 2011, Elsevier Masson SAS.

with Tetrahymena G-quadruplex sequence. An NMR spectral study revealed that distamycin A interacts with the groove of the G-quadruplex DNA.[137-139] A Dist-A derivative MEN 10716 [**71**] containing five *N*-methyl-pyrrole rings inhibits the human telomerase enzyme in a dose-dependent manner with an IC_{50} of 24 ± 3 μM.[140] Cyanine dye derivatives represent another class of G-quadruplex DNA groove binding agents. Thus the cyanine derivative, 3, 3′-diethyloxa-dicarbocyanine (DODC) [**66**] shows its binding capability with the grooves of a dimeric hairpin G4 DNA.[141] The appearance of ICD band (534–626 nm) gives direct evidence for its groove targeting. Importantly, DODC shows significantly preferential binding ability towards G4 DNA even in presence of a large amount of the duplex DNA. Among others, DTC [**67**][142] and DTDC [**68**][143] also show excellent G4 DNA binding ability predominantly through groove targeting. Recently Paul *et al.* have developed benzimidazole derivatives containing non-planar Troger's base scaffolds [**64**,**65**] and showed their significant potential in binding via targeting the groove in the G-quadruplex DNA structure and telomerase inhibition ability.[144] As a matter of the fact, a groove binder may generally have lower affinity towards the G4 DNA and may also lack in the ability to discriminate between the G4 DNA from the duplex DNA. A planar pharmacophore preferably of G-tetrad dimension attached to a groove binder may improve the efficiency of binding.

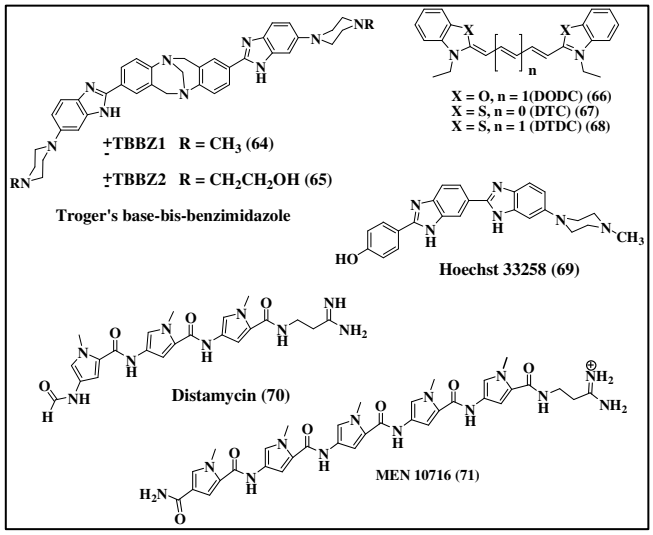


Figure 13. G-quadruplex binding ligands [VI].

Does G-quadruplex really exist *in vivo*?

Widespread data on the G-quadruplex DNA like their high kinetic and thermodynamic stability, NMR and crystal structures, characteristic CD signatures, FRET studies and DNA sequencing assays even in physiological conditions evidenced their existence *in vitro*. But there is no guarantee for the *in vitro* biomolecular structure to exist *in vivo* as well. Towards this end, quite a few G4 ligands have been developed which interfere with events in telomere biology probably through the G4 DNA binding. For instance, certain coumarin derivatives (Figure 8, ligand **2**) inflict selective DNA damages at telomeric level resulting in the apoptosis and senescence on tumor cells.[95] Additionally, telomestatin mediated telomere shortening associated with apoptosis was also evident in some freshly obtained leukemia cells from acute myeloid leukemia patients.[97] However, despite these, an unambiguous claim pertaining to their biological role may not be made with confidence.

Schaffitzel *et al.* furnished the first experimental evidence of the existence of the G-quadruplex DNA at the telomeric ends of macronuclei in stylonychia lemnae *in vivo* using G-quadruplex-specific antibodies.[23] These authors showed that G-quadruplex formation can be a mechanism for telomere capping along with

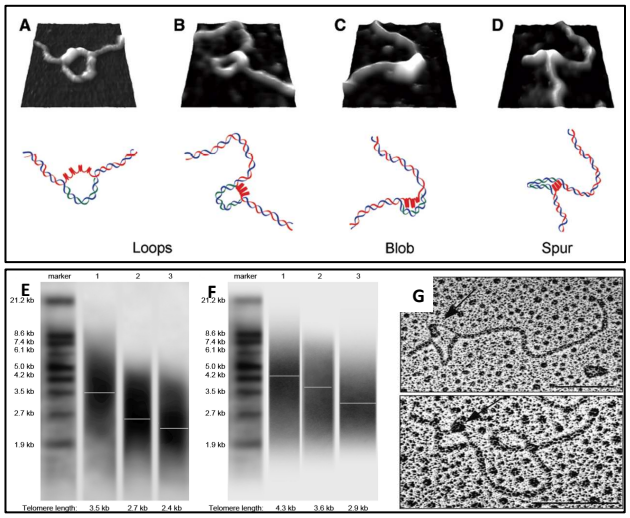


Figure 14. Zoomed AFM images of the G-quadruplex structures with corresponding diagrammatic representations of the DNA arrangements. Areas (140×140 nm^2) show regions of transcribed plasmids containing loops (A, B), a blob (C) and a spur (D), Adapted with the permission from reference (151), copyright 2007, Oxford University Press. TRF analysis of (E) HL60, (F) CA46 cells treated with increasing ligand concentrations or untreated for 16 days, Adapted with the permission from reference (70), copyright 2008, American Chemical Society. (G) G4-DNA recognized in TEM by recombinant biotinylated Nucleolin-428/streptavidin gold beads. Arrows indicate beads bound at loops. Bar, 200 nm. Adapted with the permission from reference (145), copyright 2004, Cold Spring Harbor Laboratory Press.

the t-loops and TEBP (oxytricha telomere binding protein) binding. Maizels in 2004 first reported an electron microscopic evidence of the G-loops and the G4 DNA within plasmid genomes transcribed *in vitro* in E. coli (Figure 14G).[145]

In 2008, Gu reported a few 5-*N*-methylated quindoline derivatives (Figure 9, ligand **15**) which show remarkable cessation in population growth and cellular senescence phenotype accompanied by a shortening of the telomere length (Figure 14E,F).[70] A telomeric restriction fragment (TRF) length assay showed ligand induced shortening in the telomeric length in leukemia cell HL60 and lymphoma cell CA46 which was in accordance with the cytotoxicity results obtained by MTT assay.[55,70] Regulation in the expression levels of the associated genes by the G-quadruplex binding ligands, proteins and antibody was indirect yet a strong evidence in favour of the existence of the G-quadruplex DNA structure *in vivo*.[145-150] In 2009, Edwardson and co-workers first visualized the G-quadruplex structure *in vivo* by atomic force microscopy (Figure 14A-D).[151] Afterwards, Balasubramanian and Edwardson showed a ligand-induced perturbation of G-quadruplexes in a plasmid DNA using atomic force microscopy.[152]

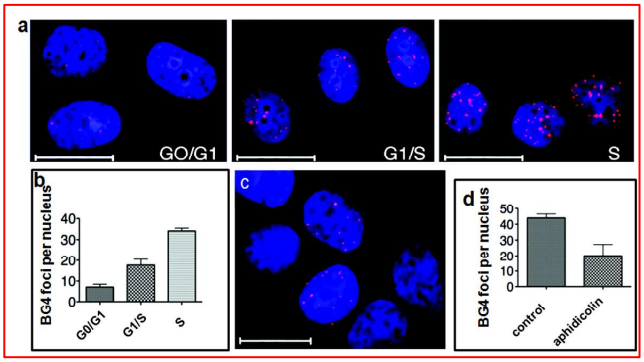


Figure 15. Modulation of G-quadruplex structures during cell-cycle progression. (a) BG4 staining in synchronized MCF-7 mammary adenocarcinoma cell populations at the G0/G1 and G1/S boundaries, and during the S phase. Nuclei are counterstained with DAPI (blue). Scale bars, 20 µm. (b) Quantification of BG4 foci number per nucleus for a. 100 nuclei were counted per stage and the s.e.m. was calculated from a set of three replicates. (c) A greater than two-fold reduction in the BG4 foci number after inhibition of DNA synthesis by aphidicolin treatment (5 µM for two hours). (d) Quantification of the BG4 foci number with or without aphidicolin treatment. 100 nuclei were counted and the S.E.M. was calculated from a set of three replicates. These experiments demonstrate that G-quadruplex structures are modulated during the cell cycle and, in particular, support the replication-dependent formation of endogenous DNA G-quadruplexes. Adapted with the permission from reference (153), copyright 2013, Nature Publishing Group.

In 2013 Balasubramanian *et al.* reported the quantitative visualization of G-quadruplex DNA structures in human cells by structure-specific antibody (BG4) and G4 ligand assisted enhancement of the G-quadruplex staining. The authors demonstrated the presence of endogenous G-quadruplex DNA also outside the telomere using TRF2 (telomere repeat-binding factor 2) co-localization. Interestingly, the relative abundance of G-quadruplex DNA in different stages of cell cycle has been reported for the first time (Figure 15).[153] The authors further established a G4 ligand, pyridostatin, mediated DNA damage which emphasised the G-quadruplex DNA formation probed by a DNA damage marker γH2AX (Figure 16A,B).[154] These findings provide significant evidence for the existence of G4 DNA *in vivo* which also justifies the G4 DNA targeted anticancer drug design strategy through telomerase inhibition pathway (Figure 16C).

G-quadruplex ligand's activity *in vivo*

The availability of cellular G-quadruplex structures and telomerase enzymes provides an opportunity to target the cancer cell selectively. As the double-stranded DNA is overwhelmingly more abundant than the G-quadruplex DNA, a potent ligand should have very high selectivity towards the G4 DNA over the normal *B*-DNA. Most of the efficient G4 ligands show excellent *in vitro* cancer cell cytotoxicity keeping the healthy cells intact. The lack of selectivity in cellular cytotoxicity affects the normal cells as well. The G4 ligand mediated telomere shortening,[70] DNA damage (Figure 17)[155] and selective cancer cell anti-proliferative activity *in vivo* provide strong boost to the telomere research. A potent G4 ligand terminates cell cycle at S, G2/M phases and enhances sub-G1 populations leading to induction of apoptosis in cancer cells (Figure 18).[156]

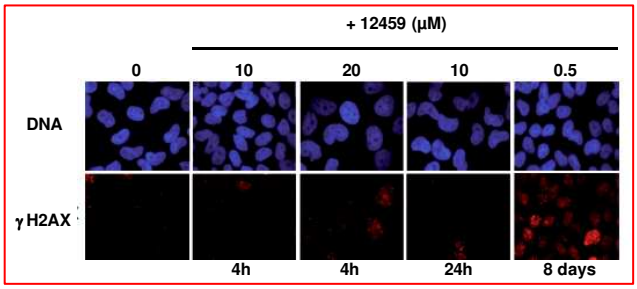


Figure 17. Delayed DNA damage signalization induced by **12459** (Figure 8, ligand **6**) in A549 cells. (A) Cells untreated or treated with 12459 for 4 h (10 and 20 µM) and for 24 h (10 µM) were examined for γ-H2AX foci (red). Hoechst staining of DNA is shown in blue. As a positive control for DNA damage, A549 cells were treated with 12459 (0.5 µM) for 8 days. Adapted with the permission from reference (155), copyright 2013, Oxford University Press.

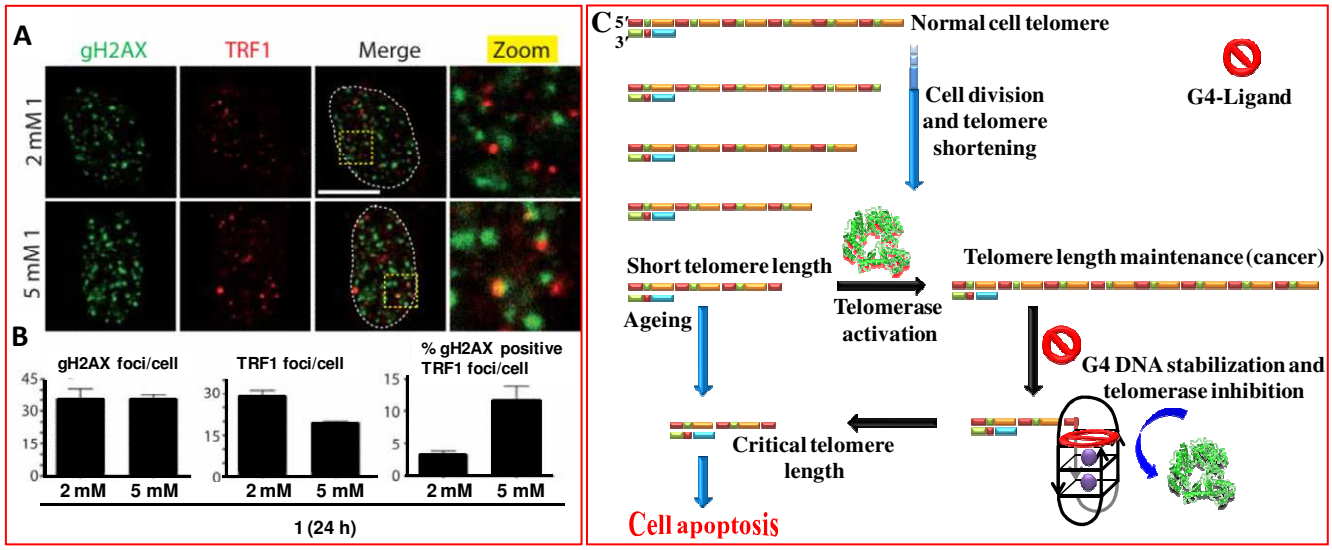


Figure 16. Visual analysis of pyridostatin targets. (A) DNA damage signals induced by pyridostatin were mainly nontelomeric and the areas within the white dotted lines indicate the locations of nuclear DNA. Scale bar, 10 µm; the zoomed images are ×4 magnifications of the main images. The yellow dotted boxes in the merged images indicate the area of magnification. (B) Quantification of the experiment shown in a. n = 3; >100 cells were scored per condition per replica; error bars represent S.E.M. Adapted with the permission from reference (154), copyright 2012, Nature Publishing Group. (C) Schematic representation of G4 ligand mediated telomerase inhibition and anticancer activity.

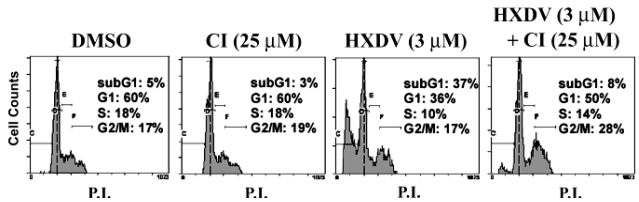


Figure 18. HXDV induces robust apoptosis. HeLa cells were treated with 3 μm HXDV in the absence or presence of the caspase inhibitor (labeled CI) for 16 h. DNA was stained with propidium iodide (P.I.) and analyzed by FACS. DMSO, dimethyl sulfoxide. Adapted with the permission from reference (156), copyright 2009, The American Society for Biochemistry and Molecular Biology.

Even though medicinal chemists have developed a number of potent G4 ligands, still the question remains, whether a G-quadruplex ligand would be an anticancer drug in the near future. To answer this question, scientists have moved forward from the *in vitro* study to appropriate *in vivo* model. Telomestatin which is among the most potent G4 ligands, induces senescence and apoptosis in a number of different tumor cells without any significant toxicity to the normal progenitor cells.

When the drug was administered in mice containing U937 xenografts, it could suppress the tumor volume significantly.[92] However, so many steps involved in its total synthesis makes it prohibitively expensive and restricts its application in real life. Among others, polyoxazole derivative HXDV and macrocyclic pyridyl polyoxazole [**46**] are potential drug candidates.[111] The pyridyl polyoxazole shows enormous tumor suppression ability in human tumor xenografts athymus nude mice model established using MDA-MB-435 breast cancer cells (Figure 19).[111]

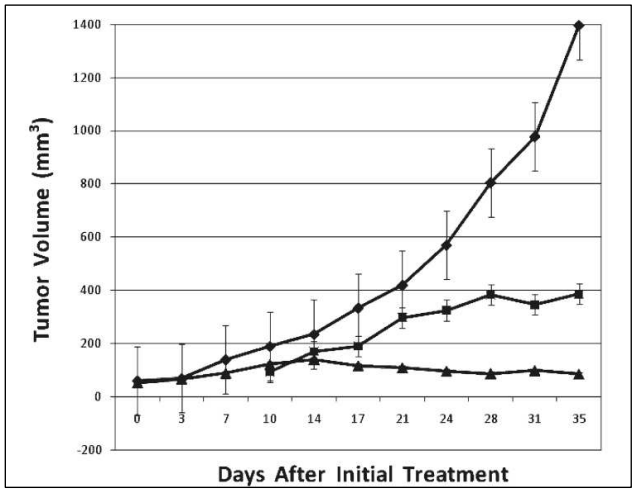


Figure 19. The test compounds, irinotecan (▲), 10 mM citrate (♦), and **46** (Figure 10) (■) were administered by ip injection to athymic nude mice with human tumor xenografts established using MDA-MB-435 breast cancer cells. Mice were injected ip 3× weekly. Negative controls (7 mice) were injected with 150 μL of 10 mM citrate. The positive control group (8 mice) received irinotecan by ip injection at a dose of 20 mg/kg, 3× weekly, for all four weeks. Compound **46** was similarly administered to seven mice, 3× weekly, at a dose of 25 mg/kg starting at week 2 with increasing doses of 32 and 42 mg/kg on weeks 3 and 4, respectively. Data are presented as the mean ±SE. The *% T/C* (average tumor volume of treated as compared to control group) is 27.7% for **46** and 6.1% for irinotecan. Adapted with the permission from reference (111), copyright 2010, American Chemical Society.

Recently Neidle reported a tetra-substituted naphthalene diimide, BMSG-SH-3[57] [**13**] as a potent G-quadruplex DNA stabilizing ligand with telomerase inhibitory activity in cells (Figure 20). The drug seems to be the first G-quadruplex ligand to show anticancer activity *in vivo* through telomerase inhibition action in a pancreatic cancer model.[57]

Another trisubstituted acridine compound BRACO-19 showed an excellent response in UXF1138L human uterine carcinoma xenograft models and showed a good hope for the future in cancer research.[56] RHSP4 [**17**] is another potent G4 ligand, which showed complete remissions in UXF1138L human uterine carcinoma xenograft tumor model in combination with established anticancer drug Taxol.[88,157-159] Quarfloxin is among the first G4 ligand which has potential to disrupt the G-quadruplex-nucleolin complexes. The G-quadruplex DNA binder has reached phase II clinical trials for the treatment of neuroendocrine/cancerous tumors.[160,161] Unfortunately for a ligand, bioactivity is not the only hurdle to cross in order to be a potent drug, other issues like targeting capability, bioavailability, cellular availability and clearance after its function *etc* are also essential. Quarfloxin could not however, proceed beyond the phase II clinical trials due to its limited bioavailability even though it showed promising drug criteria.[162]

Needs for Further Investigations

The DNA-ligand interactions have been quite extensively probed with the aid of various physical methods. However, still, there are a number of parameters from the findings from the *in vitro* studies which need to be correlated for more accurate predictability at the biological level. For instance, a study of energetics of G-quadruplex DNA-ligand interactions provides key information on the thermodynamic parameters that govern the biomolecular interactions. As opposed to popular spectroscopic methods such as UV-Vis absorption, fluorescence emission and circular dichroic spectral studies, techniques like isothermal titration calorimetry (ITC) offer a direct measurement of the binding enthalpy, stoichiometry and affinity constants.[163,164] By careful dissection of the enthalpic and entropic components of binding, it may be possible to optimize drug-G-quadruplex DNA complexation more accurately.

Detection of G4 DNA structure by visual methods *in vivo* using a ligand's emission property is a major challenge. Recently few reports have come on staining of G-quadruplex DNA *in vivo* using antibody conjugate, damage marker and G4 ligands (Figure 21).[165] However, more information has to be extracted by following this method.[166,167] 'Light-up' probes that display a strong enhancement in G4 binding and 'light-off' probes that display a quenched fluorescence upon binding in cellular environment should report involvement of specific biological functions.

On the other hand, over-expression and availability of telomerase in most of the cancer cells justified the telomerase targeted anticancer drug design provided the cytotoxicity remains minimal towards normal cells. The G4 ligands reported so far are mostly small molecules having good cell membrane permeability and high binding affinity. There are a number of reports on G4 ligands which could enter into pre-clinical or clinical trials. The number of such molecules is expected to increase in the near

future.[168] Nevertheless, a few challenges that a G4 ligand has to meet to perform as a telomere targeted anticancer drug. The G-quadruplex DNA structure which was identified primarily in the telomere region has also been found in other genomic regions.

There are over 350,000 predicted sequences in the genome which can fold into G-quadruplex structures and are believed to be involved in the genome maintenance.[168-170]

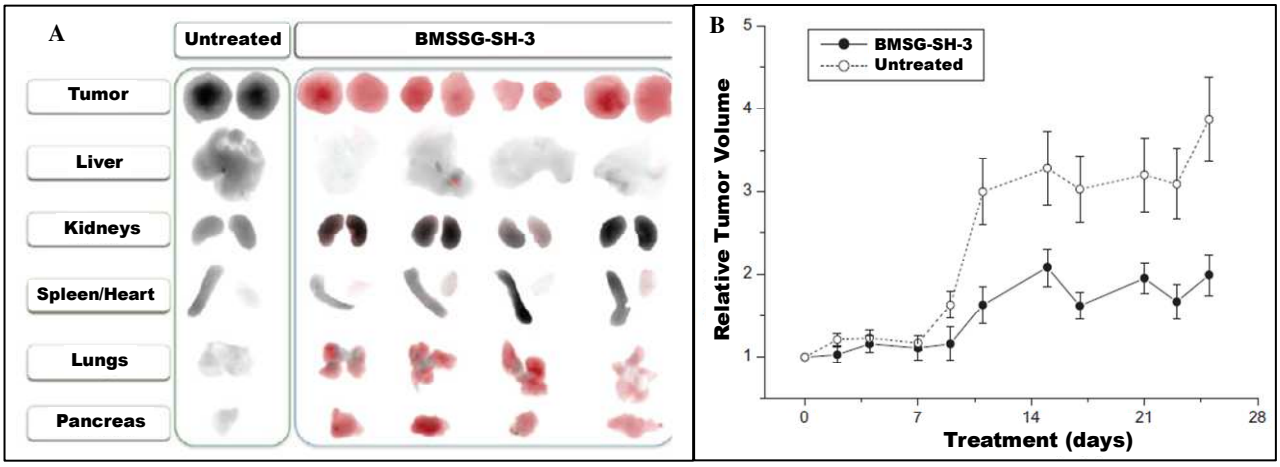


Figure 20. (A) Bio-distribution of BMSG-SH-3 in major organs. Fluorescent BMSG-SH-3 was visualized ex-vivo 48 h after the last of four cycles of 3 mg/kg given 3 times/week intra-peritoneally. (B) Development of MIA-Pa-Ca2 flank xenograft tumors volume treated with BMSG-SH-3. Animals received 3 mg/kg intraperitonally 3/week tumor. Adapted with the permission from reference (57), copyright 2011, Elsevier.

Table 1. Binding parameters and telomerase inhibition properties of various G4-ligands.

Ligand	G4-DNA affinity[a]		Telomerase inhibition (IC_{50}; μM)		Reference
	K_d (μM)	ΔT_m (°C)	TRAP	TRAP-LIG	
1	NR	NR	23.0	NR	48
3	0.063[c]	NR	0.48	6.3	55, 45
4	6.0[b]	21.5[b]	NR	NR	53
8	NR	NR	0.3	NR	121
9	0.12[d]	10.7[e]	0.1-0.2	NR	60
10	1.7[f]	NR	NR	NR	186
13	NR	28.3[g]	NR	NR	57
16	NR	6.2[h]	NR	35.0	187
18	7.5[i]	20[i]	14-28	NR	73
35	0.94[j]	NR	0.7	8.9	45, 188
37	0.2[k]	4[l]	NR	NR	191
38	1.0[k]	4[l]	NR	NR	191
39	NR	NR	0.005-0.02	0.6	102,45
40	NR	NR	0.2-0.6	NR	109
41	0.12[m]	10.5[n]	0.23	NR	192
42	0.006[o]	NR	NR	NR	118
43	12-56[p]	4.5-6.4[i]	NR	NR	189
44	NR	33.8[q]	NR	NR	190
45	NR	28[r]	0.13	NR	121
51	0.82[s]	24[s]	NR	38.6	88
52	4.92[s]	33[s]	NR	32.2	88
53	6.16[s]	38[s]	NR	5.9	88
64	7.0[t]	15[t]	NR	34.7	144
65	2.3[t]	17[t]	NR	14.5	144

[a] K_d is the dissociation constant of the telomeric G-quadruplex DNA-bound ligand complexes and ΔT_m is the extent of elevation observed in the G-quadruplex DNA denaturation temperature upon ligand binding. NR: Not reported. [b]Method: UV, ODN: d[$A(G_3T_2A)_3G_3T$]; [c]Method: SPR, ODN: (5'-Biot-d[$AG_3(TTAG_3)_3$]); [d]Method: fluorescence, ODN: d[$AG_3(TTAG_3)_3$]; [e,q]Method: FRET, ODN: 5'-(FAM-d[GGG(TTAGGG)$_3$]-TAMRA)-3'; [f]Method: SPR, ODN: 5'-SH-d[TTT TTT TTT TAG GGT TAG GGT TAG GGT TAG GG]-3'; [g]Method: FRET, ODN: (5'-FAM-d[GGG TTA GGG TTA GGG TTA GGG]-TAMRA-3'); [h]Method: FRET, ODN: 5'-(FAM-d[GGGTTAGGGTTAGGGTTAGGGTTAGGG]-TAMRA)-3'; [i]Method: FRET, ODN: (5'-FAM-d[GGG(TTAGGG)$_3$]-TAMRA-3'); [j]Method: UV, ODN: d[$AG_3(T_2AG_3)_3$]; [k]Method: UV and [l]CD, ODN: (5-biotin-d[GTTA$(GGGTTA)_4$GG]-3); [m]Method: SPR, ODN: 5'-biotin-d[$AG_3(TTAG_3)_3$]-3'; [n]Method: UV, ODN: d[$[T_2AG_3]_4$; [o]Method: FRET, ODN: (FAM-d[GGGAGGGAGGGAAGGAGGGAGGGAGG-GA]-TAMRA); [p]Method: SPR, ODN: d[5'-biotin-$GT_2A(G_3T_2A)_4G_2$]; [r]Method: FRET, ODN: 5'-(Fluo-d[$G_3(T_2AG_3)_3$]-3'-Tamra); [s]Method: UV, ODN: d$(T_2AG_3)_4$; [t]Method: UV, ODN: d[$G_3(T_2AG_3)_3$].

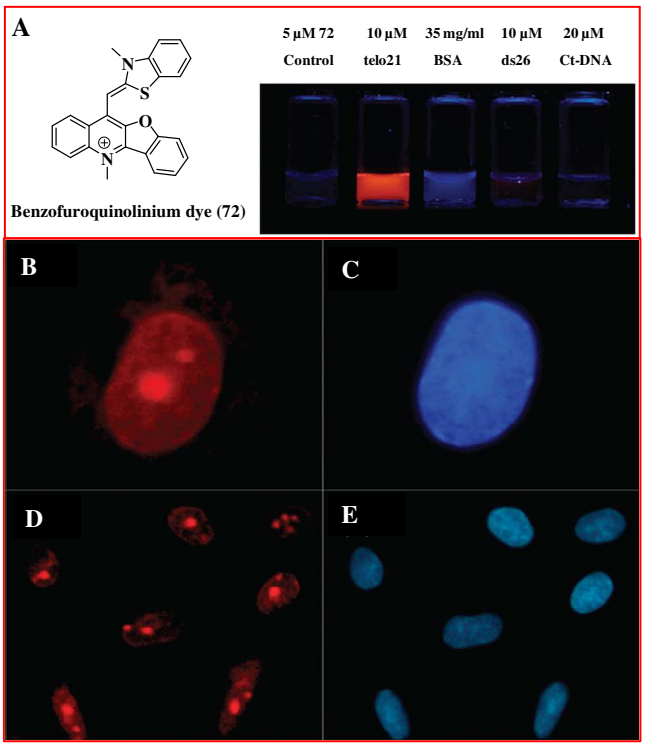


Figure 21. (A) Structure of ligand **72** and photographic images of **72** (5 µM) alone and in the presence of telomere G-quadruplex telo21, BSA protein, duplex DNA ds26 and Ct-DNA in Tris–HCl buffer containing 60 mM KCl under UV light (λ_{ex} = 302 nm). Adapted with the permission from reference (165), copyright 2011, The Royal Society of Chemistry. (B, D) Fluorescence microscopic images of fixed MCF7 cells stained with 2 µM ligand **72**for 15 min and (C, E) 2 µM DAPI for 20 min. Adapted with the permission from reference (165), copyright 2011, The Royal Society of Chemistry.

Thus a potential telomerase inhibitor should not only have the sequence and topology specificity but also have discrimination ability among different G-quadruplex DNA topologies distributed throughout the genome. Though telomerase is known mostly for its association with cancer, there are instances where telomerase plays an important role in renewal capacity of the normal stem cells.[171] Additionally, G4 ligands may also shorten telomere length in bone marrow like cells which are extremely susceptible towards telomere length shortening over time.[172] According to Harley, this kind of cells may need telomere-activation therapy in the near future which may interfere with the telomerase inhibition mediated anticancer therapy.[171] Cancer cells are known to survive in the most robust conditions and are not unexpected to escape the telomere dependent survival like recombination based alternative lengthening of telomeres (ALT).[173] So telomerase targeted anticancer therapy needs to be under scanner particularly in case of the long term treatment. Besides telomeric, oncogenic promoter (c-myc, c-kit and variant bcl-2) DNA G-quadruplexes, there is evidence for the existence of RNA G-quadruplex (from 5'-untranslated regions, 5'-UTRs) as well which could also be targeted.[174,175] Thus, telomere targeted telomerase inhibition mediated anticancer therapy needs to be looked at closely to overcome the hurdles acquiring the opportunities at the same time.

Future outlook

For the last two decades various researchers have enriched the library of telomeric G4 binding ligands with many potential candidates. Parallel research targeting telomerase or telomere binding proteins would be the other strategy for the regulation of genetic expression.[11,171,176,177] Both the findings with G4 ligands *in vitro* and *in vivo* are encouraging to carry on further investigations in search of the superior drugs. For the design of more effective and promising ligands, newer strategies have to be adopted. One possibility is to include design and synthesis of G4 ligands having the capability of inducing DNA cleavage which could be triggered through an external stimulus. Such kind of ligands may not only be able to inhibit the telomerase activity, but also the same should be able to nick an elongated telomere to restore it to the 'normal' length. In the other strategy, a DNA sequence complementary to the telomeric overhang may be conjugated with a *ds*-DNA stabilizing ligand. The attached complementary DNA can form a Watson-Crick duplex with an elongated single-stranded telomere. This may be stabilized by the duplex binding ligand available in the proximity. Favorable energy factors associated with the additional duplex formation may govern the selectivity of the conjugate to enable targeting of the single-stranded overhang. To protect the conjugated complementary DNA sequence from nucleolytic degradations one can employ a corresponding PNA as well. On the other hand, a G4 ligand may be conjugated with the complementary hTR sequence (AUCCCAAUCUGUU).[171] The conjugated ODN (TAGGGTTAGACAA) may bind with the hTR template and may thus block the telomere docking. Moreover, the conjugated G4 ligand may stabilize the telomere overhang available in the proximity. Thus, targeting both telomerase function as well as telomere stabilization may lead to higher activity.

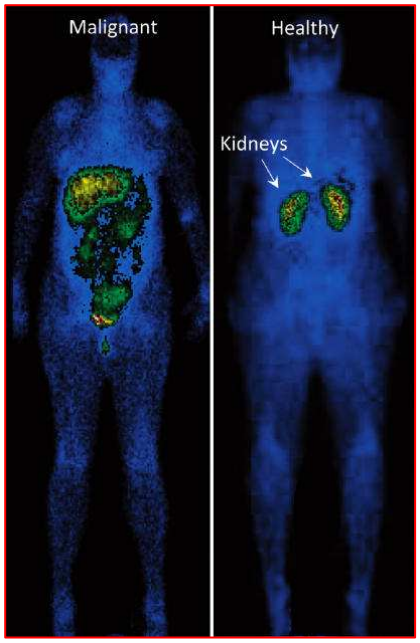


Figure 22. Comparison of [^{111}In]DTPA-folate uptake in a patient with stage III ovarian cancer (left) and that in a healthy volunteer (right). Adapted with the permission from reference (179), copyright 2010, American Chemical Society.

Another important strategy could involve receptor targeted drug design. Most of the cancer cells need higher level of nutrients to carry out telomere loss mediated '*genetic time bomb*' activity.[178] Cancer cells over-express receptors from the cell surface to enhance the endocytosis process.

The common receptors include folate, tyrosine kinase, biotin, transferrin and G protein-coupled receptors. Some of these have already been identified for over-expression in many human cancer cells.[32,179-182] Among these, the most aggressively up-regulated receptor is folate which has been detected in cancers of the ovary, lung, kidney, endometrium, breast, brain, colon, and myeloid cells of hematopoietic lineages. Folate-diethylene triamine penta acetic acid (DTPA) complex, [^{111}In]DTPA-folate, has been found to have excellent targeting ability in ovarian cancer patients. Interestingly, it did not show any accumulation in healthy human organs except kidneys before it rapidly gets excreted into the urine (Figure 22).[179] This observation raises the possibility of receptor-mediated selective targeting of conjugated G4 ligands. The G4 ligand conjugated with molecular entities targeting such receptors may enhance the cellular uptake and improve bioavailability. This in turn may lead to the reduction in normal cell cytotoxicity.[183-185] Moreover, a highly active G4 ligand with lower selectivity could be made superior with this strategy. Lastly, effort and time will provide us the right path to achieve the desired objectives.

Acknowledgements

This work was supported by a grant (J.C. Bose Fellowship grant to Prof. S. Bhattacharya) from the Department of Science and Technology (DST), New Delhi, India. BM is thankful to CSIR New Delhi for a research fellowship.

Notes and References

1 R. Dahm, *Hum. Genet* 2008, **122**, 565.
2 D. Lydall, *J. Cell Sci.* 2003, **116**, 4057.
3 Watson, J. D and F. H. C. Crick, *Nature* 1953, **171**, 737.
4 F. Baltzer, *University of California Press, Berkeley* 1967, pp. 85.
5 J. G. Gall, *Cold Spring Harbor Laboratory Press,* 1995, pp. 1.
6 C. W. Greider and E. H. Blackburn, *Cell* 1987, **51**, 887.
7 T. T. Chow, Y. Zhao, S. S. Mak, J. W. Shay and W. E. Wright, *Genes & Dev.* 2012, **26**, 1167.
8 J. Graakjaer, R. Christensen, S. Kolvraa and N. Serakinci, *BMC Mol. Biol*. 2007, **8**, 49.
9 L. Hayflick and P. S. Moorhead, *Exp. Cell Res.* 1961, **25**, 585.
10 T. R Cech, *Angew. Chem. Int. Ed.* 2000, **39**, 34.
11 L. K. White, W. E. Wright and J. W. Shay, *Trends in Biotechnology* 2001, **19**, 114.
12 R. C. Wang, A. Smogorzewska and T. D. Lange, *Cell* 2004, **119**, 355.
13 D. Miller, G. E. Reynolds, R. Mejia, J. M. Stark and J. P. Murnane, *DNA Repair (Amst)* 2011, **10**, 536.
14 J. D. Watson, *Nature, New Biol.* 1972, **239**, 197.
15 J. W. Szostak and E. H. Blackburn, *Cell* 1982, **29**, 245.
16 C. W. Greider, 1991, **9**, 4572.
17 C. W. Greider and E. H. Blackburn, *Cell* 1985, **51**, 405.
18 X. Cortez-Gonzalez and M. Zanetti, *J. Transl. Med.* 2007, **5**, 12.
19 J. Meyen, R. L. Ratlife and R. K. Moyzis, *Proc. Natl. Acad. Sci. USA* 1989, **86**, 7049.
20 E. W. Blackburn, *Nature* 1991, **350**, 569.
21 J. R. Williamson, M. K. Raghuraman and T. R. Cech, *Cell* 1989, **59**, 871.
22 E. K. Parkinson, *British J. Cancer* 1996, **73**, 1.
23 C. Schaffitzel, I. Berger, J. Postberg, J. Hanes, H. J. Lipps and A. Plückthun, *Proc. Natl. Acad. Sci. USA* 2001, **98**, 8572.
24 A. J. Zaug, E. R. Podell and T. R. Cech, *Proc. Natl. Acad. Sci.* 2005, **102**, 10864.
25 M. Lei, A. J. Zaug, E. R. Podell and T. R. Cech, *J. Biol. Chem*. 2005, **280**, 20449.
26 D. Sen and W. Gilbert, *Nature* 1990, **344**, 410.
27 R. Jin, K. J. Breslauer, R. A. Jones and B. L. Gaffney, *Science* 1990, **250**, 543.
28 E. Henderson, C. C. Hardin, S. K. Walk, I. Tinoco and E. H. Blackburn, *Cell* 1987, **51**, 899.
29 A. K. Shukla and K. B. Roy, *Biol. Chem.* 2006, **387**, 251.
30 Y. Xu, Y. Noguchi and H. Sugiyama, *Bioorg. Med. Chem.* 2006, **14**, 5584.
31 K. N. Luu, A. T. Phan, V. Kuryavyi, L. Lacroix and D. J. Patel.
32 D. Yang and K. Okamoto, *Future Med. Chem.* 2010, **2**, 619.
33 A. Ambrus, D. Chen, J. Dai, T. Bialis, R. A. Jones and D. Yang, *Nucleic Acids Res.* 2006, **34**, 2723.
34 J. X. Dai, M. Carver, C. Punchihewa, R. A. Jones and D. Z. Yang, *Nucl. Acids Res.* 2007, **35**, 4927.
35 G. N. Parkinson, M. P. H. Lee and S. Neidle, *Nature* 2002, **417**, 876.
36 A. N. Lane, J. B. Chaires, R. D. Gray, and J. O. Trent, *Nucleic Acids Res*. 2008, **36**, 5482.
37 L. Petraccone, J. O. Trent, and J. B. Chaires, *J. Am. Chem. Soc*. 2008, **130**, 16530.
38 J. B. Chaires, *FEBS J.* 2010, **277**, 1098.
39 A. D. Cian, E. DeLemos, J-L. Mergny, M-P. Teulade-Fichou and D. Monchaud, *J. Am. Chem. Soc.* 2007, **129**, 1856.
40 S. Burge, G. N. Parkinson, P. Hazel, A. K. Todd and S. Neidle, *Nucleic Acids Res*. 2006, **34**, 5402.
41 F-M. Chen, *Biochemistry* 1992, **31**, 3769.
42 L. L. Guan, J. Kuwahara and Y. Sugiura, *Biochemistry* 1993, **32**, 6141.
43 Q. Chen, I. D. Kuntz and R. H. Shafter, *Proc. Natl. Acad. Sci. USA* 1996, **93**, 2635.
44 N. W. Kim, M. A. Piatyszek, K. R. Prowse, C. B.; Harley, M. D. West, P. L. C. Ho, G. M. Coviello, W. E. Wright, S. L. Weinrich and J. W. Shay, *Science (Washington DC)* 1994, **266**, 2011.
45 J. Reed, M. Gunaratnam, M. Beltran, A. P. Reszka, R. Vilar and S. Neidle, *Anal. Biochem.* 2008, **380**, 99.
46 A. D. Cian, G. Cristofari, P. Reichenbach, E. D. Lemos, D. Monchaud, M-P. Teulade-Fichou, K. Shin-ya, L. Lacroix, Joachim. Lingner and J-L. Mergny, *Proc. Natl. Acad. Sci. USA* 2007, **104**, 17347.
47 D. Sun, B. Thompson, B. E. Cathers, M. Salazar, S. M. Kerwin, J. O. Trent, T. C. Jenkins, S. Neidle and L. H. Hurley, *J. Med. Chem.* 1997, **40**, 2113.
48 S. Bhattacharya, P. Chaudhuri, A. K. Jain and A. Paul, *Bioconjugate Chem.* 2010, **21**, 1148.
49 A. K. Jain, V. V. Reddy, A. Paul, K. Muniyappa and S. Bhattacharya, *Biochemistry* 2009, **48**, 10693.
50 A. D. Moorhouse, A. M. Santos, M. Gunaratnam, M. Moore, S. Neidle and J. E. Moses, *J. Am. Chem. Soc.* 2006, **128**, 15972.
51 X. Wang, J. Huang, Y. Zhou, S. Yan, X. Weng, X. Wu, M. Deng and X. Zhou, *Angew. Chem. Int. Ed.* 2010, **49**, 5305.
52 S. Ghosh, D. Usharani, A. Paul, S. De, E. D. Jemmis, and S. Bhattacharya, *Bioconjugate Chem.* 2008, **19**, 2332.
53 J. Dash, R. N. Das, N. Hegde, G. D. Pantoş, P. S. Shirude and S. Balasubramanian, *Chem. Eur. J.* 2012, **18**, 554.
54 J. Dash, P. S. Shirude and S. Balasubramanian, *Chem. Commun.* 2008, **26**, 3055.
55 M. Read, R. J. Harrison, B. Romagnoli, F. A. Tanious, S. H. Gowan, A. P. Reszka, W. D. Wilson, L. R. Kelland and S. Neidle, *Proc. Natl. Acad. Sci. USA* 2001, **98**, 4844.
56 A. M. Burger, F. Dai, C. M. Schultes, A. P. Reszka, M. J. Moore, J. A. Double and S. Neidle, *Cancer Res.* 2005, **65**, 1489.
57 M. Gunaratnam, M. de la Fuente, S. M. Hampel, A. K. Todd, A. P. Reszka, A. Schätzlein, and S. Neidle, *Bioorg. Med. Chem.* 2011, **19**, 7151.
58 G. W. Collie, R. Promontorio, S. M. Hampel, M. Micco, S. Neidle and G. N. Parkinson, *J. Am. Chem. Soc*. 2012, **134**, 2723.
59 Q. Huang and W. L Fu, *Clin. Chem. Lab. Med*. 2005, **43**, 841.
60 F. Koeppel, J-F. Riou, A. Laoui, P. Mailliet, P. B. Arimondo, D. Labit, O. Petitgenet, C. Helene and J-L. Mergny, *Nucleic Acids Res*.

2001, **29**, 1087.
61 Q. Li, J. F. Xiang, Q. F. Yang, H. X. Sun, A. J. Guan and Y. L. Tang, *Nucleic Acids Res*. 2013, **41**, D1115.
62 A. Paul and S. Bhattacharya, *Curr. Sci*. 2012, **102**, 212.
63 R. J. Harrison, S. M. Gowan, L. R. Kelland and S. Neidle, *Bioorg. Med. Chem. Lett.* 1999, **9**, 2463.
64 J. L. Li, R. J. Harrison, A. P. Reszka, R. M. Jr. Brosh, V. A. Bohr, S. Neidle and I. D. Hickson, *Biochemistry* 2001, **40***,* 15194.
65 R. A. Heald and M. F. G. Stevens, *Org. Biomol. Chem*. 2003, **1**, 3377.
66 J. L. Mergny, L. Lacroix, M. P. Teulade-Fichou, C. Hounsou, L. Guittat, M. Hoarau, P. B. Arimondo, J. P. Vigneron, J. M. Lehn, J. F. Riou, T. Garestier and C. Hélène, *Proc. Natl. Acad. Sci. USA* 2001, **98**, 3062.
67 R. Kieltyka, J. Fakhoury, N. Moitessier and H. F. Sleiman, *Chem. Eur. J*. 2008, **14**, 1145.
68 A. F. Larsen, M. C. Nielsen and T. Ulven, *Chem. Eur. J*. 2012, **18**, 10892.
69 L. Wang, Y. Wen, J. Liu, J. Zhou, C. Li and C. Wei, *Org. Biomol. Chem*. 2011, **9**, 2648.
70 Y. J. Lu, T. M. Ou, J. H. Tan, J. Q. Hou, W. Y. Shao, D. Peng, N. Sun, X. D. Wang, W. B. Wu, X. Z. Bu, Z. S. Huang, D. L. Ma, K. Y. Wong and L. Q Gu, *J. Med. Chem*. 2008, **51**, 6381.
71 Y. Ma, T-M. Ou, J-Q. Hou, Y-J. Lu, J-H. Tan, L-Q. Gu and Z-S Huang, *Bioorg. Med. Chem*. 2008, **16**, 7582.
72 M. Y. Kim, W. Duan, M. Gleason-Guzman and L. H. Hurley, *J. Med. Chem*. 2003, **46**, 571.
73 Y. Ma, T. M. Ou, J. H. Tan, J. Q. Hou, S. L. Huang, L. Q. Gu and Z. S. Huang, *Bioorg. Med. Chem. Lett.* 2009, **19**, 3414.
74 E. Salvati, C. Leonetti, A. Rizzo, M. Scarsella, M. Mottolese, R. Galati, I. Sperduti, M. F. Stevens, M. D'Incalci, M. Blasco, G. Chiorino, S. Bauwens, B. Horard, E. Gilson, A. Stoppacciaro, G. Zupi and A. Biroccio, *J. Clin. Invest.* 2007, **117**, 3236.
75 H. Sun, J. Xiang, Y. Tang and G. Xu, *Biochem. Biophys. Res. Commun*. 2007, **352**, 942.
76 L. P. Bai, H. M. Ho, D. L. Ma, H. Yang, W. C. Fu and Z. H. Jiang, *PLoS One* 2013, **8**, e53962.
77 L. Xue, N. Ranjan and D. P. Arya, *Biochemistry* 2011, **50**, 2838.
78 N. Ranjan, E. Davis, L. Xue, and D. P. Arya,
79 G. Zagotto, A. Ricci, E. Vasquez, A. Sandoli, S. Benedetti, M. Palumbo and C. Sissi, *Bioconjug. Chem*. 2011, **22**, 2126.
80 J. E. Redman, J. M. Granadino-Roldán, J. A. Schouten, S. Ladame, A. P. Reszka, S. Neidle and S. Balasubramanian, *Org. Biomol. Chem*. 2009, **7**, 76.
81 A. Arola-Arnal, J. Benet-Buchholz, S. Neidle and R. Vilar, *Inorg Chem*. 2008, **47**, 11910.
82 S. Bianco, C. Musetti, A. P. Krapcho, M. Palumbo and C. Sissi, *Chem. Commun*. 2013, **49**, 8057.
83 Q. Yu, Y. Liu, J. Zhang, F. Yang, D. Sun, D. Liu, Y. Zhou and J. Liu, *Metallomics* 2013, **5**, 222.
84 J-T. Wang, X-H. Zheng, Q. Xia, Z-W. Mao, L-N. Jia and K. Wang, *Dalton Trans*. 2010, **39**, 7214.
85 Y. Haijia, W. Xiaohui, F. Manliang, R. Jinsong and Q. Xiaogang, *Nucleic Acids Res*. 2008, **36**, 5695.
86 P. Chaudhuri, B. Ganguly and S. Bhattacharya, *J. Org. Chem*. 2007, **72**, 1912.
87 S. Bhattacharya and P. Chaudhuri, *Curr. Med. Chem*. 2008, **15**, 1762.
88 A. Paul, A. K. Jain, S. K. Misra, B. Maji, K. Muniyappa and S. Bhattacharya, *PLoS One* 2012, **7**, e39467.
89 A. K. Jain, A. Paul, B. Maji, K. Muniyappa and S. Bhattacharya, *J. Med. Chem*. 2012, **55**, 2981.
90 B. Maji and S. Bhattacharya, *Chimia* 2013, **67**, 39.
91 J. F. Riou, L. Guittat, P. Mailliet, A. Laoui, E. Renou, O. Petitgenet, F. Mégnin-Chanet, C. Hélène and J-L. Mergny, *Proc. Natl. Acad. Sci. USA* 2002, **9**, 2672.
92 V. Casagrande, A. Alvino, A. Bianco, G. Ortaggi and M. Franceschin, *J. Mass. Spectrom.* 2009, **44**, 530.
93 Y-L. Tsaia, C-C. Changa, C-C. Kanga and T-C. Chang, *J. Luminescence* 2007, **127**, 41.
94 R. Rodriguez, S. Müller, J. A. Yeoman, C. Trentesaux, J. F. Riou and S. Balasubramanian, *J. Am. Chem. Soc*. 2008, **130**, 15758.
95 S. Cosconati, A. Rizzo, R. Trotta, B. Pagano, S. Iachettini, S. De Tito, I. Lauri, I. Fotticchia, M. Giustiniano, L. Marinelli, C. Giancola, E. Novellino, A. Biroccio and A. Randazzo, *J. Med. Chem*. 2012, **55**, 9785.
96 K. Shin-ya, K. Wierzba, K. Matsuo, T. Ohtani, Y. Yamada, K. Furihata, Y. Hayakawa and H. Seto, *J. Am. Chem. Soc*. 2001, **123**, 1262.
97 M. Sumi, T. Tauchi, G. Sashida, A. Nakajima, A. Gotoh, K. Shin-Ya, J. H. Ohyashiki and K. Ohyashiki, *International J. Oncology* 2004, **24**, 1481.
98 T. Tauchi, K. Shin-ya, G. Sashida, M. Sumi, S. Okabe, J. H. Ohyashiki and K. Ohyashiki, *Oncogene* 2006, **25**, 5719.
99 H. Tahara, K. Shin-ya, H. Seimiya, H. Yamada, T. Tsuruo and T. Ide, *Oncogene* 2006, **25**, 1955.
100 D. Gomez, T. Wenner, B. Brassart, C. Douarre, M. F. O'Donohue, V. El Khoury, K. Shin-Ya, H. Morjani, C. Trentesaux and J. F. Riou, *J. Biol. Chem*. 2006, **281**, 38721.
101 T. Doi, K. Shibata, M. Yoshida, M. Takagi, M. Tera, K. Nagasawa, K. Shin-ya and T. Takahashi, *Org. Biomol. Chem*. 2011, **9**, 387.
102 T. Doi, M. Yoshida, K. Shin-ya and T. Takahashi, *Org. Lett*. 2006, **8**, 4165.
103 N. Endoh, K. Tsuboi, R. Kim, Y. Yonezawa and C-G. Shin, *Heterocycles* 2003, **60**, 1567.
104 J. M. Atkins and E. Vedejs, *Org. Lett*. 2005, **7**, 3351.
105 S. K. Chattopadhyay and S. Biswas, *Tetrahedron Lett.* 2006, **47**, 7897.
106 J. Deeley, A. Bertram and G. Pattenden, *Org. Biomol. Chem*. 2008, **6**, 1994.
107 M. Tera, H. Ishizuka, M. Takagi, M. Suganuma, K. Shin-ya and K. Nagasawa, *Angew. Chem. Int. Ed. Engl*. 2008, **47**, 5557.
108 R. Rodriguez, G. D. Pantoş, D. P. Gonçalves, J. K. Sanders and S. Balasubramanian, *Angew. Chem. Int. Ed. Engl.* 2007, **46**, 5405.
109 G. S. Minhas, D. S. Pilch, J. E. Kerrigan, E. J. LaVoie and J. E. Rice, *Bioorg. Med. Chem. Lett*. 2006, **16**, 3891.
110 E. S. Baker, J. T. Lee, J. L. Sessler and M. T. Bowers, *J. Am. Chem. Soc*. 2006, **128**, 2641.
111 S. G. Rzuczek, D. S. Pilch, A. Liu, L. Liu, E. J. LaVoie and J. E. Rice, *J. Med. Chem*. 2010, **53**, 3632.
112 P. S. Shirude, E. R. Gillies, S. Ladame, F. Godde, K. Shin-Ya, I. Huc, and S. Balasubramanian, *J. Am. Chem. Soc*. 2007, **129**, 11890.
113 A. Bugaut, K. Jantos, J-L. Wietor, R. Rodriguez, J. K. M. Sanders and S. Balasubramanian. *Angew. Chem. Int. Ed. Engl*. 2008, **47**, 2677.
114 I. Haq, J. O. Trent, B. Z. Chowdhry and T. C. Jenkins, Journal *J. Am. Chem. Soc*. 1999, **121**, 1768.
115 C. Romera, O. Bombarde, R. Bonnet, D. Gomez, P. Dumy, P. Calsou, J. F. Gwan, J. H. Lin, E. Defrancq, G. Pratviel, *Biochimie* 2011, **93**, 1310.
116 B. Fu, J. Huang, L. Ren, X. Weng, Y. Zhou, Y. Du, X. Wu, X. Zhou and G. Yang, *Chem Commun* 2007, **31**, 3264.
117 E. M. Rezler, J. Seenisamy, S. Bashyam, M. Y. Kim, E. White, W. D. Wilson and L. H. Hurley, *J. Am. Chem. Soc*. 2005, **127**, 9439.
118 H. Yaku, T. Fujimoto, T. Murashima, D. Miyoshi and N. Sugimoto, *Chem. Commun.* 2012, **48**, 6203.
119 K. Iida, M. Tera, T. Hirokawa, K. Shin-ya and K. Nagasawa, *Chem. Commun.* 2009, **42**, 6481.
120 A. A. Ivanov, S. A. Streltsov,T. A. Prikazchikova, M. B. Gottikh, A. L. Zhuze. *Russ. J. Bioorg. Chem.* 2008, **34**, 261.
121 M. P. Teulade-Fichou, C. Carrasco, L. Guittat, C. Bailly, P. Alberti, J-L. Mergny, A. David, J. M. Lehn and W. D. Wilson, *J. Am. Chem. Soc*. 2003, **125**, 4732.
122 P. Alberti, J. Ren, M. P. Teulade-Fichou, L. Guittat, J. F. Riou, J. Chaires, C. Hélène, J. P. Vigneron, J. M. Lehn and J-L. Mergny, *J. Biomol. Struct. Dyn*. 2001, **3**, 505.
123 D. Monchaud, C. Allain and M. P. Teulade-Fichou, *Nucleosides Nucleotides Nucleic Acids* 2007, **26**, 1585.
124 S. Nakayama, I. Kelsey, J. Wang and H. O. Sintim, *Chem Commun* 2011, **47**, 4766.
125 D. Monchaud, C. Allain, H. Bertrand, N. Smargiasso, F. Rosu, V. Gabelica, A. D. Cian, J-L. Mergny and M. P. Teulade-Fichou, *Biochimie* 2008, **90**, 1207.
126 P. L. Tran, E. Largy, F. Hamon, M. P. Teulade-Fichou and J-L. Mergny, *Biochimie* 2011, **93**, 1288.
127 M. Tera, K. Iida, K. Ikebukuro, H. Seimiya, K. Shin-Ya and K. Nagasawa, *Org. Biomol. Chem*. 2010, **8**, 2749.

128 S. Alcaro, C. Musetti, S. Distinto, M. Casatti, G. Zagotto, A. Artese, L. Parrotta, F. Moraca, G. Costa, F. Ortuso, E. Maccioni and C. Sissi, *J. Med. Chem*. 2013, **56**, 843.

129 P. Ragazzon and J. B. Chaires, *Methods* 2007, **43**, 313.

130 P. Murat, R. Bonnet, A. Van der Heyden, N. Spinelli, P. Labbé, D. Monchaud, M. P. Teulade-Fichou, P. Dumy, E. Defrancq, *Chem. Eur. J*. 2010, **16**, 6106.

131 A. K. Jain and S. Bhattacharya, *Curr. Pharm. Des*. 2012, **18**, 1917.

132 A. K. Jain and S. Bhattacharya, *Bioconjugate Chem*. 2010, **21**, 1389.

133 J. Li, X. Jin, L. Hu, J. Wang and Z. Su, *Bioorg Med Chem Lett*. 2011, **21**, 6969.

134 S. Maiti, N. K. Chaudhury and S. Chowdhury, *Biochem. Biophys. Res. Commun*. 2003, **10**, 505.

135 M. J. Cocco, L. A. Hanakahi, M. D. Huber and N. Maizels, *Nucleic Acids Res*. 2003, **31**, 2944.

136 M. J. Moore, F. Cuenca, M. Searcey and S. Neidle, *Org. Biomol. Chem*. 2006, **4**, 3479.

137 B. Pagano, I. Fotticchia, S. D. Tito, C. A. Mattia, L. Mayol, E. Novellino, A. Randazzo and C. Giancola, *J. Nucleic Acids*. 2010, 247137.

138 L. Martino, A. Virno, B. Pagano, A. Virgilio, S. D. Micco, A. Galeone, C. Giancola, G. Bifulco, L. Mayol and A. Randazzo, *J. Am. Chem. Soc*. 2007, **129**, 16048.

139 S. Cosconati, L. Marinelli, R. Trotta, A. Virno, S. D. Tito, R. Romagnoli, B. Pagano, V. Limongelli, C. Giancola, P. G. Baraldi, L. Mayol, E. Novellino and A. Randazzo, *J. Am. Chem. Soc*. 2010, **132**, 6425.

140 N. Zaffaroni, S. Lualdi, R. Villa, D. Bellarosa, C. Cermele, P. Felicetti, C. Rossi, L. Orlandi and M. G. Daidone. *Eur. J. Cancer* 2002, **38**, 1792.

141 Q. Chen, I. D. Kuntz and R. H. Shafer. *Proc. Natl. Acad. Sci. U.S.A* 1997, **93**, 2635.

142 W. M. David, J. Brodbelt, S. M. Kerwin and P. W. Thomas. *Anal. Chem*. 2002, **74**, 2029.

143 S. Paramasivan and P. H. Bolton. *Nucleic Acids Res*. 2008, **36**, e106.

144 A. Paul, B. Maji, S. K. Misra, A. K. Jain, K. Muniyappa and S. Bhattacharya, *J. Med. Chem*. 2012, **55**, 7460.

145 M. L. Duquette, P. Handa, J. A. Vincent, A. F. Taylor and N. Maizels, *Genes Dev*. 2004, **18**, 1618.

146 S. Balasubramanian, and S. Neidle, *Curr. Opin. Chem. Biol*. 2009, **13**, 345.

147 T. M. Ou, Y. J. Lu, J. H. Tan, Z. S. Huang, K. Y. Wong, and L. Q. Gu, *Chem. Med. Chem.* 2008, **3**, 690.

148 M. Isalan, S. D. Patel, S. Balasubramanian and Y. Choo, *Biochemistry* 2001, **40**, 830.

149 H. Fernando, R. Rodriguez, and S. Balasubramanian, *Biochemistry* 2008, **47**, 9365.

150 H. Fernando, S. Sewitz, J. Darot, S. Tavare, J. L. Huppert and S. Balasubramanian, *Nucleic Acids Res.* 2009, **37**, 6716.

151 K. J. Neaves, J. L. Huppert, R. M. Henderson and J. M. Edwardson, *Nucleic Acids Res*. 2009, **18**, 6269.

152 I. Mela, R. Kranaster, R. M. Henderson, S. Balasubramanian and J. M. Edwardson, *Biochemistry* 2012, **51**, 578.

153 G. Biffi, D. Tannahill, J. McCafferty and S. Balasubramanian, *Nat. Chem*. 2013, **5**, 182.

154 R. Rodriguez, K. M. Miller, J. V. Forment, C. R. Bradshaw, M. Nikan, S. Britton, T. Oelschlaegel, B. Xhemalce, S. Balasubramanian and S. P. Jackson, *Nat. Chem. Biol*. 2012, **8**, 301.

155 C. Douarre, X. Mergui, A. Sidibe, D. Gomez, P. Alberti, P. Mailliet, C. Trentesaux, and J. F. Riou. *Nucleic Acids Res*. 2013, **41**, 3588.

156 Y. C. Tsai, H. Qi, C. P. Lin, R. K. Lin, J. E. Kerrigan, S. G. Rzuczek, E. J. LaVoie, J. E. Rice, D. S. Pilch, Y. L. Lyu and L. F. Liu. *J. Biol. Chem*. 2009, **284**, 22535.

157 Q. Li, J. Xiang, X. Li, L. Chen, X. Xu, Y. Tang, Q. Zhou, L. Li, H. Zhang, H. Sun, A. Guan, Q. Yang, S. Yang and G. Xu, *Biochimie* 2009, **91**, 811.

158 T. Tauchi, K. Shin-Ya, G. Sashida, M. Sumi, A. Nakajima, T. Shimamoto, J. H. Ohyashiki and K. Ohyashiki, *Oncogene* 2003, **22**, 5338.

159 S. Neidle, *FEBS J*. 2010, **277**, 1118.

160 D. Drygin, A. Siddiqui-Jain, S. O'Brien, M. Schwaebe, A. Lin, J. Bliesath, C. B. Ho, C. Proffitt, K. Trent, J. P. Whitten, J. K. Lim, D. V. Hoff, K. Anderes and W. G. Rice, *Cancer Res*. 2009, **69**, 7653.

161 N. W. Luedtke, *Chimia* 2009, **63**, 134.

162 S. Balasubramanian, L. H. Hurley and S. Neidle, *Nat. Rev. Drug. Discov*. 2011, **4**, 261.

163 C. Giancola and B. Pagano, *Top. Curr. Chem*. 2013, **330**, 211.

164 E. Largy, A. Granzhan, F. Hamon, D. Verga and M. P. Teulade-Fichou, *Top. Curr. Chem*. 2013, **330**, 111.

165 Y. J. Lu, S. C. Yan, F. Y. Chan, L. Zou, W. H. Chung, W. L. Wong, B. Qiu, N. Sun, P. H. Chan, Z. S. Huang, L. Q. Gu and K. Y. Wong, *Chem. Commun*. 2011, **47**, 4971.

166 H. Ihmels, and L. Thomas, *Org. Biomol. Chem*. 2013, **11**, 480.

167 D. Yang, and K. Okamoto, *Future Med. Chem*. 2010, **2**, 619.

168 J. L. Huppert, and S. Balasubramanian, *Nucleic Acids Res*. 2007, **35**, 406.

169 J. L. Huppert and S. Balasubramanian, *Nucleic Acids Res*. 2005, **33**, 2908.

170 H. T. Le, M. C. Miller, R. Buscaglia, W. L. Dean, P. A. Holt, J. B. Chaires and J. O. Trent, *Org. Biomol. Chem*. 2012, **10**, 9393.

171 C. B. Harley, *Nat. Rev. Cancer* 2008, **8**, 167.

172 H. Vaziri, W. Dragowska, R. C. Allsopp, T. B. Thomas, C. B. Harley and P. M. Lansdorp, *Proc. Natl. Acad. Sci. USA* 1994, **91**, 9857.

173 Z. H. Zhong, W. Q. Jiang, A. J. Cesare, A. A. Neumann, R. Wadhwa and R. R. Reddel, *J. Biol. Chem*. 2007, **282**, 29314.

174 D. J. Patel, A. T. Phan and V. Kuryavyi, *Nucleic Acids Res.* 2007, **35**, 7429.

175 K. I. McLuckie, M. Di. Antonio, H. Zecchini, J. Xian, C. Caldas, B. F. Krippendorff, D. Tannahill, C. Lowe and S. Balasubramanian, *J. Am. Chem. Soc*. 2013, **135**, 9640.

176 K. Takahama, A. Takada, S. Tada, M. Shimizu, K. Sayama, R. Kurokawa and T. Oyoshi, *Chem Biol*. 2013, **20**, 341.

177 S. Bañuelos, B. Lectez, S. G. Taneva, G. Ormaza, M. Alonso-Mariño, X. Calle and M. A. Urbaneja, *FEBS Lett*. 2013, **587**, 2254.

178 C. B. Harley, *Mutat. Res*. 1991, **256**, 271.

179 W. Xia and P. S. Low, *J. Med. Chem*. 2010, **53**, 6811.

180 G. Russell-Jones, K. McTavish and J. McEwan. *J. Inorg. Biochem.* 2004, **98**, 1625.

181 E. S. Antonarakis and A. Emadi. *Cancer Chemother. Pharmacol*. 2010, **66**, 1.

182 S. Li, S. Huang and S-B. Peng. *Int. J. Oncol*. 2005, **27**, 1329.

183 R. J. Lee and P. S. Low, *J. Biol. Chem*. 1994, **269**, 3198.

184 P. S. Low and S. A. Kularatne, *Curr. Opin. Chem. Biol*. 2009, **13**, 256.

185 J. Gravier, R. Schneider, C. Frochot, T. Bastogne, F. Schmitt, J. Didelon, F. Guillemin and M. *J. Med. Chem*. 2008, **51**, 3867.

186 F. Pillet, C. Romerae, E. Trévisiol, S. Bellond, M-P. Teulade-Fichoug, J-M. Francois, G. Pratviel and V. A. Leberre, *Sensors and Actuators B* 2011, **157**, 304.

187 S. Sparapani, S. M. Haider, F. Doria, M. Gunaratnam and S. Neidle, *J. Am. Chem. Soc*. 2010, **132**, 12263.

188 C. Wei, G. Jia, J. Zhou, G. Han and C. Li, *Phys. Chem. Chem. Phys.* 2009, **11**, 4025.

189 K. Jantos, R. Rodriguez, S. Ladame, P. S. Shirude and S. Balasubramanian, *J. Am. Chem. Soc*. 2006, **128**, 13662.

190 P. S. Shirude, E. R. Gillies, S. Ladame, F. Godde, K. Shin-Ya, I. Huc and S. Balasubramanian, *J. Am. Chem. Soc*. 2007, **129**, 11890.

191 D. P. N. Gonçalves, R. Rodriguez, S. Balasubramaniana and J. K. M. Sanders, *Chem. Commun*. 2006, 4685.

192 L. Ren, A. Zhang, J. Huang, P. Wang, X. Weng, L. Zhang, F. Liang, Z. Tan and X. Zhou, *Chembiochem*. 2007, **8**, 775.