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2,6-Diphenylthiazolo[3,2-*b*][1,2,4]triazoles as telomeric G-quadruplex stabilizers

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ABSTRACT

The design and synthesis of 2,6-diphenylthiazolo[3,2-*b*][1,2,4]triazoles characterized by a large aromatic building block bearing cationic side chains are reported. These molecules are evaluated as telomeric G-quadruplex stabilizers and for their selectivity towards duplex DNA by competition experiments. Two compounds (**14a**, **19**) were found active with high selectivity for telomeric G-quadruplex over duplex DNA.

Telomeres are guanine-rich DNA sequences located at the end of eukaryotic chromosomes, which protect them from fusion and degradation.[1] Human somatic cells undergo erosion of telomeres after each cell division,[2] leading to replicative senescence and apoptosis.[3] In contrast, most cancer cells are able to maintain telomere length either by the activity of telomerase or by recombination between telomeres (alternative lengthening of telomeres).[4] Almost two decades ago, it was shown that the telomeric G-overhang is able to fold into G-quadruplex structures, leading to inhibition of telomerase activity.[5] The telomeric G-quadruplex building blocks (called G-quartets) are based on stacked associations of hoogsteen bonded guanines, forming square aromatic surfaces whose dimensions are larger than the duplex DNA. This difference constitutes the basis for designing selective telomeric G-quadruplex ligands that are capable of stabilizing them so as to inhibit telomerase activity and reverse tumor cell immortalization.[6,7] Indeed, prolonged treatment of various tumor cell lines with telomeric G-quadruplex ligands has been shown to provoke a telomerase-like inhibition phenotype (including telomere shortening, delayed growth inhibition and senescence induction), but also telomere uncapping (including apoptosis, telomere fusion, anaphase bridges, G-overhang degradation and DNA damage to telomeres).[8,9]

So far, several telomeric G-quadruplex interacting ligands have been described (Scheme 1), such as acridine **1**,[10] anthraquinone **2**,[6] perylene **3**,[11] and dibenzophenanthroline **4**,[12] (for a recent review see Ref. 13). Most of them include a large aromatic core suitable for π-π stacking interaction with terminal G-tetrads and possess cationic side chains able to engage electrostatic bonds with DNA phosphates.

Previous studies from our laboratory focused on the synthesis and intercalative properties of a 2-phenyl-6-thiazolyl[3,2-*b*][1,2,4]triazole (PETT).[14] Because Hoechst 33258 was shown to present G-quadruplex binding properties[15] and displayed some scaffold similarities with PETT, we planned to develop new molecules based on this bicyclic condensed system (Scheme 2), presenting these features. It confers a crescent shape to the extended aromatic structure and above all, cationic chains substituting two lateral phenyl rings.

We present here the synthesis of a series of 2,6-diphenylthiazolo[3,2-*b*][1,2,4]triazoles **12a**,**b**, **13a**,**b**, **14a**,**b**, **15–19** and the ability of some of them to stabilize telomeric G-quadruplex.

The synthesis of substituted 2,6-diphenylthiazolo[3,2-*b*][1,2,4]triazoles was performed from ethyl 4-hydroxybenzoate. The thiazolo[3,2-*b*][1,2,4]triazole scaffold was prepared as

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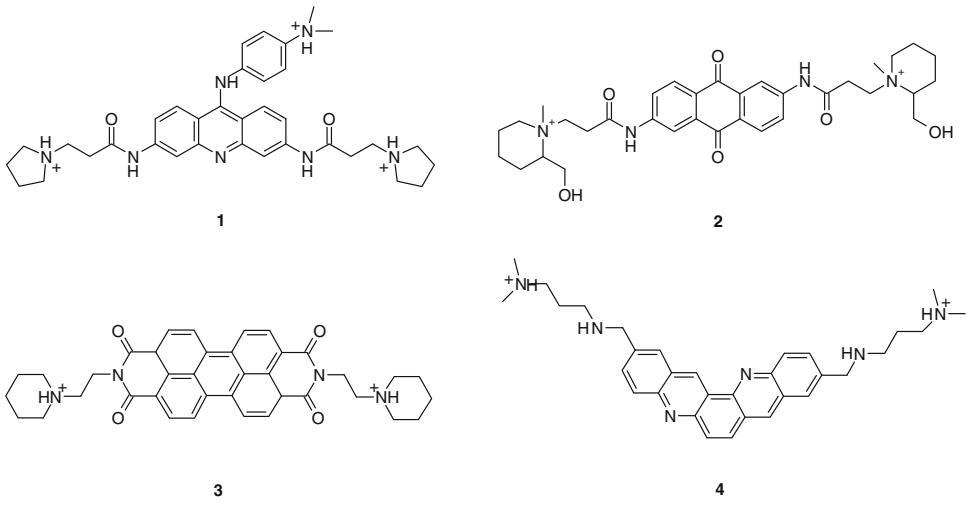
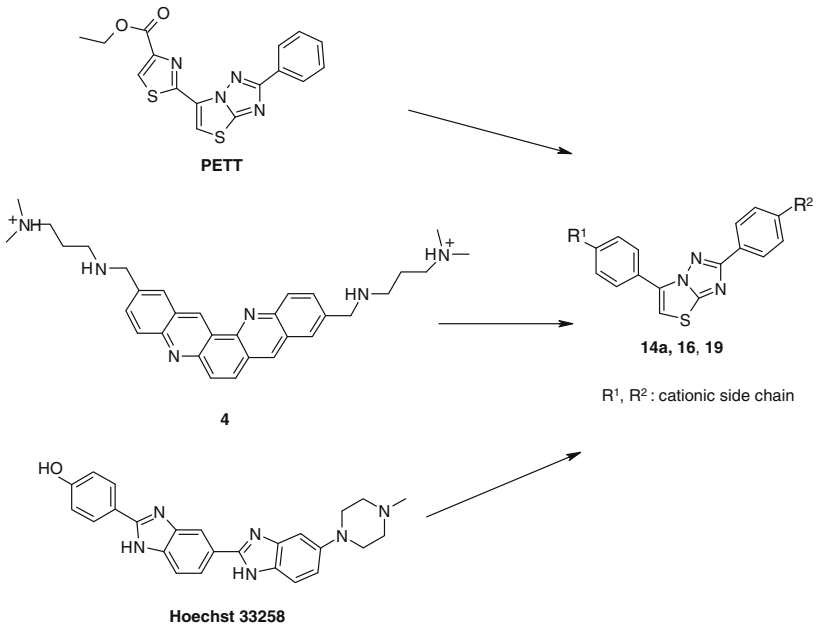
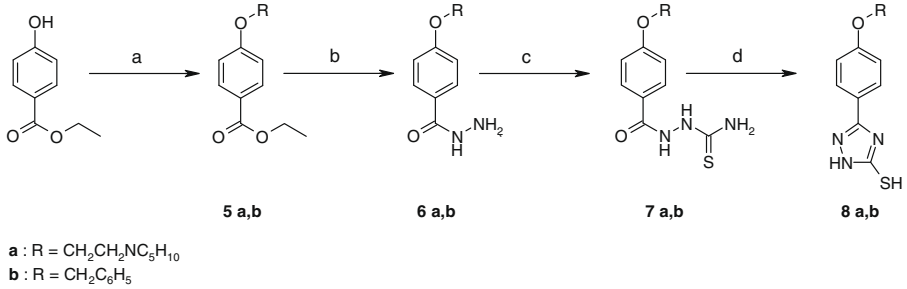


Scheme 1. Structure of some telomeric G-quadruplex ligands.

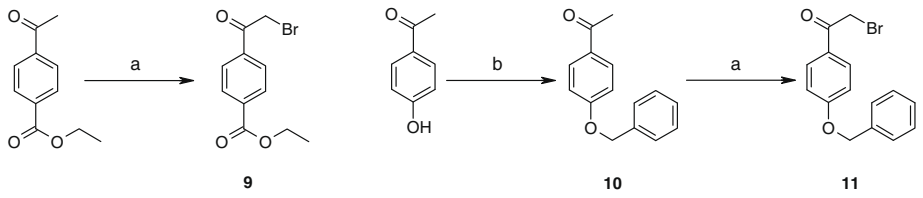


Scheme 2. Drug design of 2,6-diphenylthiazolo[3,2-*b*][1,2,4]triazoles.



Scheme 3. Reagents and conditions: (a) 1-(2-Chloroethyl)piperidine or benzyl bromide (1.2 equiv), K_2CO_3 (3.2 equiv), DMF, 80 °C, 12 h, 95%; (b) NH_2NH_2,H_2O (2.5 equiv), EtOH, reflux, 72 h, 82–88%; (c) NH_4SCN (2.5 equiv), 1 N HCl (2.5 equiv), EtOH, reflux, 60 h, 86%; (d) (i) 1% NaOH (2 equiv), 90 °C, 12 h, (ii) 1 N HCl (pH 6), 71–91%.

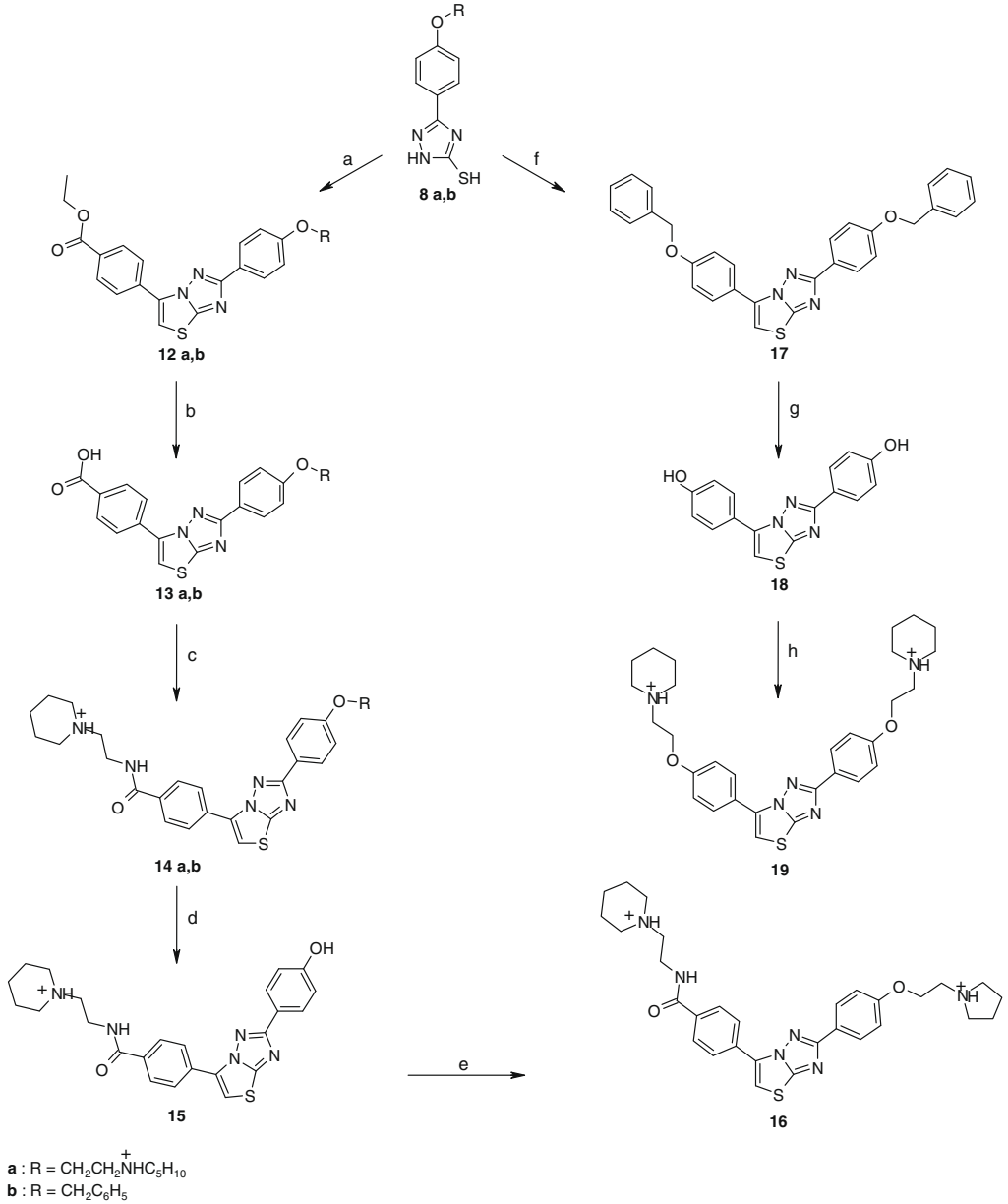
previously described,[16,17] using appropriate 3-mercapto-5-aryl-[1,2,4]-triazoles and α-bromoketones. The O-alkylated 3-mercapto-5-aryl-[1,2,4]-triazoles **8a**,**b** were obtained (Scheme 3) by O-alkylation of ethyl 4-hydroxybenzoate followed by the reaction of hydrazine monohydrate on ester (hydrazides **6a**,**b**); subsequent addition of ammonium thiocyanate in acidic conditions gave thiosemicarbazides **7a**,**b**. Cyclization in alkaline medium led to 3-mercapto-5-aryl-[1,2,4]-triazoles **8a**,**b** at very high yields.



Scheme 4. Reagents and conditions: (a) Br_2 (1.2 equiv), AcOH, rt, 12 h, 79–83%; (b) benzyl bromide (1.2 equiv), K_2CO_3 (3.2 equiv), DMF, 80 °C, 12 h, 98%.

α-Bromoketones **9** and **11** were synthesized (Scheme 4) using a modified version of the described procedure,[18] by bromination of the corresponding commercial methylketones (with previous benzylation of 4-hydroxyacetophenone).

Condensation and cyclization of mercaptotriazoles **8a**,**b** with appropriate α-bromoketones **9** and **11** led directly to 2,6-diphenylthiazolo[3,2-*b*][1,2,4]triazoles **12a**,**b** and **17** at high yields (Scheme 5). After saponification of the esters **12a**,**b** (lithium hydroxide), the carboxylic acids **13a**,**b** were engaged in an amidation reaction with aminoethylpiperidine under peptidic conditions (compounds **14a**,**b**). Further O-debenzylation of **14b** (and **17**) leading to hydroxyphenylthiazolotriazole **15** (and **18**) was found to be



Scheme 5. Reagents and conditions: (a) **9** (1 equiv), EtOH, reflux, 72 h, 86–77%; (b) (i) 0.1 N LiOH (4 equiv), THF, rt, 12 h, (ii) 1 N HCl (pH 5), 94%; (c) (i) 1-(2-aminoethyl)piperidine (1.5 equiv), HOBt (0.5 equiv), HBTU (1.5 equiv), DIPEA (2 equiv), DMF, rt, 12 h, (ii) HCl/*i*-PrOH, 75%; (d) (when **14b** in its free base form) (i) BBr_3 (1 M in CH_2Cl_2, 3 equiv), CH_2Cl_2, −78 °C, 1 h, (ii) rt, 12 h, (iii) HCl/*i*-PrOH, 86%; (e) (i) 1-(2-chloroethyl)pyrrolidine (1.2 equiv), K_2CO_3 (3.2 equiv), DMF, 80 °C, (ii) HCl/*i*-PrOH, 21%; (f) (when **8b**) **11** (1 equiv), EtOH, reflux, 72 h, 79%; (g) (i) BBr_3 (1 M in CH_2Cl_2, 6 equiv), CH_2Cl_2, −78 °C, 1 h, (ii) rt, 12 h, 81%; (h) (i) 1-(2-chloroethyl)piperidine (2.4 equiv), K_2CO_3 (6 equiv), DMF, 80 °C, (ii) HCl/*i*-PrOH, 64%.

Table 1
Experimental conditions used for debenzylation of benzyl ether **14b**

Entry	Experimental conditions	Yield (%)
1	H_2/Pd, MeOH, rt, 12 h	0
2	H_2/Pd, MeOH, 50 °C, 12 h	0
3	H_2/Pd, MeOH, 50 °C, 50 bars, 12 h	0
4	H_2/PtO_2, 50 °C, 50 bars, 12 h	0
5	BBr_3, CH_2Cl_2, rt, 16 h	18
6	BBr_3, CH_2Cl_2, -78 °C, 1 h, then rt, 12 h	86

effective with boron tribromide (low temperature), whereas classical catalytic conditions failed (Table 1). Finally, O-alkylation was completed with the appropriate chloroethylamine to give the target compounds **16**, **19**.

The planarity of the diphenylthiazolo[3,2-*b*][1,2,4]triazole system was proved thanks to the X-ray structure of ester **12a** (Fig. 1) and was essential in enabling this aromatic system to establish aromatic–aromatic stacking with G-tetrads.

Compounds **12b**, **14a**,**b**, **15–19** were evaluated for their ability to bind and stabilize a telomeric G-quadruplex structure by a fluorescence resonance energy transfer (FRET) assay using the 21-mer d[$(GGGTTA)_3GGG$] oligonucleotide end-labeled with a fluorescent donor–acceptor pair (F21T).[20,21] The change in F21T emission in the presence of the tested compounds (3 μM) was monitored as a function of temperature whether it be in Na^+ (NaCl 100 mM) or K^+ (KCl 10 mM; LiCl 90 mM) conditions.[22] The resulting ΔT_m values provide an indication of the stability of a ligand–quadruplex complex and are summarized for Na^+ and K^+ (Table 2).

These results indicate that compounds **14a**, **16** and **19** induce a significant stabilization of the telomeric G-quadruplex (ΔT_m >1 °C) in both Na^+ and K^+ conditions. In agreement with previous studies, these compounds were found more active in K^+ than in Na^+[22,23] and presented two cationic side chains able to interact with the groove and/or the negatively charged phosphate backbone of a G-quadruplex.[13] Compounds **12b**, **14b**, **17** and **18** did not present significant G-quadruplex stabilizing properties. The presence of a piperidinoethylaminocarbonyl chain (compound **15**) in place of one hydroxyl group of the inactive derivative **18** produced moderate stabilizing properties in K^+ conditions. Replacement of the remaining hydroxyl of molecule **15** by a benzyl ether (compound **14b**) did not improve activity. Replacement of this benzyl group by a piperidinoethyl group (compound **14a**) or by a pyrrolidinoethyl group (compound **16**) significantly improved the biochemical properties. Finally, the presence of an oxygen (compound **19**) instead of the amide group (compound **14a**) did not significantly reduce activity. In contrast, the replacements of the two piperidinic chains of compound **19** by two benzyl groups (compound **17**) completely eliminated G-quadruplex stabilization and highlight the importance of two cationic side chains.

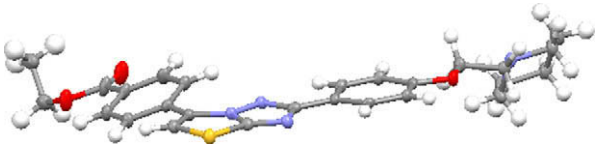


Figure 1. Perspective view of **12a**.[19]

Table 2
F21T FRET-based ΔT_m values[a] (°C) for compounds **12b**, **14a**,**b**, **15–19** (3 μM) at the indicated ionic conditions

Compounds	Na^+	K^+
12b	<1	<1
14a	5.0 (±1.0)	11.2 (±1.2)
14b	<1	2.7 (±0.5)
15	<1	1.8 (±2.3)
16	5.9 (±1.8)	14.6 (±0.6)
17	<1	<1
18	<1	<1
19	2.1 (±0.2)	8.7 (±0.3)

[a] Values are means of three experiments, standard deviation is given in parentheses (<1 = inactive compound).

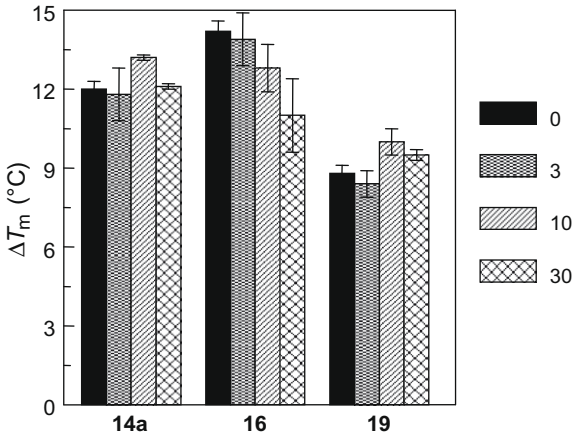


Figure 2. Stabilization (ΔT_m for the F21T telomeric G-quadruplex at 0.2 μM) for compounds **14a**, **16** and **19** (3 μM) in the presence of ds26 competitor (0, 3, 10 and 30 μM).

To determine the selectivity of the interaction for the telomeric G-quadruplex relative to duplex DNA, the melting temperature of F21T (0.2 μM) in the presence of compounds **14a**, **16** and **19** (3 μM) was monitored (Fig. 2) with a 26-oligonucleotide duplex (ds26) competitor (at 3, 10 and 30 μM). In the presence of 30 μM ds26 oligonucleotide (i.e., a 150-fold molar excess), the stabilization induced by compounds **14a** and **19** was not significantly lowered, demonstrating that these two compounds display excellent selectivity for G-quadruplex relative to duplex DNA. In contrast, the stabilization induced by compound **16** decreased by about 3 °C in the presence of 30 μM ds26, indicating that this compound displays lower selectivity for G-quadruplex. This concords well with the work[24] which showed that the replacement of the tertiary amine piperidine by pyrrolidine provoked a slight increase of G-quadruplex stabilization but also a decrease of selectivity. The importance of the steric factor as well as the nature of the amine substituent at the termini of the side chain and the ligand core conformation have already been found critical in terms of selectivity and affinity for G-quadruplex.[25–27]

The putative binding modes of compounds **14a** and **16** to the telomeric G-quadruplex structure were then investigated by a docking study carried out with Gold 4.0.1.[28] Both compounds behaved similarly, yielding a single conformation forming a large stacking with the bases of the terminal G-quartet (Fig. 3).

Comparison of the G-quadruplex stabilization by **14a** and **19** with previously published derivatives such as telomestatin[29] or pyridine dicarboxamide 360A[30,31] indicated that the ligands tested here are, respectively, 5- and 30-fold less potent (based on the concentration of ligand necessary to obtain the same stabilization).[32]

The data presented here indicated that the melting profile of **14a** and **19** was almost unaffected by the presence of 150 molar equivalent of unlabeled ds-DNA competitor and represented one of the highest levels of selectivity that we are aware of for the telomeric G-quadruplex over duplex DNA,[13,22] although ΔT_m values are not the highest reported for G-quadruplex interacting ligands. Therefore, these compounds represent interesting molecular

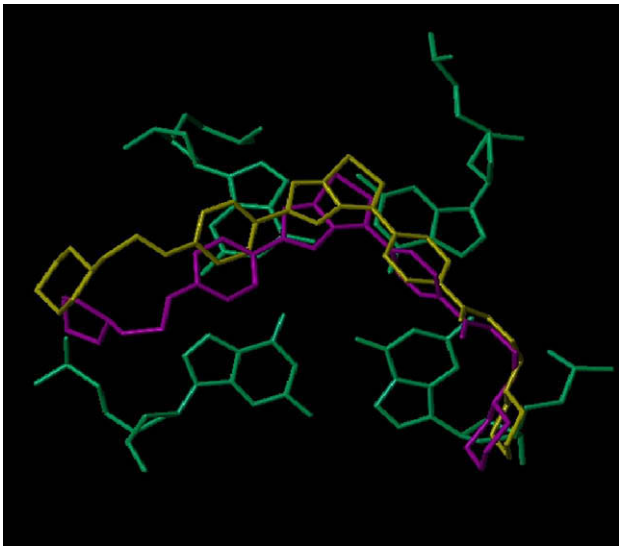


Figure 3. Docking of compounds **14a** (yellow) and **16** (magenta) on the G-quartet structure (green).

probes or tools to analyze the biological processes affected by G-quadruplex stabilization. This work has revealed the interest of substituted diphenylthiazolo[3,2-*b*][1,2,4]triazole as a promising scaffold for the design of telomeric G-quadruplex stabilizers. Further studies -with isomeric diphenylthiazolo[2,3-*c*][1,2,4]triazoles conferring an orientation to various charged side chains close to the one recently described[26,27]—are ongoing and will be presented in due course.

Acknowledgments

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