Nucleic Acids as Targets for Antitelomerase Agents

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Abstract: Telomeric DNA progressively erodes with each round of cell division in cells that do not express telomerase, a specialized reverse transcriptase necessary to fully duplicate the chromosomal ends. Telomerase is expressed in tumor cells but not in most somatic cells and thus telomeres and telomerase may be proposed as attractive targets for the discovery of new anticancer agents. In this paper we will present different strategies to inhibit telomerase activity via an interaction with a telomere/telomerase nucleic acid component, with a special emphasis on quadruplex ligands.

Keywords: Telomeres, Telomerase, Cancer, Proliferation, G-quadruplex.

INTRODUCTION

Telomeres protect chromosomal ends from fusion events and provide a mean for complete replication of the chromosome. Human telomeric DNA consists of a few kilobases of a short repetitive motif which is double-stranded, except for a terminal 3' G-rich overhang [1-3]. In the absence of a specific replication machinery at the telomere ends, it was predicted [4] and later demonstrated [5] that gradual sequence loss due to the incomplete replication of the lagging strand would eventually lead to critically short telomeres which would ultimately trigger replicative senescence. In order to compensate for this loss, different mechanisms for the addition of new telomere sequences have evolved. In humans, telomere maintenance is mainly performed by a specific reverse transcriptase, telomerase. Human telomerase is a ribonucleoprotein [6] composed of a catalytic subunit, hTERT [7-9], and a 451 nucleotide long RNA (hTR) [10] which acts as a template for the addition of a short repetitive motif d(GGGTTA)n.

Telomerase is inactive in most somatic cells. It is active in the germ line, in some stem cells and in a large majority of cancer cells. Furthermore, recent key experiments demonstrated that: (i) telomerase is sufficient for the immortalization of many cell types [11] and sufficient to allow transformed cells to escape from crisis [12]. However, telomerase alone does not induce changes associated with a transformed phenotype [13,14]. (ii) Inhibition of telomerase limits the growth of human cancer cells [15]; and (iii) the ectopic expression of the telomerase catalytic subunit (hTERT) in combination with two oncogenes results in tumourigenic conversion of normal human cells [16,17]. All these results point out the key role of telomerase in the tumourigenic process; its manipulation becomes a challenge for the design of future anti-oncogenic approaches. In this review, we will present the different strategies that have been proposed in order to inhibit telomerase in cancer cells via an interaction with a telomere/telomerase nucleic acid component.

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INHIBITING TELOMERASE

Telomerase is over-expressed in a large number of tumors whereas it is not expressed in most somatic cells that usually have longer telomeres. This characteristic differential gives a rational for further evaluation of telomerase as a target for new anticancer drugs. Several reviews concerning telomerase inhibitors have been published in the last few years [18-26]. For this reason we will mainly focus on recent developments in the field. The search for telomerase inhibitors was made possible by the introduction of enzymatic tests that allow the semi-quantitative measurement of telomerase activity in cell extracts. Different strategies have been developed in order to inhibit telomerase activity and interfere with tumor development. In this review, we will focus on targeting a nucleic acid component, such as hTERT or hTR RNAs, or telomeric DNA (Fig. (**1**)).

1) Targeting the Catalytic Subunit (hTERT).

Normal human diploid cells transiently expressing hTERT acquire telomerase activity, demonstrating that hTERT is the limiting component necessary for restoration of telomerase activity in these cells [27,28]. There have been a few reports of short antisense oligonucleotides targeted to the catalytic subunit mRNA. 20-22 base-long phosphorothioate anti-hTERT oligomers (5-15 μM) induce delayed inhibition of cell viability in the DU145 prostate cancer cell line [29]. However, no reduction in telomere length is observed even after 45 days of treatment. Among 4 different antisense oligonucleotides directed against the mouse TERT mRNA, a 19 mer overlapping the translation initiation codon inhibits the production of the protein in developing brain neurons [30]. On the other hand, ribozymes

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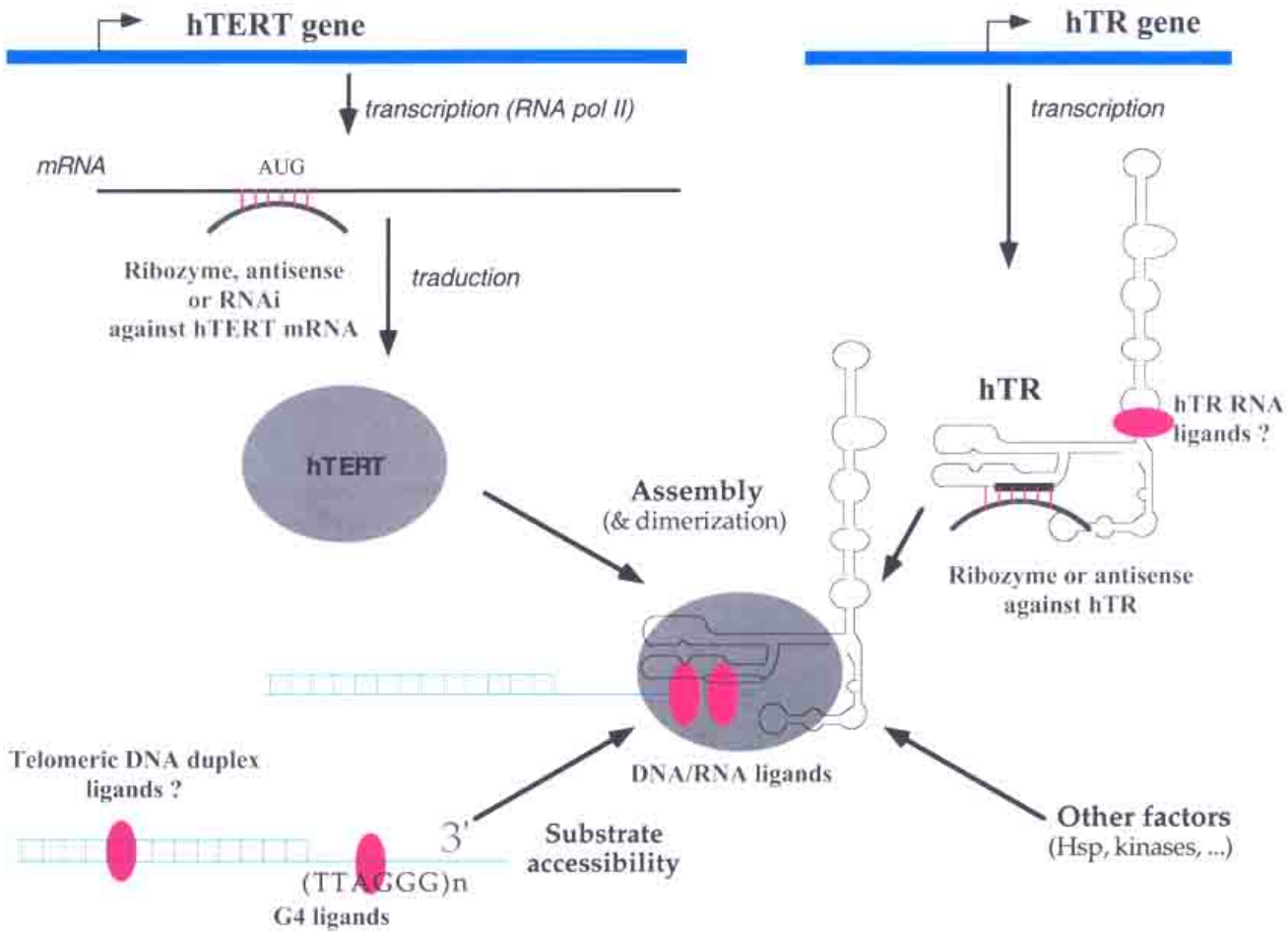

Fig. (1). Nucleic acids targets.
Possible pathways of pharmacological inhibition of telomerase involving nucleic acids. DNA is shown in blue, RNA in black. Telomerase is composed of two major components: the catalytic subunit (hTERT) and the template RNA (hTR). Pharmacological agents that interfere with telomerase assembly or activity are shown in red. See text for details.

targeting 13 nucleotides downstream from the 5'-end of hTERT mRNA exhibit a strong telomerase-inhibitory activity. A stable transfection study confirmed that this ribozyme suppresses telomerase [31]. Ribozyme cleavage of telomerase mRNA also sensitizes breast epithelial cells to inhibitors of topoisomerase [32].

2) Targeting RNA (hTR)

A mutation in hTR has recently been demonstrated to be involved in a progressive bone-marrow failure syndrome called dyskeratosis congenita (DKC) [33], and a mutation of another telomerase component is involved in the X-linked form of the disease [34]. The RNA component of telomerase hTR is absolutely required for telomerase reverse transcription and is therefore a natural target for antitelomerase agents.

The antisense approach has widely been exploited against the 451 nucleotide-long human telomerase RNA. The hTR target has some original characteristics: (i) it is not mRNA and will not be translated into protein: an antisense oligomer will not have to compete with the ribosomal machinery. As a consequence, RNAse H-independent inhibition of telomerase activity should be possible. (ii) hTR provides a template (nucleotide 46-56; r $^{5'}$CUAACCCUAAC$^{3'}$) for reverse transcription. Therefore, this region of the RNA is expected to be highly accessible. A recent comparison of vertebrate telomerase RNA genes from a variety of species shows an evolutionary conservation of the global architecture of telomerase RNA, and would help in the identification of other regions that may be targeted by antisense oligomers [35].

The original antisense approach used an expression vector that allowed the synthesis of a long antisense RNA. This key experiment demonstrated that hTR was indeed the RNA component of telomerase [10]. Short oligomers have also been targeted to the hTR RNA. Peptide nucleic acids (PNAs), in which the sugar-phosphate backbone has been replaced by N-(2-aminoethyl)glycine, recognize the RNA component of human telomerase (hTR) [36]. In contrast, phosphorothioate oligonucleotides (PS) inhibit telomerase in a non-sequence-selective fashion [36] and probably act by

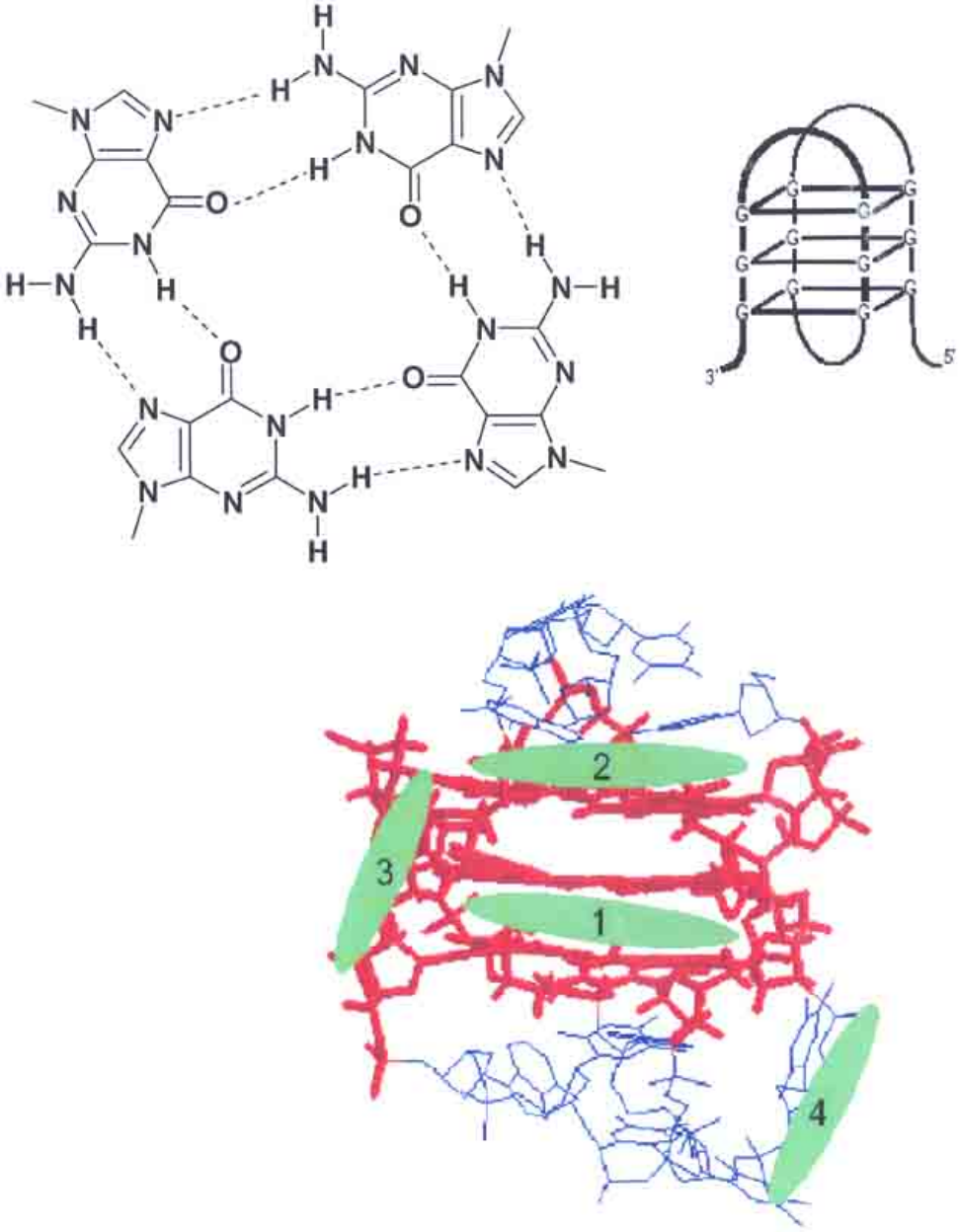

Fig. (2). G-quadruplexes.
Top Left: A G-quartet involving 4 guanines.
Top Right: The G-rich telomeric strand may fold into an intramolecular G-quadruplex leading to the formation of three adjacent G-quartets.
Bottom: Tertiary structure of the human telomeric G-rich strand (d-AGGGTTAGGGTTAGGGTTAGGG) based on PDB structure 143d [51]. Four possible binding modes of a G4-interacting molecule (in green) have been superimposed : 1. " True " intercalation; 2. End stacking; 3. " Minor " groove binding; 4. Binding to the loops.

interacting with the catalytic subunit rather than the RNA. 2'-O-methyl-RNA (2'-O-MeRNA) inhibits telomerase with potency superior to those possessed by analogous peptide nucleic acids (PNAs), despite a lower binding affinity for complementary RNA [37,38]. Various phosphoramidate derivatives, including 2' deoxy, hydroxy, methoxy or fluoro N3'-P5' phosphoramidates, have recently been tested against telomerase *in vitro*. These compounds demonstrate sequence specific and dose-dependent activities with IC_{50} below 1 nM [39]. Hammerhead ribozymes directed against the RNA component of human telomerase show a specific cleavage activity for the telomerase RNA component and inhibit telomerase activity in cell extracts [40], endometrial carcinoma cells [41] and melanoma cells [42].

3) Targeting Telomeric DNA Rather than Telomerase

Targeting the *substrate* of an enzyme is an original way to inhibit its activity. There are fundamental differences

between the targeting of telomeres and the targeting of telomerase subunits (hTR, hTERT or associated factors). Telomeres exist in the absence of telomerase activity, and play at least one fundamental role in telomerase-negative cells: the protection (capping) of chromosome ends. Telomere interacting molecules might then have an effect on immortal cells that regulate telomere length via the ALT pathway (a potential benefit) but also on normal/mortal cells that do not maintain telomere length leading to undesired toxicity.

Chromosomal DNA of ciliates, yeasts and vertebrates end in a 3' single stranded overhang. These overhangs are relatively long and may be involved in different DNA conformations such as T-loops [43], triplexes [44] or G-quadruplexes [45-48]. The presence of telomeric antiparallel quadruplexes has recently been demonstrated in the macronucleus of a ciliate, *Stylonychia lemnae* [49]. A DNA strand carrying at least four blocks of consecutive guanines may fold into an intramolecular G4' structure schematically presented in Fig. (**2**). In the case of the human telomeric overhang, this motif is compatible with the formation of 3 adjacent G-quartets. Each G-quartet involves 4 coplanar guanines (Fig. (**2**), left). The intramolecular telomeric G-quadruplex is fairly stable under physiological conditions [50]. The 3-dimensional structure of the telomeric quadruplex has been solved [51]. In the presence of sodium, this G-tetraplex is stabilized by three stacked G-tetrads which are connected by two lateral loops and a central diagonal loop (Fig. (**2**), bottom). The detailed structure of the loops remains to be elucidated in order to rationalize loop recognition (see below). Of the four grooves that are formed, one is wide, two are of medium width and one is narrow. Three of the four adenines stack on top of adjacent G-tetrads while the majority of the thymines experience multiple conformations. The potassium crystal structure of an identical oligonucleotide has been solved recently and is fundamentally different from the sodium solution conformation [99]. There are a number of proteins that either bind to preformed quadruplex DNA, induce its formation, unwind or cleave it (for a review: [52]). Some of these proteins play a role in telomere maintenance or other key processes such as meiosis or immunoglobulin switch recombination.

In vitro folding of the telomeric G-rich single strand quadruplex DNA has been found to inhibit telomerase activity [53] (Fig. (**3**)). It was deduced from this observation that a molecule that favors quadruplex formation locks the telomeric substrate into an inactive conformation which is no longer recognized nor extended by the enzyme. Stabilization of G-quadruplexes can then be considered an original strategy to achieve antitumor activity [54-56]. G4 ligands require a structural selectivity, i.e. preferential binding to quadruplexes over duplexes and single strands. DNA structure-specific (rather than sequence-specific) ligands have been identified previously [57]. The quadruplex itself, which is very different from classical double stranded B-DNA, provides a good structural basis for selective recognition, and such assumption has been shown to be correct [58-62].

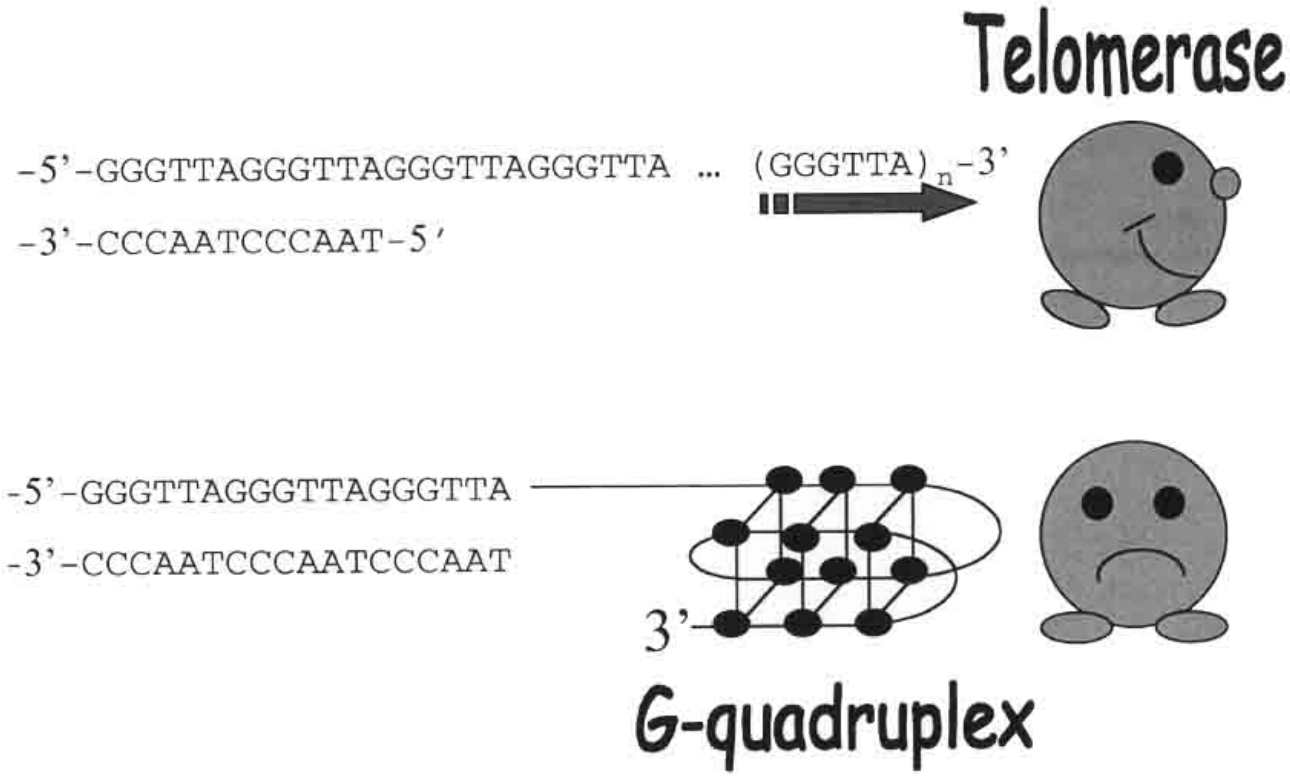

Fig. (3). Principle of telomerase inhibition by G-quadruplexes.
Telomerase requires a 3' protruding single stranded substrate. Folding of the G-rich strand into a quadruplex inhibits its activity.

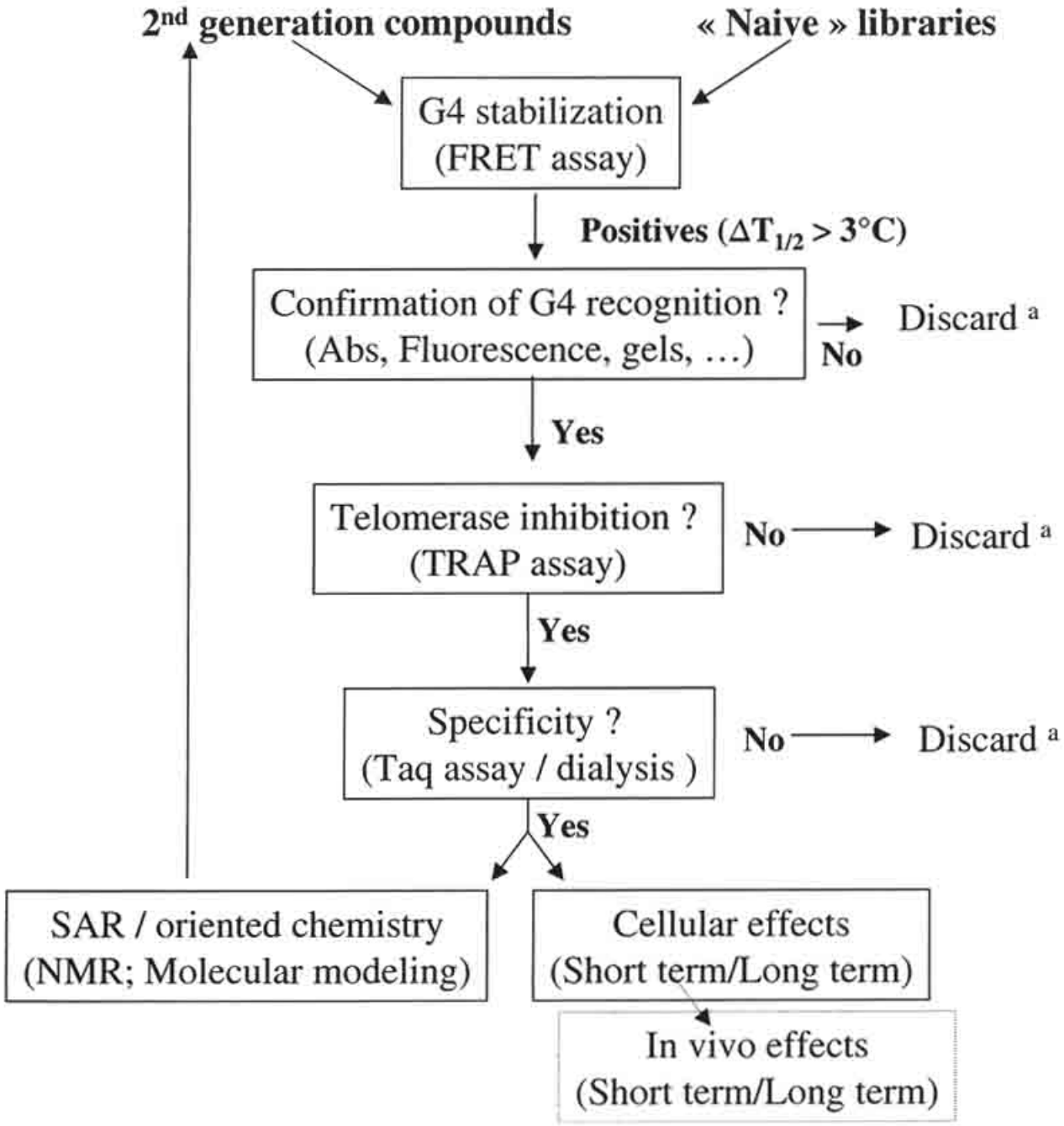

Fig. (4). Strategy for identification of G4-based telomerase inhibitors.
Over 1 000 molecules have been tested for their G4 stabilization potential by the FRET assay (see Fig. (**5**) for an example). G4 affinity is then confirmed by spectroscopic or biochemical methods. *Bona fide* ligands are then tested for telomerase inhibition (TRAP assay), and possible interference with PCR (Taq assay).
[a]) Discard : although these products will probably not be selected for *in vivo* inhibition of telomerase activity, they may be used for comparison purposes, SAR studies, …

These pioneering studies have opened up a new field in the area of ligand-DNA interactions. The strategy we have developed to isolate G4-based telomerase inhibitors is presented in Fig. (**4**). We use a fluorescence resonance energy transfer (FRET)-based method to discover new G4 based telomerase inhibitors [63,64]. An example of such an experiment is presented in Fig. (**5**). At least five independent families of ligands have been evidenced (ethidium derivatives [65], dibenzophenanthrolines [64], triazines [66], bisacridines [67] and benzoindoloquinolines [68]). Once stabilization is obtained with a given compound, we check that this molecule does interact with quadruplex structures. Various spectroscopic or biochemical methods are available to confirm this interaction. For example, some of these molecules

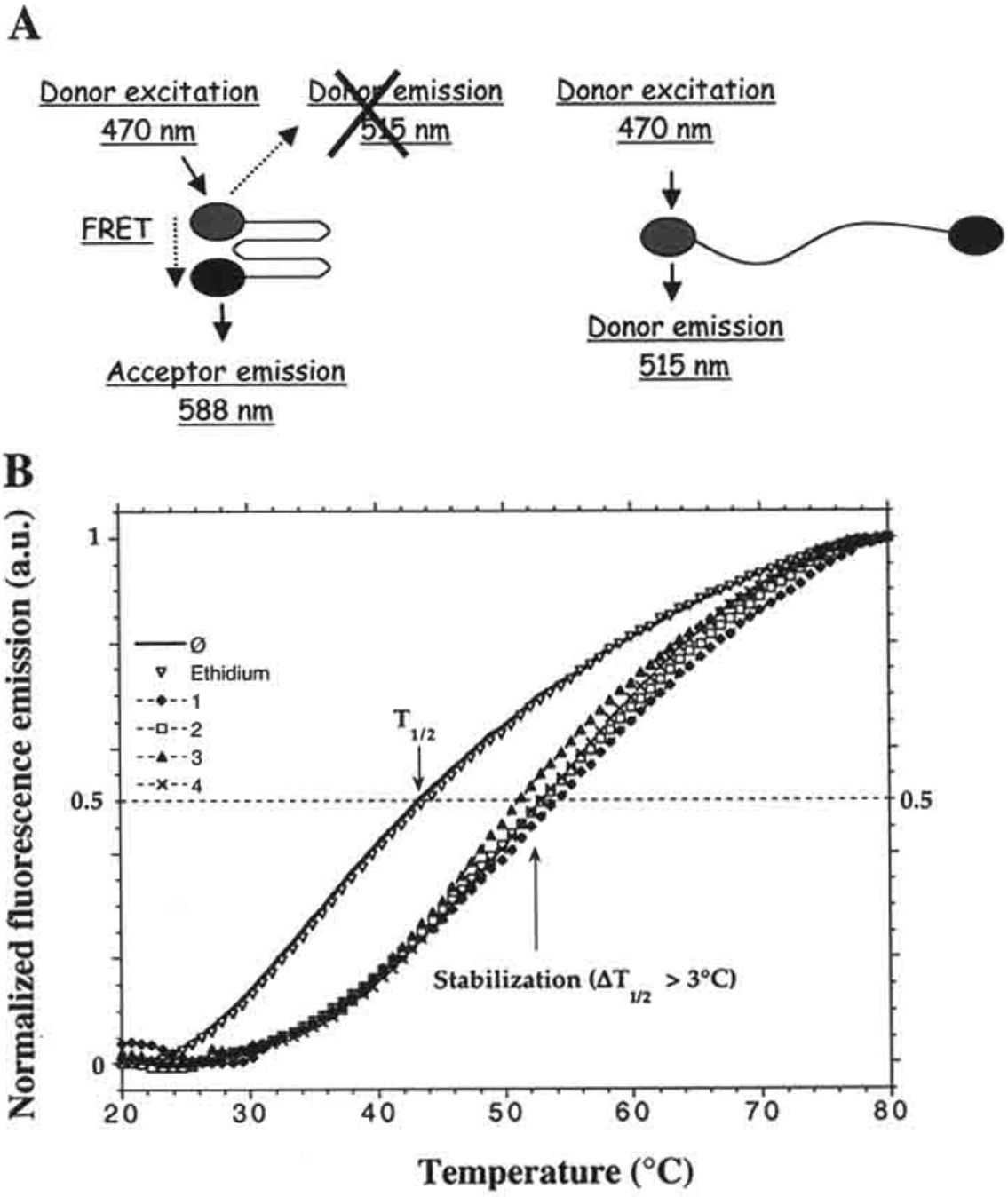

Fig. (5). A FRET assay for G4 binding.

A. Principle of the experiment. At low temperature, the G-rich oligonucleotide is folded into a G-quadruplex, leading to a juxtaposition of the two dyes (grey and black ovals). This close proximity leads to FRET from the donor (fluorescein) to the acceptor (either tetramethylrhodamine or DABCYL). When the temperature increases, the G-quadruplex unfolds and FRET disappears, leading to an increase in the emission intensity at 515 nm, which allows the determination of a half denaturation temperature ($T_{1/2}$).

B. Example of FRET denaturation profiles obtained with a series of ethidium derivatives. Solid line: oligonucleotide alone. Open triangles: ethidium (negative control ; no stabilization). All four other curves correspond to 4 different ethidium derivatives that provide a 7-11°C thermal stabilization of the quadruplex.

may act as quadruplex probes, as their fluorescence is altered in the presence of quadruplex DNA [65,67]. Their affinities for quadruplex DNA range from 10^6 to 10^8 M^{-1}. Besides demonstrating an interaction with a preformed quadruplex, it might be useful to test whether such molecules may accelerate or induce the formation of quadruplexes. Fig. (**6**) presents an example of quadruplex induction by a benzophenanthroline derivative, based on an assay designed by Hurley and Coll. [69].

The next step is to establish whether the *bona fide* G4 ligands inhibit telomerase. The now famous TRAP assay

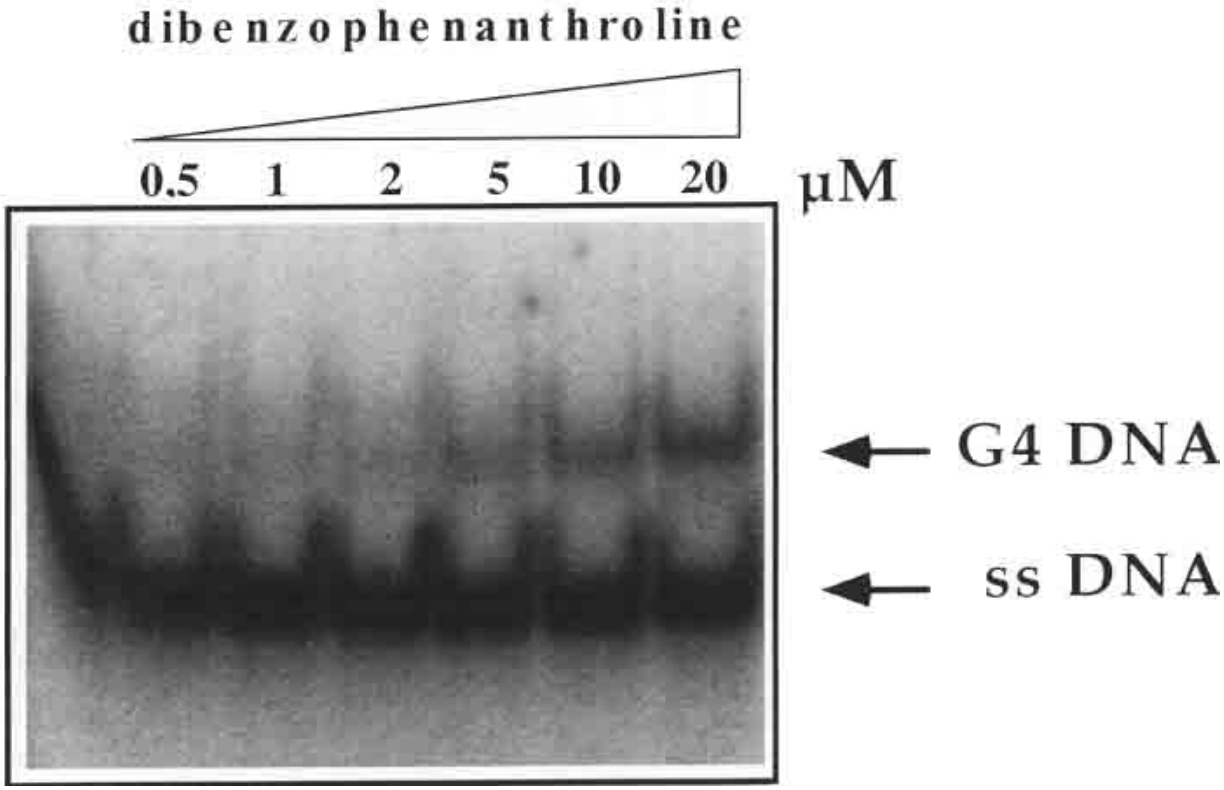

Fig. (6). G-quadruplex induction.

Induction of bimolecular G4-DNA by a dibenzophenanthroline [64]. The TRr2 oligonucleotide (d-TCAGATAGTTAGGGTTAGGGTTA, 8 μM strand concentration) was incubated at 20°C in a 1X Tris-EDTA buffer containing 0.1 M KCl. Compound **1** was added at a final concentration ranging from 0 (left lane) to 20 μM (right lane). All mixtures were loaded on a non-denaturing 12% polyacrylamide gel (0.5x TBE, 20 mM KCl) and run at 4°C for 6 hours (50V). ss-DNA corresponds to the single-stranded species, G4-DNA to the quadruplex. The amount of quadruplex species increases with benzophenanthroline concentration.

uses a polymerase amplification step after telomerase extension of a primer [70]. Many variations and improvements of this test have been proposed and many laboratories use related but not identical protocols for telomerase activity measurement. For these reasons a direct comparison of the concentrations that inhibit 50% of telomerase activity (IC_{50}) should be made with caution. Various G4-based telomerase inhibitors (identified by FRET or other methods) have an IC_{50} down to 0.02 μM [64-66,71].

Several crucial experiments dealing with the specificity of these molecules should not be neglected. First, the TRAP assay involves an amplification of the products by PCR. Some molecules may act on a TRAP assay by interfering with the second step of the test (the PCR amplification) rather than with telomerase. A classical PCR " Taq assay " with a plasmid substrate should be performed in parallel to analyze this possibility. Second, the specificity towards quadruplexes (vs. duplexes) is a major issue. In our hands, it has relatively been simple to find quadruplex ligands, and much harder to evidence quadruplex-*specific* ligands. A standard dialysis assay may help to evaluate this specificity [72,73]. Other techniques, such as fluorescence spectroscopy or surface plasmon resonance are also possible [71]. In all cases, the binding of a ligand to a quadruplex structure is compared to the binding to duplexes or single strands under identical conditions. Some compounds do exhibit a moderate specificity for quadruplexes. Unfortunately, this preferential binding is often unsufficient ([73]; Alberti & Mergny, unpublished observations). In the selection scheme proposed on Fig. (**4**) this specificity test would lead to the elimination of a large number of ligands. Such observation incite us to reconsider our strategy, and include a strong " selectivity requirement" at the very beginning of the process. For example, one may perform the FRET assay in the presence of a large excess of double-stranded DNA [63]. This excess duplex would trap unspecific G4 ligands, and only G4-specific molecules would induce a significant stabilization under these conditions. Another potential problem arises from the fact that only a few ligands exhibit a significant preference for the human telomeric intramolecular quadruplex as compared to other parallel or antiparallel quadruplexes. Large molecules such as antibodies may

distinguish these two conformations [49]. In contrast, triazines, ethidiums, bisacridines, and many other ligands discovered by FRET exhibit similar affinities for all types of G-quadruplexes studied by equilibrium dialysis (to be presented elsewhere).

A large number of quadruplex ligands have been found such as porphyrins [74-76], perylenes [77], amidoanthracene-9,10-diones [60], 2,7-disubstituted amidofluorenones [61] and indoloquinolines [78] (for a review, [52]). Most quadruplex ligands are polyaromatic molecules bearing one or more positive charge(s) (Fig. (**7**) and (**8**)). A notable exception to that rule is NMM [75] (compound #12 in Fig. (**8**)), an anionic porphyrin, which binds rather exclusively to quadruplexes. Equilibrium dialysis assay allows one to conclude that this derivative, despite a relatively low affinity perhaps thanks to its negative charge is the most selective quadruplex ligand studied so far [79].

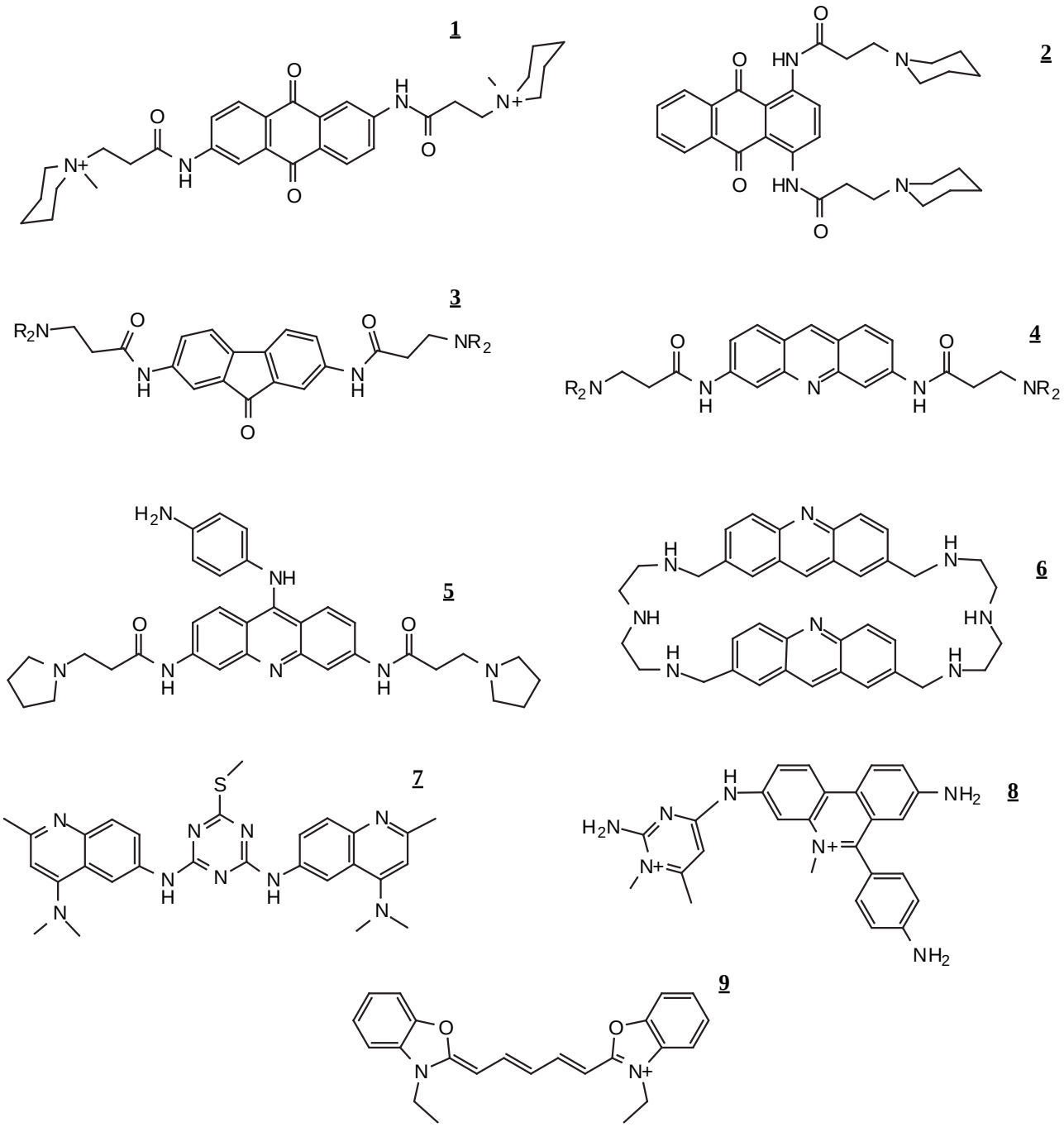

Fig. (7). " Small "G4 ligands.

Formula of some G4 ligands with 3 conjugated aromatic cycles or less: **1.** BSU-1051 (2,6 diamidoanthraquinone) [58,60], **2.** BSU-1071 (1.4 bis-piperidino amidoanthraquinone) [60], **3.** 2,7 disubstituted amidofluorenenone [61], **4.** 3,6 acridines [62] **5.** 3,6,9 trisubstituted acridine [71] **6.** Bisacridine [67] **7.** Triazine [66], **8.** Ethidium [65], **9.** DODC (carbocyanines) [96,97]. The properties of these molecules are detailed in Table I.

Table I. Telomerase Inhibitors

Family[a]	IC_{50}[b](μM)	Target [c]	Compound[d] #	References
Ribozymes	?	hTR	-	[42]
PNA	<0.001	hTR	-	[38,92,93]
2'OMe (oligonucleotide)	?	hTR	-	[37,38]
2'MOE (oligonucleotide)	0.005	hTR	-	[94]
2'-5'A- oligonucleotide	?	hTR	-	[95]
Phosphoramidates	<0.001	hTR	-	[39]
Dibenzophenanthrolines	0.03	G4	15	[64]
Benzoindoloquinolines	0.50	G4	16	[68]
Triazines	0.04	G4	7	[66]
Bisacridine	0.75	G4	6	[67]
Acridines	0.06	G4 (D/R)[e]	4-5, 18-20	[71,87]
Ethidiums	0.03	G4 (D/R)[e]	8, 17	[65,87]
Carbocyanines (DTC)	≥ 50	G4	9	[96]

a) Only the most active compound of each family is presented. 2'OMe and 2'MOE are oligoribonucleotides with a modified sugar on the 2' position.
b) IC_{50} of the most active compound belonging to that family.
c) Mechanism of action /target: G4: quadruplex ligands. hTR: the RNA component of telomerase is targeted. hTERT: the catalytic subunit is targeted.
d) The formula of some compounds are shown on Fig. (**7**), (**8**) and (**9**).
e) The mechanism of action may vary depending on the nature of the derivative. For example, ethidium does not act by a G4 induction, but is more likely to inhibit telomerase via a stabilzation of the DNA/RNA hybrid. On the other hand, an ethidium derivative such as compound 8 on Fig. (**7**) stabilizes quadruplexes. In the same line, acridine orange/acridine yellow may act by DNA/RNA recognition, whereas other acridine derivatives stabilize quadruplexes.

The next logical step would be to analyze structure-activity relationships (SAR) within a family of G4-ligands. Unfortunately, little structural data is available on the mode of interaction of these molecules with quadruplex DNA [77]. Possible binding modes are shown in Fig. (**2**), bottom. The geometry of many compounds suggests that they interact by stacking on a quartet. The surface of a quartet is much larger than the surface offered by a base pair, explaining in part how a large aromatic molecule may have a preference for quadruplex DNA, thanks to the favorable stacking interactions (see for example the compounds shown in Fig. (**8**)). True intercalation between adjacent quartet might be disfavored as a result of the important energetic penalty required to unstack these two quartets and to eject a monocation [80]. Nevertheless, some results suggest that this mode of binding might be observed in specific cases [81]. Experimental observations, by either NMR [77] or site-specific cleaving studies [82], tend to favor a related mode of binding, i.e. external stacking on a terminal quartet. However, interactions such as groove binding have to be considered as well since G-quartets are likely to form 4 different grooves and/or to expose adenine/thymine loops that may be specifically recognized by ligands. Loop recognition remains to be demonstrated. The paucity of structural data somewhat impairs a proper drug design approach and the synthesis of second generation ligands is often based on assumptions that cannot be easily verified.

The main goal of such an approach is to obtain agents that would target telomerase in cells and eventually *in vivo*. Little data is available on the cellular effects of quadruplex ligands. Cationic porphyrins are readily absorbed into tumor cell nuclei in culture and exert their antiproliferative effects via chromosomal destabilization [83,84]. We have performed long term culture assays in the presence of triazines. A delayed proliferation arrest was associated with a modest but significant telomere shortening [66].

4) Telomere Mimics

Studies have been carried out to inhibit telomerase activity using a series of PS-oligonucleotides with telomere sequence motifs of various lengths and sequences. The role of the 3' end and the secondary structure of telomere mimics have also been analyzed, showing that telomerase inhibition requires guanine nucleotides on the 3' end [85]. The best telomere mimic is a 9-base long phosphorothioate oligodeoxynucleotide (GGGTTAGGG) with an IC_{50} of 0.3 μM.

5) Agents Specific for Double-stranded Telomeric DNA

DNA minor groove-binding compounds (polyamides) that specifically target vertebrate telomeric repeats have recently been synthesized [86]. Epifluorescence microscopy studies show that fluorescent derivatives of these polyamides stain insect or vertebrate telomeres of chromosomes and nuclei sharply, allowing the rapid estimation of relative telomere length. A possible interference of these compounds with the binding of telomeric proteins and/or regulation of telomere length remains to be tested.

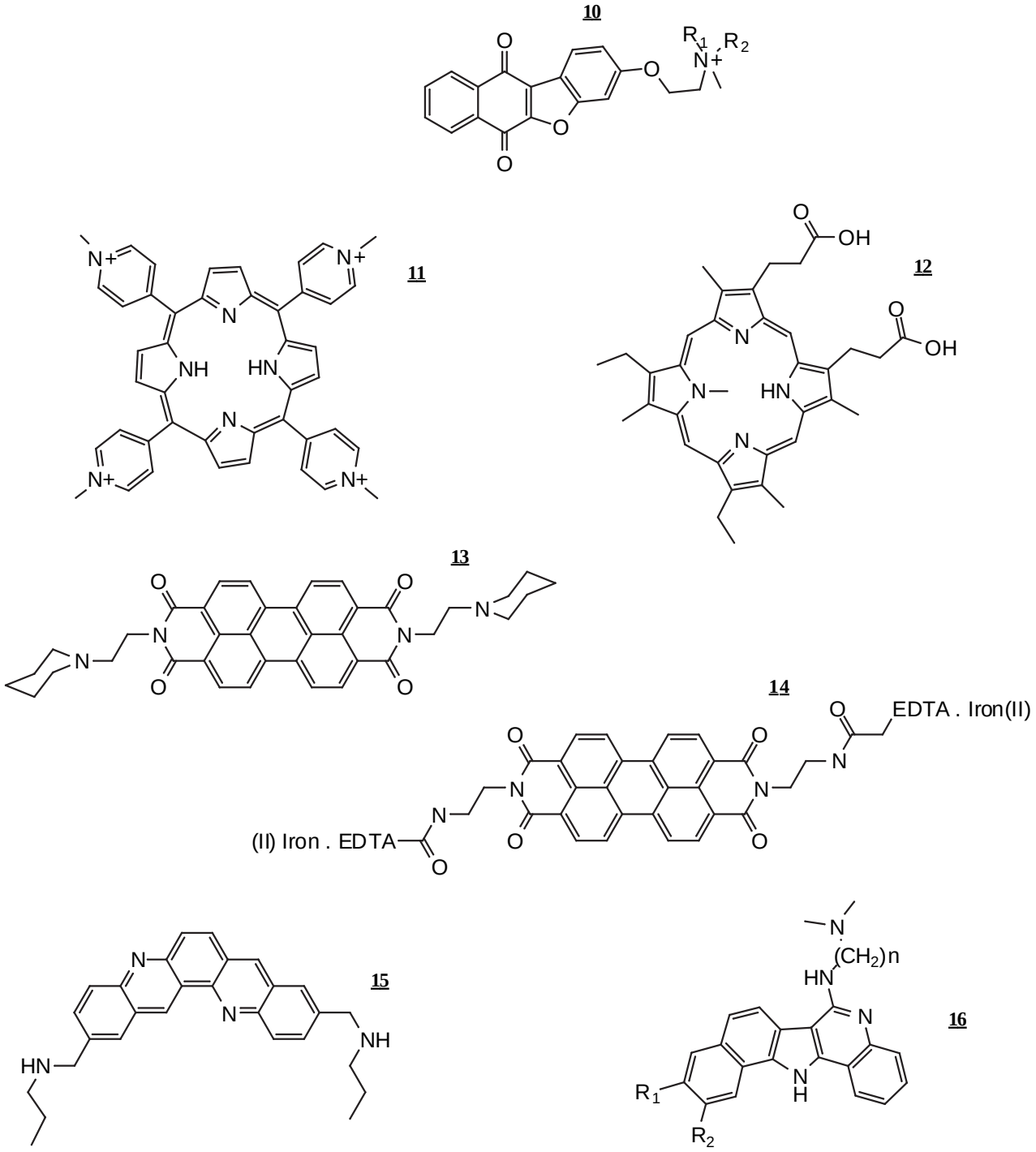

Fig. (8). " Large " G4 ligands.

Formula of some G4 ligands: **10.** benzo[b]naphto[2,3-d]furan-6,11-dione [98], **11.** TMPyP4 (cationic Porphyrin) [59,74,76,81], **12.** NMM (anionic porphyrin) [75], **13.** PIPER (perylenetetracarboxylic diimide derivative) [69,77] **14.** PIPER-EDTA [82] **15.** dibenzophenanthroline [64] **16.** Benzoindoloquinoline [68]. The properties of these molecules are detailed in Table I.

6) Targeting the RNA/DNA Duplex

Telomeric DNA synthesis by telomerase reverse transcription involves the formation of a transient DNA/RNA duplex of up to 11 base pairs. Molecules that bind to this duplex could inhibit the enzyme by either preventing strand dissociation or by sufficiently distorting the substrate, thereby causing a misalignment of key catalytic residues. These agents do not strictly target hTR, but its interaction with the substrate. Four intercalators show promising antitelomerase activity in the low micromolar range [87] and are shown in Fig. (**9**). Equilibrium dialysis [79] or affinity chromatography [88] should help to discover ligands that preferentially bind to this heteroduplex. A

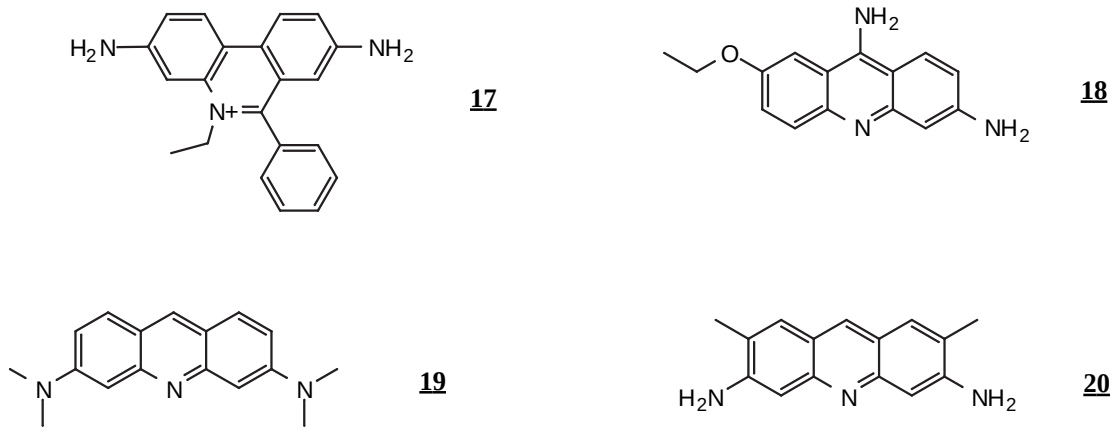

Fig. (9). DNA/RNA duplex ligands.

Formula of some DNA/RNA ligands [87]: **17.** Ethidium, **18.** Rivanol (6,9 diamino 2-ethoxy acridine), **19.** Acridine orange, **20.** Acridine Yellow. The properties of these molecules are detailed in Table I.

comparison of the binding of 84 different compounds to the polyrA.polydT hybrid led to the discovery of five compounds with higher than average affinity [89]. However, other key cellular processes involve RNA/DNA duplexes, such as Okasaki fragments occurring during the replication of the lagging strand, and these processes should be affected as well: RNase H activity is also inhibited by these ligands [89].

DISCUSSION

There are several potential (or verified) problems with the antitelomerase approach against cancer. Beside these specific problems, telomerase inhibitors may encounter the usual or classical obstacles found for all types of pharmacological agents, such as cellular uptake and localization, binding to other intra- or extra-cellular components, biodistribution, metabolism, hematological, renal or hepatic toxicities, *in vivo* half-life and pharmacokinetics. Furthermore, some organisms use telomerase-independent mechanisms to maintain their telomeres. In humans, some cancer cells (up to 15%), as well as some immortalized cells, do not express telomerase. For all these tumor cells, an approach targeting telomerase activity is unlikely to succeed.

Even for cells that use telomerase as the sole mechanism of telomere maintenance, one cannot exclude that the long term exposure to antitelomerase agents can lead to the selection of mutants that resist the drug through a variety of ways. Besides multidrug resistance phenotypes, which are not specific to telomerase inhibitors, the tumor cell could adapt by overexpressing telomerase or producing a mutated protein that is no longer sensitive to the drug, in a manner similar to the resistance to HIV-reverse transcriptase catalytic subunit inhibitors. The main cause of worry is the prediction that telomerase-dependent cells can spontaneously become telomerase-independent if a strong selection pressure is exerted by an antitelomerase agent. The likehood of such an event is difficult to predict.

According to the initial paradigm for telomerase inhibitors these agents should not affect growth rate initially but induce progressive telomere shortening. A decreased proliferation should only be observed when telomeres reach a critically short length. This paradigm has been verified by several key experiments that were used for target validation purposes. Unfortunately, only a handful of telomerase inhibitors induce telomere shortening and delayed cell growth [38]. Measuring telomere length of the tumor cells before starting a treatment might help to determine whether telomerase inhibition can be helpful or not.

Germ cells and some important somatic cells do possess an active telomerase, and telomerase inhibitors are expected to be effective on these cells as well. This opens several crucial questions: is this telomerase activity necessary for these cells ? What would be the effect of a transitory inhibition ? A few observations indicate that such toxicity can be tolerated: (i) these normal cells (and especially germ cells) generally have a longer initial telomere length than cancer cells; (ii) normal cells usually divide less frequently than tumor cells. Therefore, critical telomere shortening can be achieved in cancer cells before concerning normal cells.

Finally, the nucleic acids motifs presented here may also be present in other regions of eukaryotic genomes. As a result quadruplex ligands or RNA/DNA ligands could then have cellular effects independently of telomeres. Other DNA interacting enzymes also appear to be inhibited by quadruplex ligands, such as RecQ family helicases [90,91].

CONCLUSION

Fundamental advances have been performed in the comprehension of telomere physiology during the last few years. Nevertheless, important points remain to be elucidated, illustrating the undiminished need for strong basic science in that field. Major results have been obtained in the applied field of pharmalogical inhibition of telomerase, and various potent inhibitors have been recently isolated. They can tentatively be classified into two major classes: telomerase- or telomere-interacting molecules. Fundamental differences in terms of cell specificify, delayed *vs* immediate effects may be foreseen, and various

advantages or pitfalls may result from these differences. It is not yet possible to support the claim that the telomere is a better target than telomerase or vice versa. Many experiments presented in this review have been performed on a number of different cellular systems, using various markers to measure the effects of the compounds, which makes a comparison of these molecules difficult. Perhaps the choice of a few " standard " models should be made in order to rationalize this area of research. Nevertheless, the recent discovery of potent and specific agents of both classes should permit further target validation experiments in tumour-bearing mice and ultimately in cancer patients.

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ABBREVIATIONS

ALT = Alternating Lengthening of Telomeres.

hTERT = The human catalytic subunit of telomerase.

Also known as hEST2 or hTRT

hTR = The human RNA component of telomerase.

Also known as hTER, or hTERC

PNA = Peptide Nucleic Acid

FRET = Fluorescence Resonance Energy Transfer

TRAP = Telomere Repeat Amplification Protocol

SAR = Structure-Activity Relationships

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