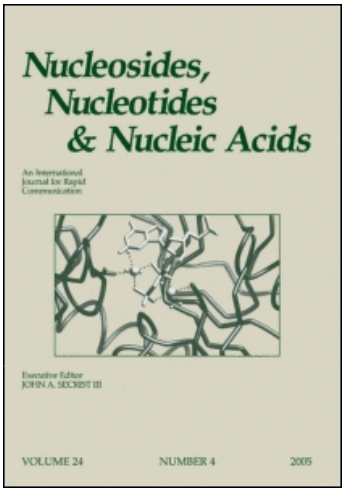

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Novel Short Oligonucleotide Conjugates as Inhibitors of Human Telomerase

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ABSTRACT

A series of oligonucleotide conjugates were designed and synthesized as novel inhibitors of human telomerase. These compounds contain a relatively short (6–7-mer) oligonucleotide domain, with an N3′ → P5′ phosphoramidate (np) or *thio*-phosphoramidate (nps) backbone, targeted to the template region of the RNA component of the enzyme and various pendant groups attached to either their 5′- or preferably to the 3′- termini. The most potent compounds in the series inhibited telomerase with low nM IC_{50} values in biochemical assays whereas the cognate oligonucleotides without the pendant groups were significantly less active having IC_{50} values 100-1000-fold higher.

Key Words: Telomerase inhibitors; Oligonucleotide thio-phosphoramidates.

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RESULTS AND DISCUSSION

Telomerase is a specialized reverse transcriptase whose main function is telomere maintenance. The vast majority of tumor cells have been shown to express telomerase and its inhibition can induce cell senescence, cell crisis and apoptosis.[1] The RNA component (hTR) of human telomerase can be targeted with oligonucleotides comprising various sugar-phosphate backbones. We have previously shown that 11-15-mer oligonucleotides, N3′ → P5′ phosphoramidate (np) and *thio*-phosphoramidate (nps), targeted to template and non-template regions of hTR, have good inhibitory activity in both biochemical assays and in cells.[2] Here we report the synthesis and anti-telomerase activity of relatively short (6–7-mer) oligonucleotide phosphoramidate conjugates, containing dyes or polyaromatic groups. The IC_{50} values for these compounds with sequences TTAGGG, GGGTTAG or GTTAGG and various pendant groups are shown in Table 1. Oligonucleotide conjugates with *thio*-phosphoramidate backbones (nps) exhibit better inhibitory activity than their phosphoramidate (np) counterparts. Comparison of the various dyes shows that the presence of hydroxyl groups in their aromatic systems (fluorescein vs. TAMRA) improves their inhibitory activity, while introduction of a 3′- flexable linker between the dye and the oligonucleotide results in decreased activity. Conjugates containing the DNA/RNA intercalator acridine are inferior to the dye containing molecules. Lastly, the 3′-trityl conjugates of sequence TTAGGG showed better inhibitory activity than those of GGGTTAG.

In brief summary, telomerase inhibitory activity of short oligonucleotide conjugates is determined by the oligonucleotide sequence, their sugar-phosphate backbone (np vs. nps backbone), the attachment site (5′ vs. 3′), the type of the linker

Table 1. Structures and IC_{50} values in biochemical assays for oligonucleotide N3′ → P5′ phosphoramidate (np) and *thio*-phosphoramidate (nps) conjugates.

Compound	IC_{50} (μmM), np	IC_{50}(μmM), nps
TTAGGG	10	1.36
TTAGGG-*fluorescein*-3′	0.055	0.015
TTAGGG-L-*fluorescein*-3′	1.9	0.017
TTAGGG-(L-*fluorescein*)$_2$-*3′*	0.023	n.d.
TTAGGG-*TAMRA*-3′	0.51	0.044
TTAGGG-L-*TAMRA*-3′	15	n.d
TTAGGG-*Oregon Green*-3′	0.046	n.d.
TTAGGG-*trityl*-3′	0.011	0.005
TTAGGG-*pyrene*-3′	0.856	n.d.
5′-*acridine*-TTAGGG	0.593	0.085
GGGTTAG	0.48	0.008
GGGTTAG-*fluorescein*-3′	0.153	0.004
GGGTTAG-L-*fluorescein*-3′	0.059	0.004
GGGTTAG-*trityl*-3′	0.684	0.053
GTTAGG	0.644	0.205
GTTAGG-*fluorescein*-3′	0.259	0.040
GTTAGG-L-*fluorescein*-3′	1.66	0.014

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(rigid thio-urea vs. relatively flexible hydrocarbon chain), and the structure of the pendant group. While the parent short oligonucleotides do not bind very strongly to the template region of hTR, their conjugation with the pendant groups may provide for additional stabilizing interactions with the telomerase protein component (hTERT) resulting in significant (by up to ~1000 fold) increases in their inhibitory activity.

The dyes were attached either via a thio-urea group directly to the terminal 3′-amino group of the oligonucleotides (–), or through $(CH_2)_5$-$(CH_2)_6$ containing phosphoramidate linkers (L).

Cellular activity was assessed for a selected group of *thio*-phosphoramidate conjugates using a cell-based telomerase detection TRAP (telomere repeat amplification protocol) assay in several cancer cell lines. The telomerase inhibition activity was found to be in the low μM range. Experiments using *thio*-TTAGGG-*fluorescein* have also demonstrated cellular uptake of this compound in A431, HME50-5E and HT-3 cells, and in the presence of oligofectamine, efficient nuclear delivery was detected.

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